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POTENCY OF CAFFEIC ACID COMPOUNDS IN OIL LEAVES (MORINGA OLEIFERA) AGAINST CARBONATE ANHYDRASE ENZYME AS ANTI-CANCER IN SILICO

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ABSTRACT

Moringa (Moringa oleifera) is a plant that can grow in tropical and subtropical areas. Cranberries are used as food and medicines to cure a variety of diseases. Cello contains a variety of active compounds, one of which is caffeic acid compound. The main mechanism of action of the caffeic acid compound is as an inhibitor of the enzyme carbonate anhydrase, which is being developed as an anti-cancer mechanism. The study aims to identify the potential of the caffeic acid compound in the Moringa oleifera leaf against the enzyme carbonate anhydrase as an anti-cancer in silico. Tests are carried out in silico (Molecular Docking) using a computing device with Pyrx software. Silico tests are conducted to predict pharmacokinetics and physiochemical properties, predict bioavailability, toxicity and continue with molecular docking. The inhibitory activity of carbonate anhydrase enzymes in silico is seen from the binding affinity values as well as the ligan-protein interaction. It was concluded that caffeic acid has the same potent potential as acetozolamide as an inhibitor of carbonate anhydrase, which can be used as a candidate anti-cancer drug.

Keywords: anti-cancer; caffeic-acid; carbonate-anhydrase; in-silico; moringa-oleifera

BACKGROUND

Moringa oleifera leaves are famous for their high nutritional content, including vitamins, minerals, proteins, and bioactive compounds. Several studies have shown that coriander leaf extract contains compounds with antioxidant potential, which can help protect cells from oxidative damage. Oxidative damage can contribute to the progression of diseases, including cancer. (Ferreira et al., 2015)(Mumtaz et al., 2021).

The plant has phenolic compounds such as flavonoids, tannins, terpenoids, alkaloids and saponins. Several studies have proven that the compounds contained in Moringa oleifera L are potentially medicinal and have bioactivity, including anti-inflammatory, antifungal, antibiotic and anti-cancer and antioxidant activity. Testing the antibacterial activity of calorie leaf extract against staphylococcus aureus and E.coli bacteria using diffusion methods by way of wells (Stohs & Hartman, 2015)(Stohs & Hartman, 2015).

The purpose of this research is to find out the binding affinity value of caffeic acid compounds to carbonate anhydrase enzyme proteins compared to the acetazolamide comparator ligans; to know the link between the compound caffic acid to the carbonate enzymes carbonate protein compared with the lignan comparator Acetzolamid;

The benefit of this research is that it can provide scientific information about the anti-cancer mechanisms of the caffeic acid compounds found in the leaves of the moringa.

METHODS

The design of the research we conducted was a study using the insilico (Molecular Docking) method on the Caffeic acid compound from the moringa plant (Moringa oliefera) that can be used as an anti-cancer and antiseptic medicine. The research location was carried out at the Medical Chemistry Laboratory, S1 Pharmaceutical Study Program, STRADA Health Sciences Institute Indonesia. (Sekretariatan : Jl. Manila No.37, Sumberece, Kec. Pesantren, Kota Kediri). Research is carried out in February – June 2024 for the preparation of research plans, data collection and processing, up to reporting.
The research tools used are intel core i5 gen 12 RAM 8 gb, Chemdraw, swissADME, pyrx, discovery studio, RSCB PDB, pubchem, chembl. The ingredient used is a caffeic acid compound from the Moringa oleifera, as a comparison using acetazolamide, a protein of the enzyme carbonate anhydrase that acts as an anti-cancer receptor that is being developed extensively.

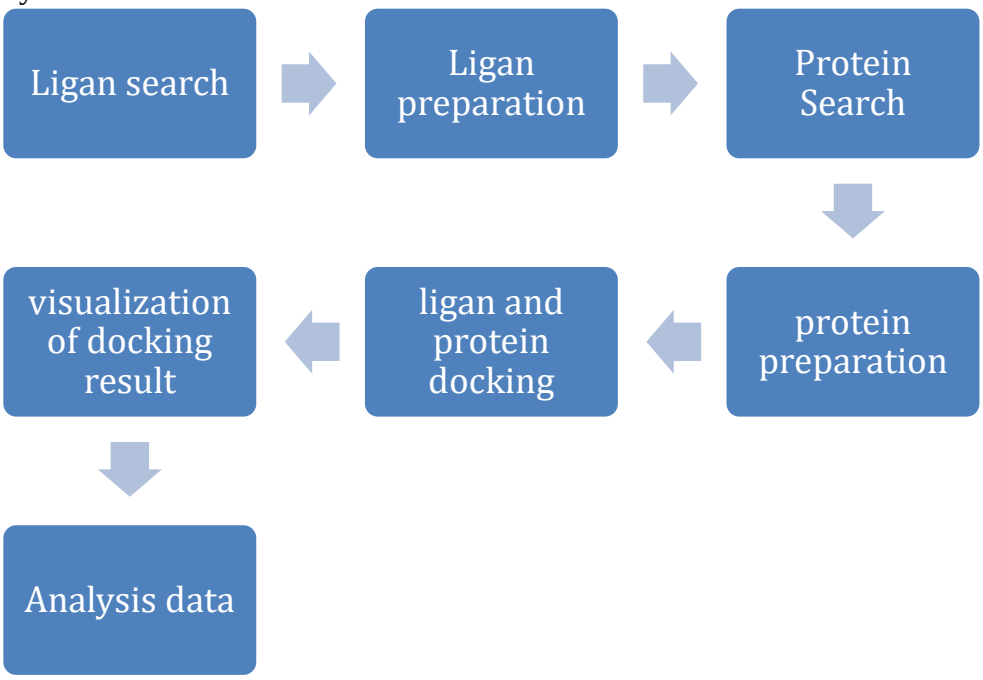

Figure 1. Block diagram of the research method

The first step is search for a ligan by way of opening PUBCHEM and find a storage compound used to be a ligant compounds in the form of convert 3d SDF. Next, the preparation of the ligan. A ligan prepared by open Discovery studio and choose the ligans in the format sdf. Then the ligans saved in the format pdf. This is the format that can be openned in the pyrx docking. The next step is searched the protein by copying SMILLES from the compound in SwissADME. After that we paste and choose the carbon anhydrase 2. To find out the comparison, select CHEMBL which is next to Unipro on the SwissADME website then go to RSCB PDB. This step is to find the carbon anyhydrase 2 protein and save it in GDP form. The fourth step is protein preparation. Open the discovery studio to make preparations of unnecessary ingredients, such as traces in water protein and so on, then store in form of Pdb. The fifth step is ligan and protein docking. This step is done by opening Pyrex then opening the protein and selecting insert new molecule then the ligand will enter. Next, to activate macro molecular autodock, you can do this by right clicking on the protein text then selecting auto dock, selecting macromolecular and selecting auto dock ligand. Then select minimize all and start vina wizard then the binding of the ligand and protein will come out, do a comparison between the compound and acetazolamide. The last step is Visualization

of docking result. To see the visualization of the docing results, you can open the Biovia Discovery Studio software. Next, select file, open, and select the target protein file that has been prepared. Next, select file, insert from, file, and select the test ligand complex file. Then select Non Bound Interaction, select all the boxes in the Favorable option, select intermolecular in the molecular space option, press OK scroll down select "Show 2D Program" then the chemical structure of the autodocking results will appear.
In Silico data analysis using Discovery Studio work. This analysis is carried out to determine the results of interactions between ligands and macromolecules in proteins, amino acid residues, bond types and bond distances. Visualization with discovery studio software aims to determine the interactions that occur between ligands and proteins. Discovery Studio can display the ligand interactions that occur as well as what Moringa Leaf (Moringa oleifera) residues are involved in the binding process between ligands and proteins. The advantage of discovery studio software is that it can display interaction results in 3D or 2D (Faqih et al., 2019). Pharmacokinetic prediction uses the SwissADME application and the results obtained later are bioavailability radar. physicochemical properties, lipophilicity, pharmacokinetics (ADME).

RESULTS

Prediction results of pharmacokinetics and physicochemical properties of Caffeic Acid compounds via the web page http://www.swissadme.ch/index.php

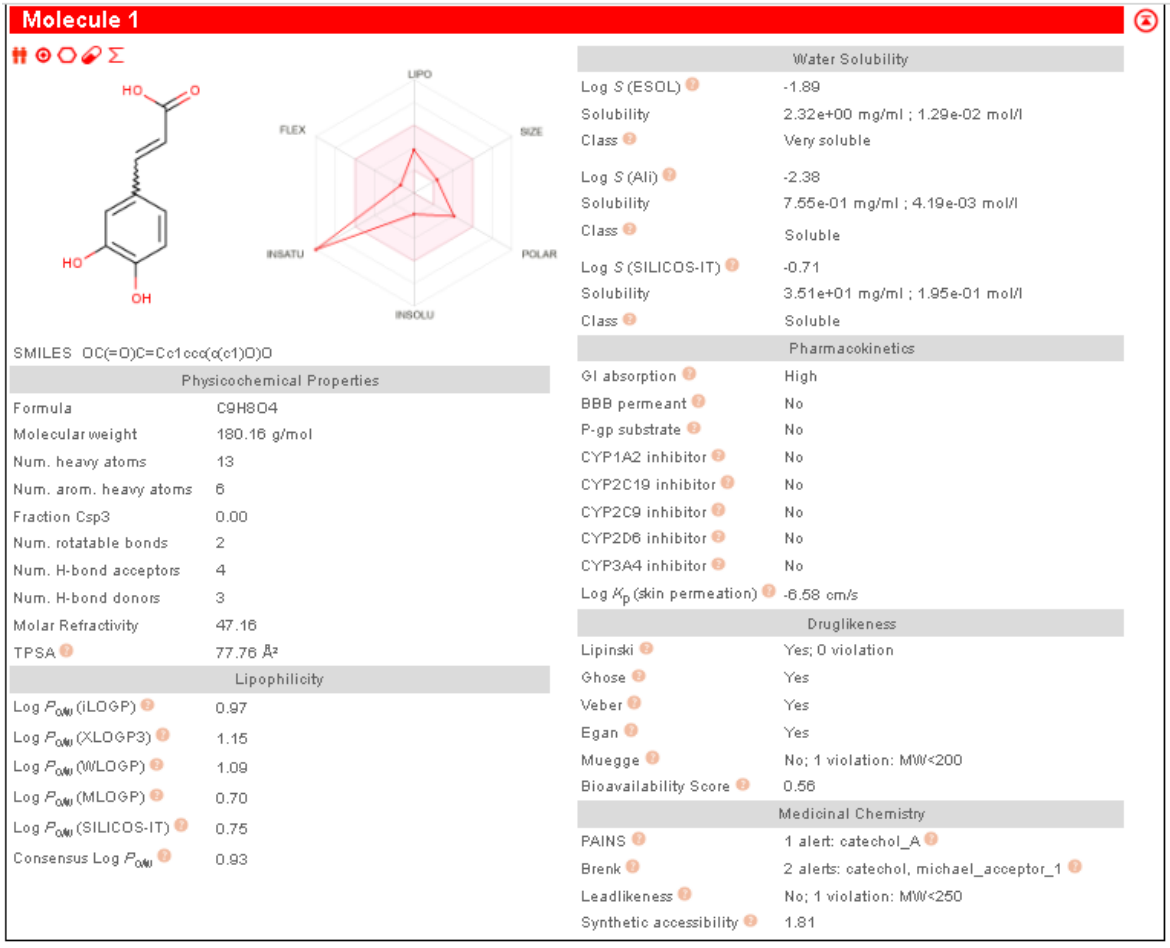

Figure 2. Cafeic Acid Profil

Determining the suitability of a compound to be used as an oral drug for a molecule can be continued with a docking simulation if it meets the Lipinski criteria as follows:

Table 1. Suitability of the caffeic acid.

Criteria	Caffeic Acid	Lipinski Criteria	Conclusion
Molecule weight	180,16 g/mol	Less than 500 Da or g/mol	Meets the Lipinski Conditions
Log P value	0,97	Less than 5 (XLOGP)	Meets the Lipinski Conditions
Number of hydrogen bonds	3	Less than 5	Meets the Lipinski Conditions
number of hydrogen bond acceptors	4	Less than 10	Meets the Lipinski Conditions

Table 2. Pharmacokinetic Prediction of Caffeic Acid Compounds

No	Parameters	Prediction results	Criteria	Information
1	GI Tract Adsorption	High	High, Medium, Low	High compounds are adsorbed in the digestive tract
2	Permeability of the blood brain barrier (BBB)	No	Yes/No	The compound does not penetrate the Blood Brain Barrier
3	P-gp substrate	No	In the intestine P glycoproteins pump the drug back into the lumen thereby reducing adsorption	The compound does not contain p glycoprotein so that adsorption in the intestine does not decrease
4	Metabolisme CYP1 A2 inhibitor CYP2 C19 inhibitor CYP2 C9 inhibitor CYP2 D6 inhibitor CYP3 A4 inhibitor	 No No No No No	• CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 are 5 isoenzymes that play a role in drug elimination. • Inhibition of this isoenzyme causes drug-drug interactions. • Inhibition of this isoenzyme causes toxic effects or other undesirable side effects due to lower elimination	The Caffeic acid compound does not inhibit the 5 isoenzymes so it does not reduce drug elimination and does not cause toxicity and side effects
5	Permeability of the skin Log Kp cm/s (centimeters/second)	-6.58 cm/s	If log Kp> -2.5, it means that the compound has little penetration into the skin	The log Kp value of the Caffeic acid compound is smaller than -2.5, meaning that penetration into the skin is large

Table 3. Bioavailability prediction

No	Parameter	Prediction result	Criteria	information
1	LIPO (Lipophilicity)	1,15	-0,7 < Log P (XLOGP3) < 5,0	
2	SIZE (molecule size)	180,16 g/mol	< 500 Da atau g/mol	
3	POLAR (Polarity)	77,76 Å²	20 Å² < TPSA (Topological Polar Surface Area) < 130 Å²	
4	INSOLU (insolubility)	-1,89	0 < Log S (ESOL) < 6	
5	INSATU (Insaturation)	0,71	0,25 < Fraction Csp3 < 1	
6	FLEX (Flexibility)	2	0 < number of rotation bonds < 9	
	Bioavaibility score	0,56		Caffeic acid compounds have good bioavailability

Prediction of phytochemical bioactivity of Caffeic acid compounds via the web http://www.way2drug.com/PASSOnline/predict.php.

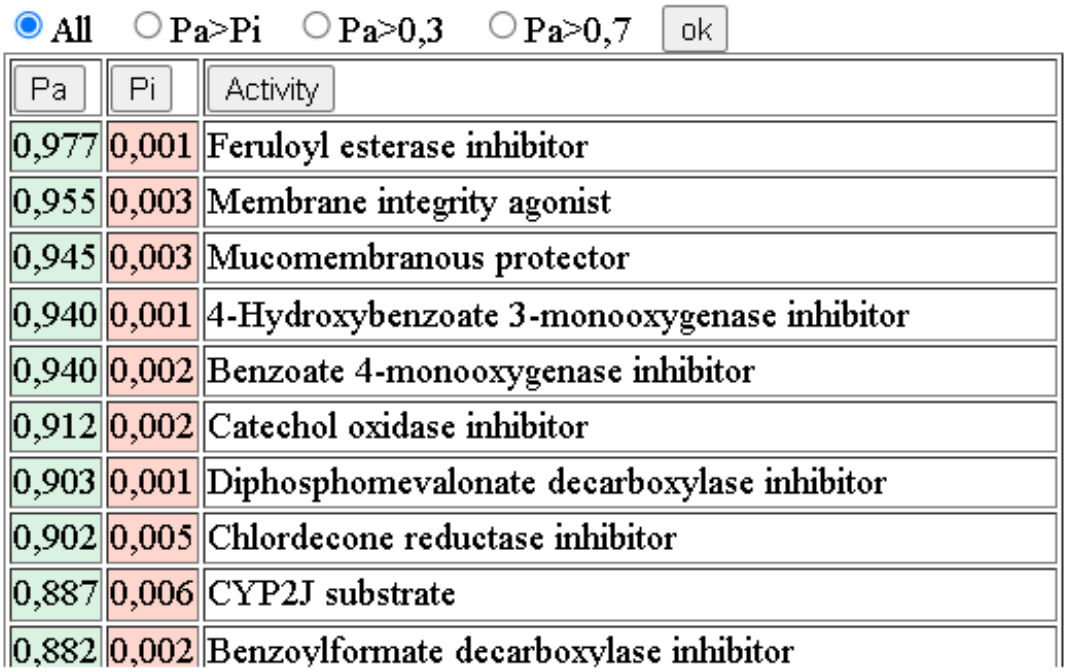

Figure 3. Prediction of phytochemical bioactivity of Caffeic acid compounds

According to the figure 3, Compounds have high potential to actually become bioactive compounds in in vitro and/or in vivo experimental tests.

Prediction of Phytochemical Target Protein Candidates via the web page http://www.swisstargetprediction.ch/found the highest potential as an inhibitor of the Carbonic anhydrase II enzyme.

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase		33 / 13
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase		1 / 29
Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase		11 / 19
Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase		28 / 16
Carbonic anhydrase VI	CA6	P23280	CHEMBL3025	Lyase		8 / 15
Matrix metalloproteinase 9	MMP9	P14780	CHEMBL321	Protease		2 / 35
Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase		19 / 14
Matrix metalloproteinase 1	MMP1	P03956	CHEMBL332	Protease		2 / 34
Matrix metalloproteinase 2	MMP2	P08253	CHEMBL333	Protease		2 / 36
Protein-tyrosine phosphatase 1B	PTPN1	P18031	CHEMBL335	Phosphatase		18 / 2
Carbonic anhydrase XIV	CA14	Q9ULX7	CHEMBL3510	Lyase		10 / 18
Carbonic anhydrase IX	CA9	Q16790	CHEMBL3594	Lyase		15 / 18
Carbonic anhydrase VB	CA5B	Q9Y2D0	CHEMBL3969	Lyase		5 / 13
Carbonic anhydrase VA	CA5A	P35218	CHEMBL4789	Lyase		6 / 14
Carbonic anhydrase III	CA3	P07451	CHEMBL2885	Lyase		4 / 3

Figure 4. Prediction of Phytochemical Target Protein Candidates

Prediction of Protein Network Construction from Carbonic Anhydrase protein via the web http://www.swisstargetprediction.ch

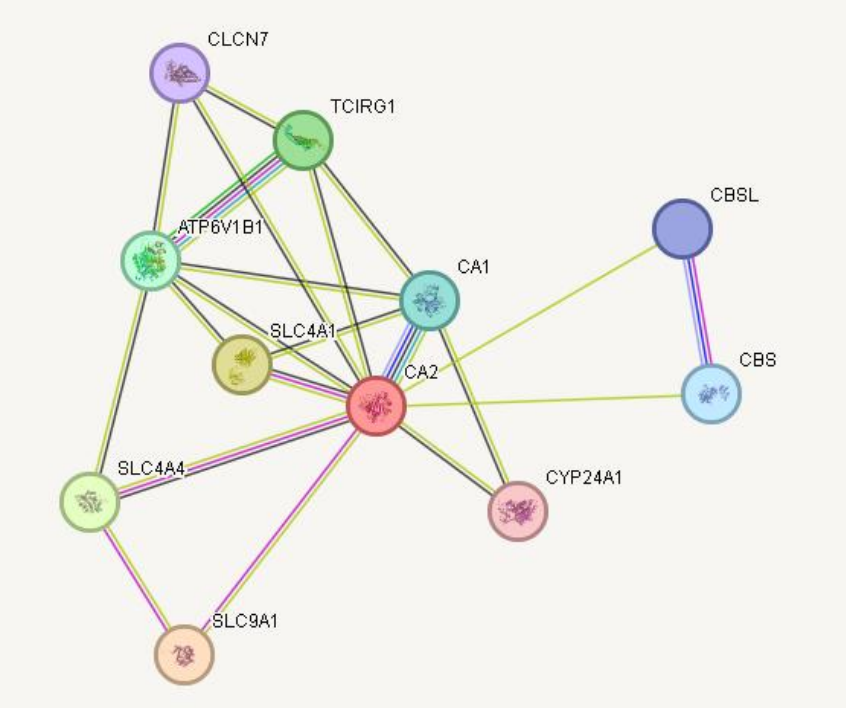

Figure 5. Prediction of Protein Network Construction from Carbonic Anhydrase protein

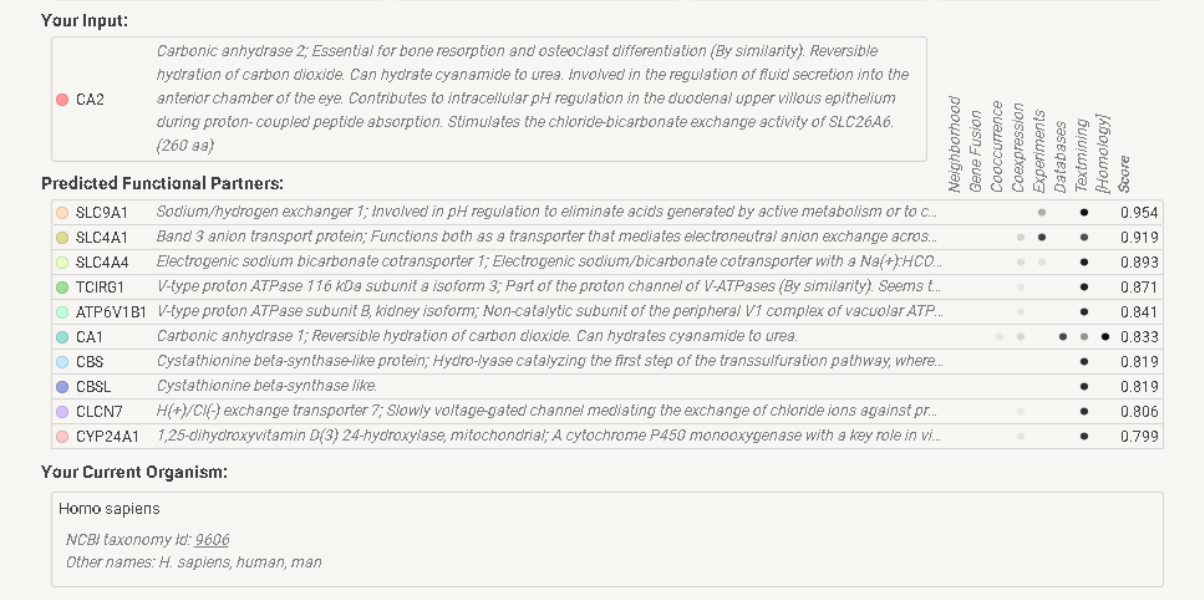

Figure 6. Predicted Functional Partners

After preparation of the Caffeic acid ligand and the reference ligand acetazolamide as well as preparation of the carbonic anhydrase enzyme protein, it was followed by molecular docking between each ligand and protein.

Table 4. Results of molecular docking between caffeic acid and protein carbonic anhydrase from Protein Data Bank 1G5C obtained binding affinity values as follows.

Ligand	Binding afinity	Mode	RMSD lower bound	RMSD upper bound
1g5c_caffeic acid_uff_E= 98,70	-6,4	0	0,0	0,0
1g5c_caffeic acid_uff_E= 98,70	-6,2	1	47.063	48.414
1g5c_caffeic acid_uff_E= 98,70	-6,1	2	21.372	23.307
1g5c_caffeic acid_uff_E= 98,70	-6,1	3	62.179	63.921
1g5c_caffeic acid_uff_E= 98,70	-6,0	4	48.469	48.769
1g5c_caffeic acid_uff_E= 98,70	-5,9	5	92.16	93.808
1g5c_caffeic acid_uff_E= 98,70	-5,7	6	47.435	49.093
1g5c_caffeic acid_uff_E= 98,70	-5,6	7	91.386	93.598
1g5c_caffeic acid_uff_E= 98,70	-5,5	8	90.925	93.094

Meanwhile, the results of molecular docking of the comparative ligand acetazolamide with protein carbonic anhydrase from Protein Data Bank 1G5C obtained binding affinity values as follows:

Table 5. molecular docking of the comparative ligand acetazolamide with protein carbonic anhydrase from Protein Data Bank 1G5C

Ligand	Binding afinity	Mode	RMSD lower bound	RMSD upper bound
1g5c_acetazolamid_uff_E= 98,70719,93	-6,2	0	0,0	0,0
1g5c_acetazolamid_uff_E= 98,70719,93	-6,1	1	59.976	61.949
1g5c_acetazolamid_uff_E= 98,70719,93	-6,1	2	15.774	17.461
1g5c_acetazolamid_uff_E= 98,70719,93	-6,0	3	48.575	49.337
1g5c_acetazolamid_uff_E= 98,70719,93	-5,9	4	48.294	49.331
1g5c_acetazolamid_uff_E= 98,70719,93	-5,7	5	49.926	50.783
1g5c_acetazolamid_uff_E= 98,70719,93	-5,5	6	15.188	16.276
1g5c_acetazolamid_uff_E= 98,70719,93	-5,5	7	48.999	50.042
1g5c_acetazolamid_uff_E= 98,70719,93	-5,4	8	14.415	15.589

Visualization of the interaction of the caffeic acid ligand with the carbonic anhydrase protein can be depicted in 3D as follows:

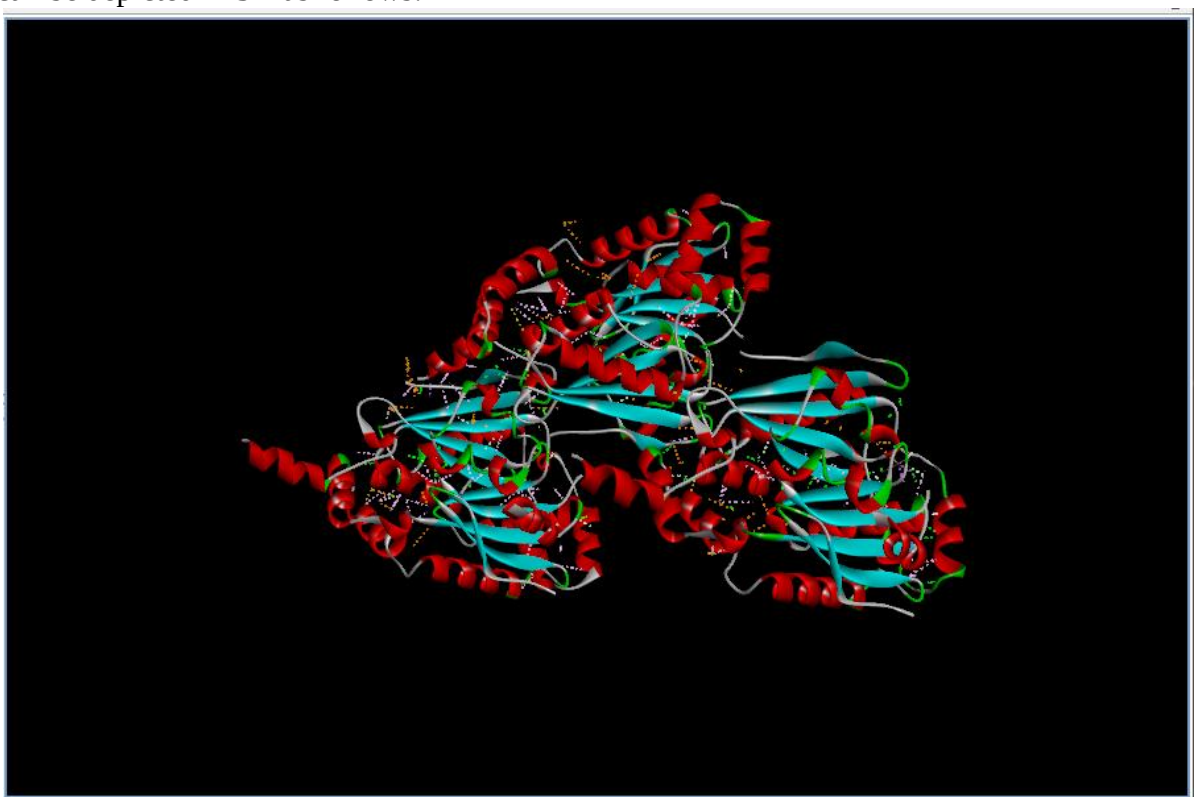

Figure 7. 3D Visualization of the interaction of the caffeic acid ligand with the carbonic anhydrase protein

Meanwhile, the visualization of the interaction of the caffeic acid ligand with the carbonic anhydrase protein can depict the type of bond that occurs in 2D as follows:

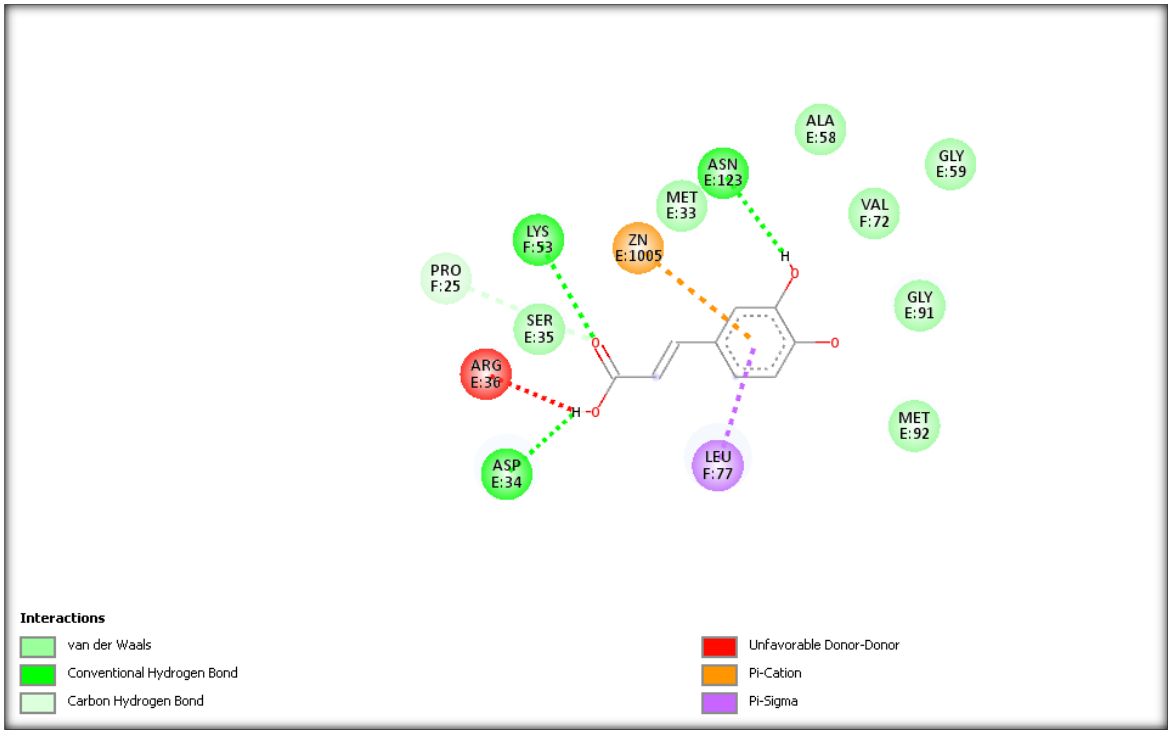

Figure 8. 2D Visualization of the interaction of the caffeic acid ligand with the carbonic anhydrase protein

Visualization of the interaction of the reference ligand acetazolamide with the carbonic anhydrase protein can be depicted in 3D as follows:

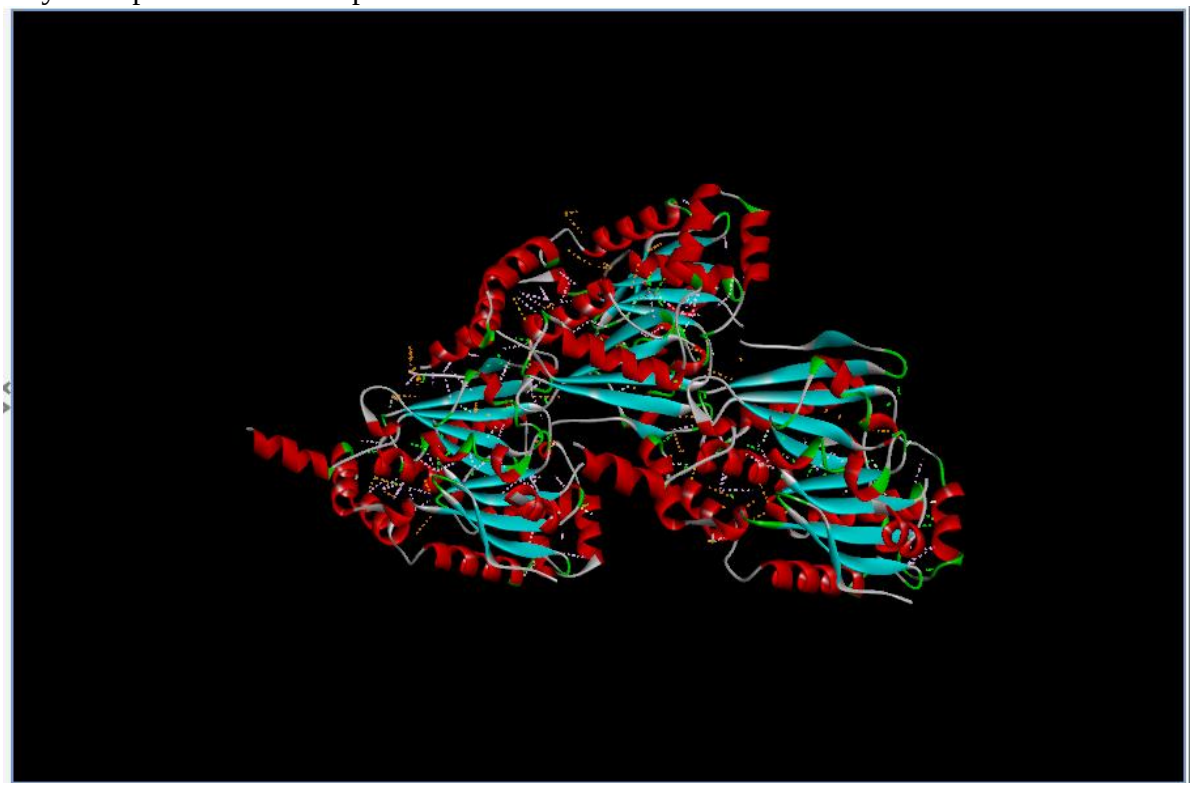

Figure 9. 3D Visualization of the interaction of the reference ligand acetazolamide with the carbonic anhydrase protein

Meanwhile, visualization of the interaction of the reference ligand acetazolamide with

carbonic anhydrase protein can be described as the type of bond that occurs in 2D as follows:

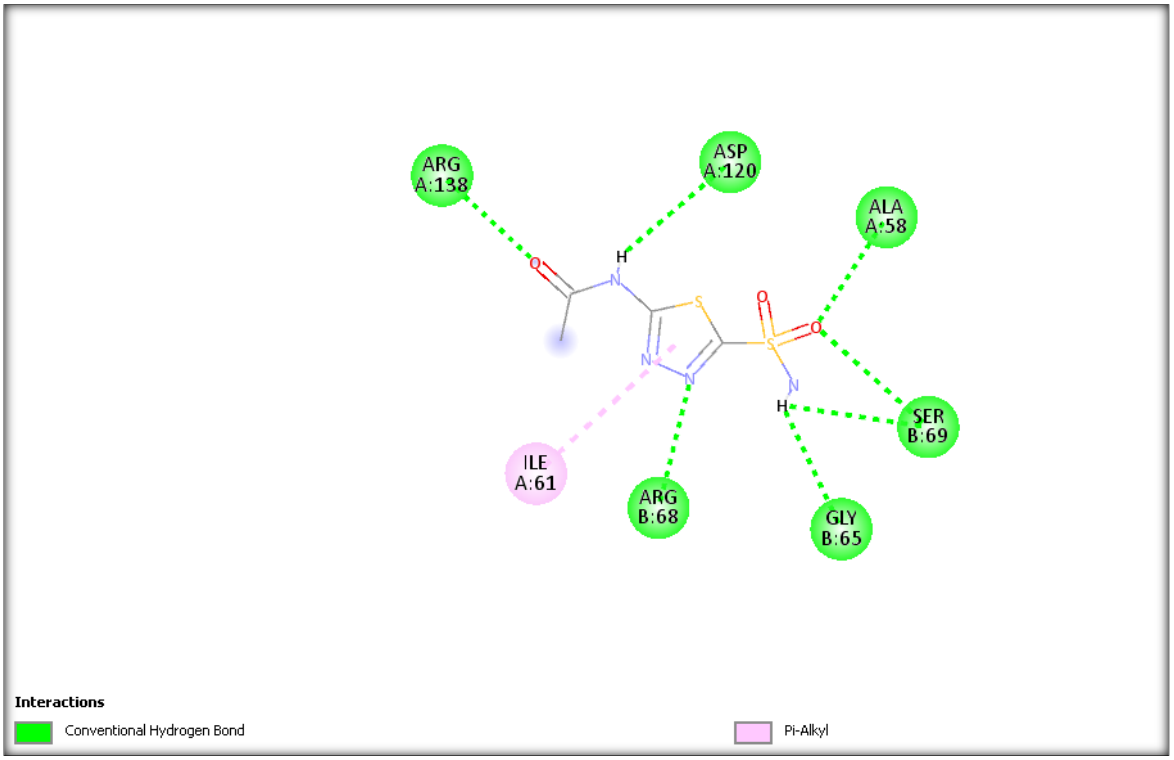

Figure 10. 2D Visualization of the interaction of the reference ligand acetazolamide with the carbonic anhydrase protein

DISCUSSION

Computational chemistry is an in silico method widely used in drug discovery, both in universities, the pharmaceutical industry, and research and development institutions for new drugs. Therefore, docking plays an important role in rational drug design . (Ferreira et al., 2015).

The pharmacokinetic and physicochemical properties prediction of Caffeic Acid through the website http://www.swissadme.ch/index.php shows that Caffeic Acid from Moringa oleifera meets Lipinski's criteria and can proceed with molecular docking. It has been found that Caffeic Acid is highly absorbed in the gastrointestinal tract, does not penetrate the blood-brain barrier, and does not contain P-glycoprotein, hence its absorption in the intestine is not reduced. Caffeic Acid does not inhibit the five isoenzymes, ensuring no reduction in drug elimination and no toxicity or side effects. Caffeic Acid has good bioavailability with a score of 0.56, indicating high potential as a bioactive compound in vitro and/or in vivo experimental tests. The prediction of the phytochemical protein target candidate through the website http://www.swisstargetprediction.ch/shows that Caffeic Acid has the highest potential as a carbonic anhydrase II enzyme inhibitor, which can be used as an anticancer drug candidate.

Molecular docking was performed using Pyrx software. Before docking, the test compounds and target molecules were prepared. The target molecules were obtained from the RCSB site. Protonation or hydrogen ion addition was done to the test compounds and target molecules to add atomic charges and observe the hydrogen bonds formed. Molecular docking is important for drug development as it can reveal interactions between ligands and receptors with affinity energy, helping to shorten time, reduce costs, and sharpen research scope (Ferreira et al., 2015).

Based on the visualization results of the molecular docking of Caffeic Acid with carbonic anhydrase protein, several types of bonds were found, including van der Waals bonds,

hydrogen bonds, and π bonds. Hydrogen bonds are a key factor affecting protein stability. Van der Waals interactions occur when two atoms are close to each other, forming weak non-specific attractive forces (Fu et al., 2018).

In the visualization, hydrophobic van der Waals interactions for Caffeic Acid were observed at amino acid residue PRO 25. Hydrogen bonds were formed at residues LYS53, ASP34, and ASN123, while π bonds were observed at residues Zn1005 and LEU77. The reference ligand acetazolamide showed conventional hydrogen bonds with residues ARG138, ASP120, ALA58, SER69, GLY65, and ARG68, and π bonds with residue ILE61.

The binding affinity value obtained from the molecular docking between Caffeic Acid and carbonic anhydrase protein was -6.4, while the reference ligand acetazolamide had a binding affinity of -6.2. Lower binding affinity values indicate better interaction stability between the ligand and receptor (Muttaqin et al., 2019).

From the visualization results, only one amino acid residue matched, showing hydrogen bonding with aspartate (ASP). However, the position differs: ASP34 in Caffeic Acid and ASP120 in acetazolamide within the polypeptide chain. This positional difference impacts the interaction with the biological target, influencing the extent to which each compound binds. The similarity in aspartate bonding suggests a comparable biological activity, effect, or response. This indicates that Caffeic Acid has the same potency as acetazolamide as an anticancer drug due to the same amino acid residue bonding.

Current research is conducted to determine the therapeutic effects of M. oleifera-based compounds on cancer. Other compounds such as quercetin, gallic acid, p-coumaric acid, and 4-hydroxy-3-methoxy cinnamic acid have shown potent anticancer activity and therapeutic potential against cancer. Based on the research, Moringa oleifera can be considered a leading plant in cancer treatment (Jung, 2014).

CONCLUSION

Caffeic acid compounds in oil leaves (*Moringa oleifera*) have potenly against carbonate anhydrase enzyme as anti-cancer in silico.

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