Jurnal Akta Kimia Indonesia
INDONESIA CHIMICA ACTA

PAPER

View Article Online
View Journal View Issue

Cite this: *Indo. Chim. Acta.*, 2020, **13**, 2.

Received Date:
14th October 2020
Accepted Date:
12nd December 2020

Keywords:
docking;
ATPase c;
Moringa oleifera;
fatty acids.

DOI:
http://dx.doi.org/10.20956/ica.v13i2.11536

Molecular Docking of Chemical Compounds from n-Hexane Extract of *Moringa oleifera* Seeds with *Escherichia coli* and *Staphylococcus aureus* ATP Synthase Subunit C

Fredy Z. Saudale[1*], Apriono Sutiono[1] , Luther Kadang[1], Early G. Boelan[2], Origenes B. Kapitan[3]

Abstract. The development of new antibiotics is an urgent need amidst the reported increase in bacterial resistance. The n-hexane extracts of *Moringa oleifera* seeds have been known to have antibacterial effects against *E.coli* and *S.aureus*. However, the mechanism of inhibition and the protein from these bacteria targeted by the chemical compounds from n-hexane extracts of Moringa seeds remains elusive. ATP synthase subunit c (ATPase c) plays an important role in the synthesis and hydrolysis of ATP providing energy for bacterial growth. It is an attractive for the new antibiotic development. This study aims to computationally study the interaction between the chemical compounds from n-hexane extract of Moringa seeds and ATPase c from *E. coli* and *S. aureus* that may shed the light on their inhibitory potentials using molecular docking method. The compound trans-9-octadecenoic acid shows the highest affinity energy with ATPase c from *E. coli* (-4.1 kcal/mol). Meanwhile, 9-cis-octadecanoic acid and glycerol-1,3-dioctadecanote show the highest interaction (-4.3 kcal/mol) with ATPase c from *S.aureus*. Nonetheless, these interaction energies are lower than a positive control Tomatidine on ATPase c *E.coli* (-6.9 kcal/mol) and *S. aureus* (-6.2 kcal/mol). Further *in vitro* and *in vivo* assays are needed to validate their potential as novel antibacterial candidates.

Introduction

Nowadays, people tend to use alternative natural ingredients as medicine. One of the natural ingredients that are known to have medicinal properties is *Moringa oleifera* plants. Moringa is one of the plants that are found in tropical countries such as Indonesia. Moringa plants are known for their use as bactericides and/or antibacterial (Fahey, 2005; Viera et al., 2010). . One part of Moringa which is used as an antibacterial is the seeds. Non-polar and polar extracts from Moringa seeds from the island of Timor in Eastern Nusa Tenggara province of Indonesia are known to inhibit *S. aureus* dan *E. coli* bacterial growth (Saudale & Boelan, 2018). Non-polar extracts with n-hexane solvent from Moringa seeds contains a majority of 80% oleic acid and a small portion of fatty acid derivatives, glycerols and esters (Saudale & Boelan, 2018). Non-polar extract containing the majority of oleic acid is able to inhibit bacterial growth of *S. aureus* dan *E. coli* (Saudale & Boelan., 2018). The results of this research support previous studies showing the potentialactivity of fatty acids as gram-positive and negative antibacterials (Abalaka et al., 2012; Abdull et al., 2014; Fahey, 2005; Nadeem & Imran, 2016; Vinoth et al., 2012). In addition, Moringa seeds which are rich in fatty acids with antibacterial activity can be a source of local natural ingredients for the development of new antibiotics amidst the problem of bacterial resistance to antibiotic capabilities that exist in the market nowadays, which has not been

[1]*Department of Chemistry, Faculty of Science and Engineering, University of Nusa Cendana, Kupang , NTT, 85001;* ***Email: fredy_saudale@staf.undana.ac.id***
[2]*Department of Chemistry Education, Faculty of Teacher Training and Education, University of Catholic Widya Mandira, Kupang, NTT, 85225*
[3]*Department of Agrotechnology, Faculty of Agriculture, University of Timor, Kefamenanu, NTT, 85616*

overcame yet (Zaman et al. 2017). However, these studies have not been able to explain the interaction patterns or mechanisms of activity between compounds specifically the fatty acids found in Moringa seed extract and the target proteins of bacteria. In addition, it has never been known the proteins and/or enzymes from *S. aureus* and *E. coli* that may act as molecular targets of the antibacterial activity of lipids and free fatty acids. ATP synthase is an essential enzyme that plays an essential role in the energy metabolism of almost all living cells (Lu et al., 2014). This enzyme utilizes stored energy due to differences in electrochemical potential between the outer and inner cell membranes for the production of ATP. The ATP synthase structure of bacteria consists of two segments, the F0 hydrophobic segment consisting of proteins embedded in cell membranes with a composition of 1 subunit a and 8 to 10 subunits c and F1 hydrophilic segment consisting of a protein complex composed of α3β3γδε subunits (Figure. 1).

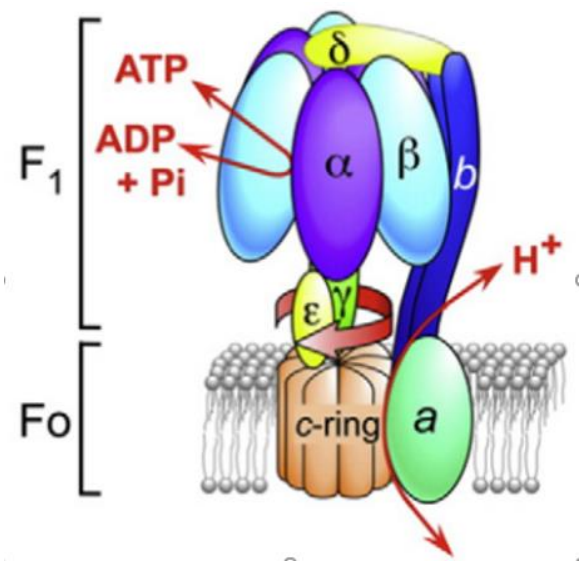


Figure 1. ATP synthase. General composition of ATP synthase structure as found in E.coli The F0 dan F1 segments are linked by a and b proteins.

ATP synthase is the only enzyme in nature that has a dual catalytic mechanism which is the function of ATP synthesis and hydrolysis (Nesci et al., 2016). The catalytic function of ATP synthase is related to intrinsic features that resemble the ring of the c subunit protein, so it is often called the c-ring which is the core of the F0 domain contained in the membrane. ATP synthase subunit c or c-ring comes into contact with protons or H^+ ions that enter the cell membrane due to differences in the electrochemical potential of the inner and outer cell membranes. The flow of proton H^+ between these membranes through subunit a then triggers the c-ring to rotate mechanically with a high energy level determining whether to synthesize or hydrolyze ATP in the F1 segment. If there are large numbers of H^+ protons that pass through the membrane that moves the c subunit protein, then there will be a lot of energy from the c-ring rotation that can be converted and used for ATP synthesis. And conversely if only a small amount of H+ protein passes, then energy needs to be generated through ATP hydrolysis. Therefore, the c-ring subunit protein from ATP synthase (ATPase c) determines the life and death of bacterial cells based on the regulation of metabolic energy. Recently ATP synthase has been used as a molecular target for the development of new selective antibiotic drugs such as tomatidine, diarilquinolin, and bedaquiline (Biuković et al., 2013; de Jonge et al., 2007; Lamontagne Boulet et al., 2018; Lobritz et al., 2015; Lu et al., 2014; Nesci et al., 2016). This reinforces the potential role of the ATPase c enzyme as an attractive antibiotic drug target (Barker, 2006; Simmons et al., 2010).

Therefore, the purpose of this research was to study the interaction of compounds from n-hexane extract of Moringa seeds with ATPase c from *S. aureus* and *E. coli* bacteria *in silico*. The amount of affinity energy (kcal/mol), accuracy of orientation and conformation (pose), interactions of hydrogen and hydrophobic formed between the ATP synthase target protein complex and chemical compounds identified at active sites were analyzed following the molecular docking process.

Experimental

Material and Methods

This research was conducted using a computational method (*in silico*) based on the structure of protein targets or known as Structure Based Drug Design (SBDD) which consists of homology modeling, evaluation and validation, molecular docking, analysis of energy affinity, poses and interaction analysis (Barker, 2006; Saudale, 2020).

Hardware, Software and Data

Laptop with Desktop-DL1LUOU operating system specifications, Intel® CoreTM i3-6006U CPU @ 2.00GHz 1.99 GHz, 4GB of memory (RAM), and 64-bit Windows 10 was used. Software used were UCSF Chimera, PyMOL, Discovery Studio, and PyRx. The structural data employed in this study is the ATPase subunit c protein from *E. coli* and *S. aureus* bacteria in PDB (Protein Data Bank) file format. As ligands to be studied their interaction with ATPase c are chemical compounds resulting from previous GC-MS analysis of n-hexane extract from Moringa seeds (Saudale & Boelan, 2018). The ATPase c inhibitors, tomatidine and bedaquiline, are used as positive controls that are prepared in SMILE (Simplified Molecular-Input-Line-Entry System) file format (Table 1 and Figure 2).

Preparation and Protein Modelling

The amino acid sequence of the ATPase subunit c protein was downloaded from the UniProt database with accession number P68699 for *E. coli* and A0A077UX57 for *S. aureus*. The 3D structure of the ATPase subunit c protein was built using Swiss-Model webserver and the structural data was saved in the PDB file format. The 3D structure of the ATPase subunit c model was then evaluated, validated and refined using ProSA (Wiederstein & Sippl, 2007), SAVES 5.0, and GalaxyWeb (Heo et al., 2013), respectively.

Table 1. The n-hexane extract compounds and inhibitor prepared and used from PubChem database

No	Compounds	PubChem code	SMILE notation
1	Methyl palmitate	8181	CCCCCCCCCCCCCCCC(=O)OC
2	*trans*-9-Octadecenoic acid	637517	CCCCCCCC/C=C/CCCCCCCC(=O)O
3	9-cis-Octadecenoic acid	965	CCCCCCCC/C=C\CCCCCCCC(=O)O
4	Glycerol-1,3-di Octadecanoate	101269	CCCCCCCCCCCCCCCCCC(=O)OCC(COC(=O)CCCCCCCCCCCCCCCC)O
5	Di-(9-Octadecenoyl)-glycerol	5316908	CCCCCCCCC=CCCCCCCCC(=O)OCC(COC(=O)CCCCCCCC=CCCCCCCCC)O
6	Bedaquiline (inhibitor)	5388906	CN(C)CCC(C1=CC=CC2=CC=CC=C21)(C(C3=CC=CC=C3)C4=C(N=C5C=CC(=CC5=C4)Br)OC)O
7	Tomatidine (inhibitor)	65576	CC1CCC2(C(C3C(O2)CC4C3(CCC5C4CCC6C5(CCC(C6)O)C)C)C)NC1

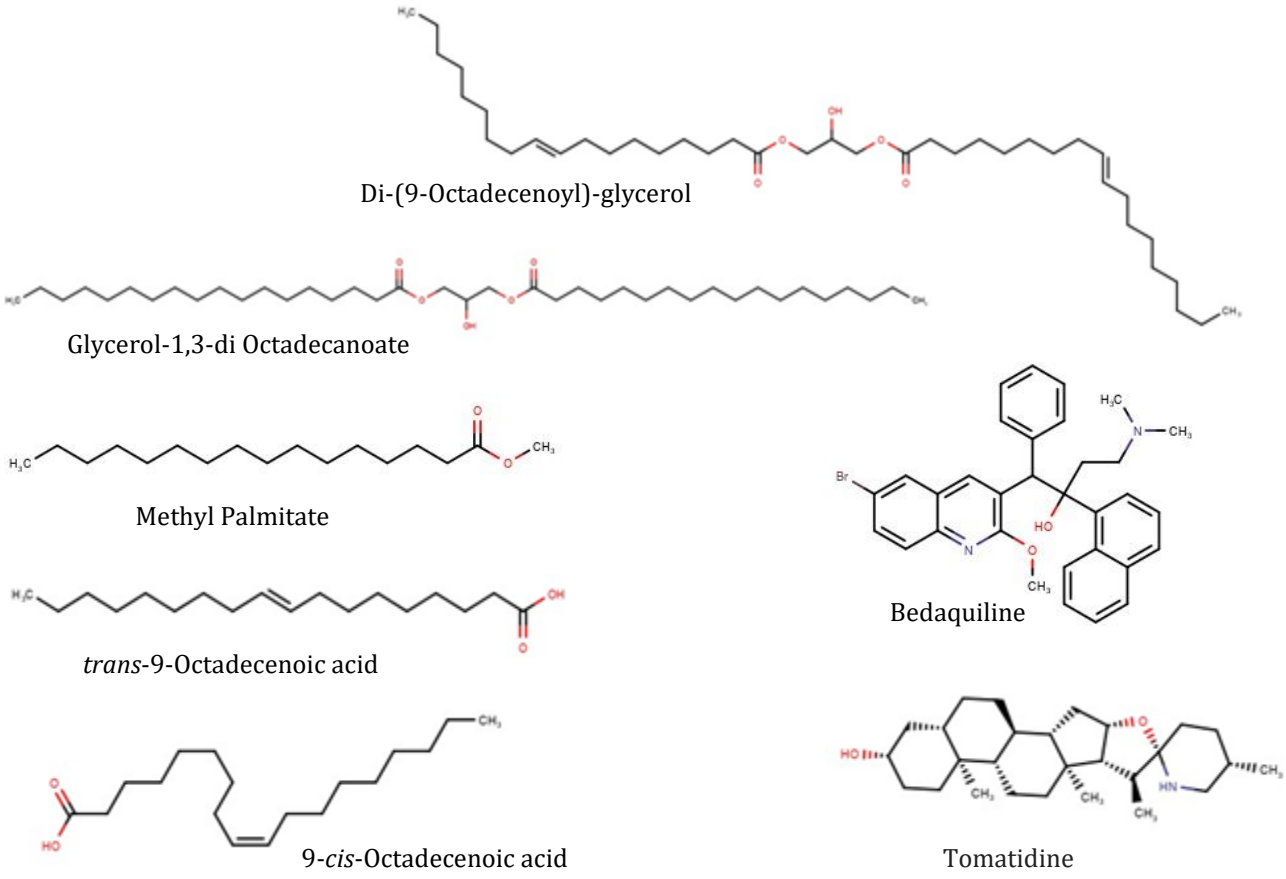


Figure 2. Structure of ligand compounds. Methyl palmitate, trans-9-Octadecenoic acid, 9-cis-Octadecenoic acid, Di- (9-Octadecenoyl) - glycerol, Glycerol-1,3-di Octadecanoate are chemical compounds in the n-hexane extract of Moringa seeds resulting from GC-MS analysis (Saudale & Boelan, 2018). Bedaquiline and Tomatidine are ATPase c inhibitors used as positive controls.

Ligand Preparation

The 2D structures of ligands and inhibitors as positive controls were downloaded from the PubChem database and then added with charges, hydrogen atoms, and minimization using the USCF Chimera software. Afterwards, we prepared the 20 conformations of the ligands and inhibitors using Frog2 (Miteva et al., 2010).

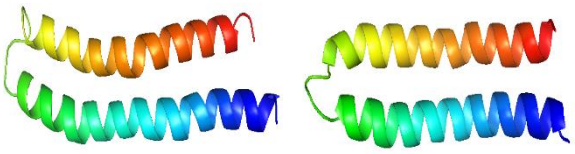
Docking and Interaction Analysis

Proteins and ligands that have been prepared are then docked using PyRx software to generate PDBQT output files for interaction and pose analysis (Dallakyan & Olson, 2015). The result of docking such as pose and hydrogen bonding distance was analyzed for 2D and 3D interactions using Discovery Studio and PyMOL software (Seeliger & de Groot, 2010), respectively.

Result and Discussion

Homology Modelling

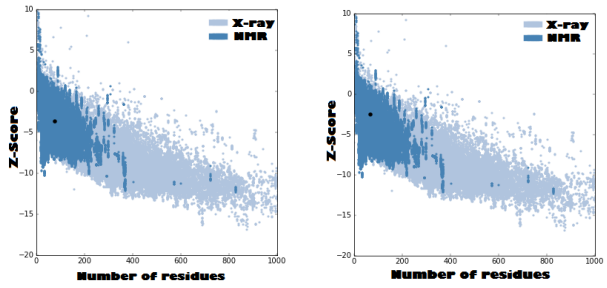
The sequence and structural information of ATPase c protein was searched using several servers and databases such as BLAST Protein, UniProtKB, PDB and Swiss-Model. The amino acid sequence and 3D structure of ATPase c protein for *E. Coli* was obtained directly from the UniProt KB database (Accession number: P68699) and Protein Data Bank (PDB ID: 1A91), respectively. Meanwhile, the 3D crystal structure of ATPase c of *S. aureus* is not available in Protein Data Bank yet because it has not been elucidated using either X-ray crystallography or NMR. Therefore, we built it by using homology modelling method by searching for the template based on sequence similarity ≥ 30% from other bacteria. We found that ATPase c of *S. aureus* shows a high percent identity of amino acid sequence with ATPase c from *Bacillus* SP3, which is 70.59 %. There are a total of 8-10 c subunits of ATPase that are identical in the term of amino acid sequence and structure. For the purpose of modelling and docking, we only employed 1 subunit (monomer) and removed the other. Figure 3 shows the 3D model of ATPase c structure of *E.coli* and *S.aureus* built using the Swiss-Model webserver.



(a) (b)

Figure 3. The 3D model of ATPase c monomer, (a) *E. coli* (b) *S. Aureus*

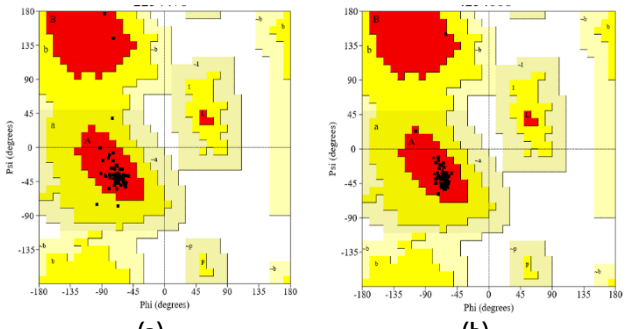
The output of ProSA assessment is the z-score showing the quality and measurement of energy deviations of the model compared to experimental proteins. As shown in Figure 4, the z-score of the 3D model of ATPase c for *E. coli* and *S. aureus* is -3.63 and -2.54, respectively. From the graph of z-score on figure 4, it graphically displays the z-score from different proteins experimentally obtained by X-ray crystallography (light blue) and NMR (dark blue) that are stored in Protein Data Bank. It showed that z-score of the 3D model of ATPase c from *E.coli* and *S.aureus* falls in the range of z-score from experimental proteins indicated by the black dot. It can be suggested that the 3D model of ATPase c of *E.coli* and *S.aureus* are energetically acceptable and can be used for interaction analysis with the chemical compounds of n-hexane extract of Moringa seeds by molecular docking.



(a) (b)

Figure 4. Graphical *z-score* from ProSa-web for the 3D model of ATPase c (a) *E. coli* (b) *S. aureus.* Black dot (●) indicates the *z-score* of the model.

The quality of a model can also be assessed using Ramanchandran plot (Figure 5). A good protein model is expected to have amino acid residues located in the most preferred area (most favored region) as much as above 90% (areas indicated with the red color).



(a) (b)

Figure 5. Ramancandran Plot of 3D model of ATPase c (a) *E. coli* (b) *S. aureus.* The black dots (●) represents the amino acid position based on the combination of dihedral angles. The region in red is the most favoured region. The yellow is the still favoured region. The white is the non-favoured region where it is forbidden for amino acids to occupy this area due to possible steric clashes.

The more is amino acids located on the most favoured region; the better is the 3D model. The percentage of amino acid in most favored region is above 90% for both 3D model of ATPase c of *E coli* and *S.aureus* as indicated by the black dots occupying the red region based on the combination values of dihedral angles (Figure 5). From these results, it can be said that the 3D model of ATPase c protein of *E. coli* and *S. aureus* is acceptable.

In addition to the assessment of 3D model using Ramachandran plot and ProSA, we also did evaluation based on the amino acid stereochemistry with Verify3D, non-bonded atomic interaction with ERRAT, atomic volume with PROVE and potential steric clashes with MolProbity that together they are part of SAVES 5.0 webserver. The results are presented in Table 2.

Table 2. Evaluation and Validation of the 3D Model of ATPase c before Refinement

Model	Verify 3D	ERRAT (Quality)	PROVE (Warning)	Mol Probity	Clash Score
E. coli	Fail	66,197	4,1%	1,90	0,84
S. aureus	Fail	90%	4,4%	2,54	10,1

The 3D model of ATPase c of both *E. coli* & *S. aureus* bacteria failed to pass the Verify 3D. The quality score of ERRAT shows that ATPase c of *E. coli* is lower than *S. aureus*. Meanwhile, MolProbity score of the 3D model of ATPase c from *E. coli* is also lower than *S.aureus*. The MolProbity score describes the total atomic interaction including the hydrogen bonds and takes into account the potential steric clashes that affects the quality of 3D protein structures (Chen et al., 2010). The MolProbity of good protein structures is expected to have score closer to zero. Therefore, the greater the MolProbity score; the worse the quality of the 3D model. MolProbity scores go hand in hand with clash scores that suggest the possible collision between atoms. Based on this evaluation and validation, it is then necessary to refine the quality of the 3D model of ATPase c of *E. coli* & *S. aureus* to see if there are any improvement in stereochemistry, MolProbity and Clash scores.

The refinement of the 3D model structure was conducted using the GalaxyRefine (Heo et al., 2013). GalaxyRefine helps to improve the quality of the 3D model of ATPase c from *E. coli* & *S. aureus* by rearrangement of the side chains of the protein via short molecular dynamics that further may reduce the clash scores. Afterwards valuation and validation were conducted again to assess the quality of 3D model as presented in Table 3.

Table 3. Evaluation and Validation of the 3D Model of ATPase c after Refinement

Model	Verify 3D (Passed)	ERRAT (Quality)	PROVE (Warning)	Mol Probity	Clash Score
E. coli	Fail	100%	4,0%	0,69	0,7
S. aureus	Fail	100%	4,3%	1,44	11,8

As shown in Table 3, the 3D model of ATPase c of *E.coli* and *S.aureus* are still fail in Verify 3D assessment. We learned that this might be a mere methodological problem. Verify 3D measures the protein models using a database of cytoplasmic proteins that have been experimentally elucidated by NMR and X-ray crystallography (Eisenberg et al., 1997). Hence, the assessment of Verify3D assumes the protein environment to be cytoplasmic and aqueous. Meanwhile, ATPase c is a membrane protein that has a hydrophobic segment of its amino acids exposing toward and interacting with non-polar environment of the membrane. Therefore, the 3D model of ATPase c of *E.coli* and *S.aureus* may be considered by Verify3D as in forbidden stereochemical positions that result in many of its amino acid residues being scored below the cut-off value of 0.2 that are reported as fail. Therefore, it is necessary to verify ATPase c protein with a specific predictor for membrane proteins within non-polar environments. Nevertheless, the 3D model of ATPase c from *E.coli* and *S.aureus* gains an improvement after refinement using GalaxyRefine on non-bonded atomic interaction and potential steric clashes as indicated by ERRAT, MolProbity and Clash Score.

Docking and Binding Analysis

To study the potential interaction between the already prepared chemical compounds of n-hexane extract from Moringa seeds with the ATPase c from *E. coli* & *S. aureus* we conducted molecular docking study on which the results are presented in Tables 5 and 6.

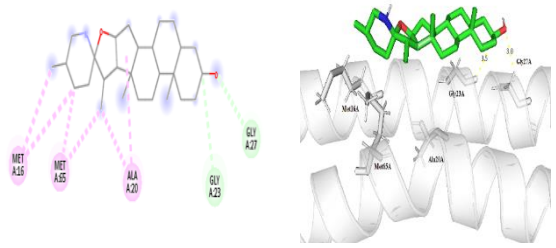
Table 5. *Docking* results with ATPase c of *E. coli*

Compounds	Affinity Energy (kkal/mol)
Tomatidine	-6.9
Bedaquiline	-5.2
9-*cis*-octadecenoic acid	-4.1
trans-9-octadecenoic acid	-4
Glycerol 1,3-dioctadecanoate	-3.8
Methyl Palmitate	-3.6
Di-(9-octadecenoyl)-glycerol	-3.5

Table 6. *Docking* results of ATPase c of *S. Aureus*

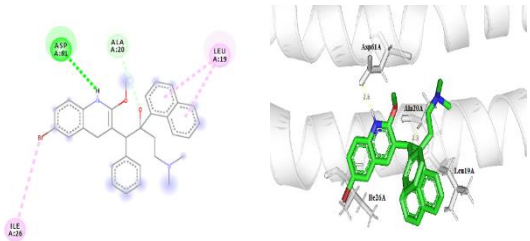
Compounds	Affinity Energy (kkal/mol)
Tomatidine	-6.2
Bedaquiline	-5.6
Glycerol 1,3-dioctadecanoate	-4.3
trans-9-octadecenoic acid	-4.3
Di-(9-octadecenoyl)-glycerol	-4.2
9-*cis*-octadecenoic acid	-4
Methyl Palmitate	-3.8

It is shown that the chemical compounds from n-hexane extract of Moringa seeds have a lower affinity energy than tomatidine and bedaquiline inhibitors as positive controls. This implies that the chemical compounds from the n-hexane extract of Moringa seeds may form a less strong or stable interaction with the ATPase c from *E. coli* & *S. aureus*.



(a) (b)

Figure 6. The interaction between Tomatidine and ATPase c from *E. coli* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and ligand in green backbone.

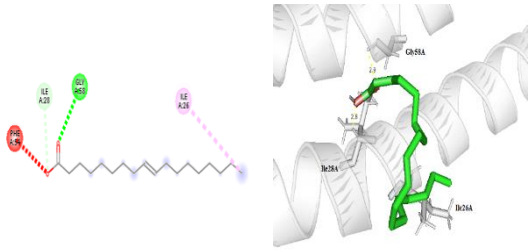


(a) (b)

Figure 7. The interaction between Bedaquiline and ATPase c from *E.coli* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and ligand in green backbone.

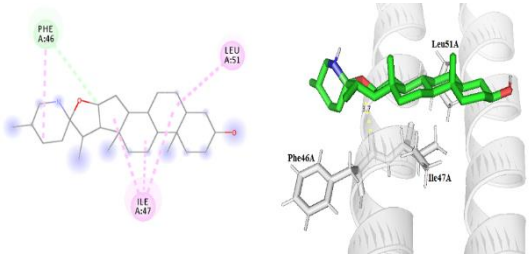
Interaction analysis between protein and ligand was performed using PyMOL and BIOVA Discovery Studio software. This analysis was carried out with the aim to search the hydrogen bonding and hydrophobic interactions formed between the chemical compounds and ATPase c from *E.coli* and *S.aureus* after the docking process. It was also conducted to analyse the suitability of conformation and orientation (pose) whether the chemical compounds that are docked to ATPase c enzymes are found at the active site of the protein.

Analysis of the interaction between tomatidine and bedaquiline with the ATPase c of *E. coli* shows the presence of hydrophobic (Alkyl) interactions and hydrogen bonds (Figures 6 and 7). Hydrophobic interactions are formed between tomatidine with Met16, Ala20, Met65 and bedaquiline with Leu19, and Ile26. Meanwhile, hydrogen bonds are formed between tomatidine and Gly23 (3.5Å), Gly27 (3.0Å), and tedaquiline with Ala20 (2.3Å), Asp61 (2.6Å).



(a) (b)

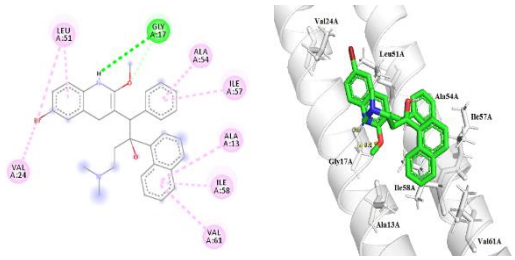
Figure 8. Interaction between *trans*-9-octadecenoic acid and ATPase c from *E. coli* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and the ligand in green color.



(a) (b)

Figure 9. Interaction between Tomatidine and ATPase c from *S. aureus* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and ligand in green backbone.

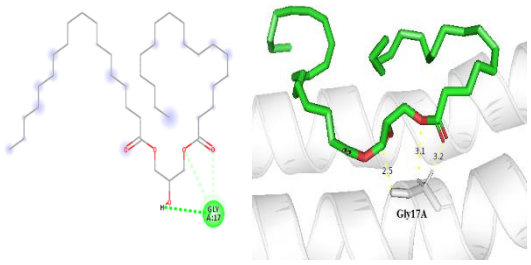
The interaction between *trans*-9-octadecenoic acid from the n-hexane extracts of Moringa seeds, which has the highest affinity energy, and the ATPase c from *E. coli* also shows the presence of hydrophobic (Alkyl) interactions and hydrogen bonds (Figure 8). Hydrophobic interactions are formed between *trans*-9-octadecenoic acid and Ile26 and hydrogen bonds are formed with Ile28 (2.8Å), Gly58 (2.9Å). The hydrophobic interactions with Ile26 that appears in bedaquline as control also shows with *trans*-9-octadecenoic acid. This suggests that *trans*-9-octadecenoic acid may have a similar interacting molecular behavior as bedaquiline. Whether *trans*-9-octadecenoic acid in particular and fatty acids in general may have similar inhibitory acitivites as bedaquilines is an interesting subject of further research.



(a) (b)

Figure 10. Interaction between Bedaquiline and ATPase c from *S. aureus* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and ligand in green backbone.

Analysis of tomatidine and bedaquiline interactions with the ATPase c of *S. aureus* shows hydrophobic interactions (Alkyl, p-Alkyl) and hydrogen bonds (Figures 9 & 10). Hydrophobic interactions are formed between tomatidine and Ile47, Leu51, and bedaquiline with Ala13, Val24, Leu51, Ala54, Ile57, Ile58. Meanwhile, a hydrogen bonds are formed between tomatidine and Phe46 (3.7Å) and bedaquiline with Gly17 (3.1Å).



(a) (b)

Figure 12. Interaction between glycerol-1,3-dioctadecanoate and ATPase c from *S. aureus* (a) 2D and (b) 3D representation. Protein is depicted as white ribbon and ligand in green backbone.

Meanwhile, the hydrogen bond between chemical compounds from the n-hexane extract of Moringa seeds and ATPase c of *S. aureus* is only formed with glycerol-1,3-dioctadecanoate that has the highest affinity energy (-4.3 kkal/mol) but not with the other compounds (Figure 11). The amino acid residue of ATPase c of *S.aureus* that binds to the glycerol 1,3-dioctadecanoate is Gly17 (2.5 Å, 3.1 Å, 3.2Å). This hydrogen bond interaction may be one of the reasons for the high affinity energy of glycerol 1,3-dioctadecanoate compared to the rest of chemical compounds derived from n-hexane extract of Moringa seeds. This may also point to the potential inhibitory activites of glycerol-1,3-dioctadecanoate.

Conclusion

The chemical compounds from the n-hexane extracts of Moringa seeds are predicted to interact with ATPase c protein of *E. coli* and *S. aureus* based on *in silico* study using molecular docking method. The *trans*-9-octadecenoic acid shows the highest affinity energy (-4.1 kkal/mol) with ATPase c of *E.coli*. This binding is stabilized by hydrophobic interactins with Ile26 and hydrogen bonds with Ile28 (2.8 Å), Gly58 (2.9Å). Meanwhile, 9-*cis*-octadecenoic acid and glycerol-1,3-di octadeanoate show the highest affinity energy (-4.3 kkal) with ATPase c of *S.aureus*. This interaction is stabilized by the formation of hydrogen bonds with Gly17 (2.5Å, 3.1Å, 3.2 Å). These results may provide computational and mechanistic support toward the growing knowledge on the antibacterial activities of glycerol and free fatty acid compounds from non-polar extracts of Moringa seeds that may guide its further validation assays *in vitro* and *in vivo*.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Abalaka, M. E., Daniyan, S. Y., Oyeleke, S. B., & Adeyemo, S. O. (2012). The antibacterial evaluation of Moringa oleifera leaf extracts on selected bacterial pathogens. *Journal of Microbiology Research*, *2*(2), 1–4.

Abdull, R., Ahmad, F., Ibrahim, M. D., & Kntayya, S. B. (2014). Health benefits of Moringa oleifera. *Asian Pacific Journal of Cancer Prevention*, *15*(20), 8571–8576.

Barker, J. J. (2006). Antibacterial drug discovery and structure-based design. *Drug Discovery Today*, *11*(9–10), 391–404.

Biuković, G., Basak, S., Manimekalai, M. S. S., Rishikesan, S., Roessle, M., Dick, T., Grüber, G. (2013). Variations of Subunit ε of the Mycobacterium tuberculosis F1Fo ATP Synthase and a Novel Model for Mechanism of Action of the Tuberculosis Drug TMC207. *Antimicrobial Agents and Chemotherapy*, *57*(1), 168–176.

Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., & Richardson, D. C. (2010). MolProbity: All-atom structure validation for macromolecular crystallography. *Acta Crystallographica Section D: Biological Crystallography*, *66*(1), 12–21.

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In *Chemical biology* (pp. 243–250). Springer.

De Jonge, M. R., Koymans, L. H. M., Guillemont, J. E. G., Koul, A., & Andries, K. (2007). A computational model of the inhibition of Mycobacterium tuberculosis ATPase by a new drug candidate R207910. *Proteins*, *67*(4), 971–980.

Eisenberg, D., Lüthy, R., & Bowie, J. U. (1997). [20] VERIFY3D: Assessment of protein models with three-dimensional profiles. In *Methods in enzymology* (Vol. 277, pp. 396–404). Elsevier.

Fahey, J. W. (2005). Moringa oleifera: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for Life Journal*,

1(5), 1–15.

Heo, L., Park, H., & Seok, C. (2013). GalaxyRefine: Protein structure refinement driven by side-chain repacking. *Nucleic Acids Research*, *41*(W1), W384–W388.

Lamontagne Boulet, M., Isabelle, C., Guay, I., Brouillette, E., Langlois, J.-P., Jacques, P.-É., Malouin, F. (2018). Tomatidine Is a Lead Antibiotic Molecule That Targets Staphylococcus aureus ATP Synthase Subunit C. *Antimicrobial Agents and Chemotherapy*, *62*(6).

Lobritz, M. A., Belenky, P., Porter, C. B. M., Gutierrez, A., Yang, J. H., Schwarz, E. G., Collins, J. J. (2015). Antibiotic efficacy is linked to bacterial cellular respiration. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(27), 8173–8180.

Lu, P., Lill, H., & Bald, D. (2014). ATP synthase in mycobacteria: Special features and implications for a function as drug target. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1837*(7), 1208–1218.

Miteva, M. A., Guyon, F., & Tuffi?` $1/2$ry, P. (2010). Frog2: Efficient 3D conformation ensemble generator for small compounds. *Nucleic Acids Research*, *38*(suppl_2), W622–W627.

Nadeem, M., & Imran, M. (2016). Promising features of Moringa oleifera oil: Recent updates and perspectives. *Lipids in Health and Disease*, *15*(212).

Nesci, S., Trombetti, F., Ventrella, V., & Pagliarani, A. (2016). The c-Ring of the F1FO-ATP Synthase: Facts and Perspectives. *The Journal of Membrane Biology*, *249*(1–2), 11–21.

Saudale, F., & Boelan, E. (2018). Aktivitas Antibakteri Ekstrak Polar dan Non Polar Biji Kelor (Moringa oleifera) Asal Pulau TImor NTT. *Jurnal Sains Dan Teknologi*, *7*(1), 67–76.

Saudale, F. Z. (2020). Pemodelan Homologi Komparatif Struktur 3d Protein dalam Desain dan Pengembangan Obat. *Al-Kimia*, *8*(1), 93-103.

Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of Computer-Aided Molecular Design*, *24*(5), 417–422.

Simmons, K. J., Chopra, I., & Fishwick, C. W. G. (2010). Structure-based discovery of antibacterial drugs. *Nature Reviews. Microbiology*, *8*(7), 501–510.

Viera, G. H. F., Mourão, J. A., Ângelo, Â. M., Costa, R. A., & Vieira, R. H. S. dos F. (2010). Antibacterial effect (in vitro) of Moringa oleifera and Annona muricata against Gram positive and Gram negative bacteria. *Revista Do Instituto de Medicina Tropical de São Paulo*, *52*(3), 129–132.

Vinoth, B., Manivasagaperumal, R., & Balamurugan, S. (2012). Phytochemical analysis and antibacterial activity of Moringa oleifera Lam. *Int J Res Biol Sci*, *2*(3), 98–102.

Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research*, *35*(suppl_2), W407–W410.