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In silico Evaluation of LUT#3 ssDNA Aptamer against Tricetin

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Abstract

Tricetin is one of many flavonoids, a polyphenolic compound that is synthesized as a secondary metabolite in the pollen of Myrtaceae members such as guava. Flavonoids are commonly consumed as part of the human diet, and they are associated with health-promoting benefits. Due to their structure, they have the ability to act as antioxidants, protecting cells from the damage caused by Reactive Oxygen Species. However, the amount of flavonoids produced by each plant or fruit varies. Ecological, environmental, and species factors may influence flavonoid production in plants. Identification and extraction are required to obtain a consistent amount of flavonoids. The identification and extraction process using chromatography yielded promising results, but the procedure laborious. As a result, aptamer could be used as an alternative in the identification and extraction of flavonoids. Aptamers are short oligonucleotide DNA or RNA molecules with a high affinity for a specific molecule or ligand. In this case, no database contains aptamers that specifically bind to tricetin. However, a previous study discovered a ssDNA aptamer called LUT#3 that was originally designed to bind to luteolin and could be used in this study. Both luteolin and tricetin have similar structures, and the ssDNA aptamer LUT#3 is selected. We were able to evaluate the interaction of aptamer against tricetin by using a computational approach and the ssDNA aptamer from the previous study. The 3D structure of the LUT#3 ssDNA aptamer is successfully constructed using UNAFOLD webserver, and the tricetin 3D structure were retrieved from the PubChem database. Both structures were energy minimized prior conducting the molecular docking of the tricetin and the LUT#3 ssDNA. The result shows the binding affinity of -4.9. Deeper analysis were conducted to further understands the interaction between the nucleic acid aptamer and the bioactive compound tricetin, by adding variables such as DNA truncation.

Keywords *in silico*; Tricetin; Flavonoid; ssDNA aptamer; Molecular Docking

Introduction

Tricetin is one of many flavonoids, a polyphenolic compound that synthesized in the pollen of Myrtaceae member such as guava as secondary metabolite. Flavonoids is commonly consumed as part of human dietary, associating it with health promoting benefits. Due to their structure, they have a characteristic acting as antioxidant, protecting cells against the damage caused by Reactive Oxygen Species. According to Pietta, (2000), the major structural characteristic of flavonoids that contribute to antioxidant properties is due to the presence of catechol group in one of its ring which help donate electron to stabilize ROS. By regulating the production of ROS in human body flavonoids is commonly associated with another bioactive characteristic such as anticancer and inflammatory. Hence, this natural synthesized compound holds a potential economical value in the market to be commercialized as a supplement for dietary, or as raw material for the industries.

Aptamers are oligonucleotide that have high affinity and specificity in binding against a target through folding into 3D structure (Kalra et al., 2018; Anthony et al., 2010). The target is a ligand which can be any molecules or compound ranging from ions to toxic compound, making it to have a wide potential application such as therapeutic, diagnostic, and extraction tools. According to Zhou and Rossi
(2016) the binding of aptamer is similar of that antibody binding to antigen. In fact, aptamer can be called as chemical antibodies due to its same functionalities as the antibodies but with several advantages Zhou and Rossi (2016). Aptamer has wide spectrum of potential binding ligands due to infinite possible of oligonucleotide sequence can be synthesized and screened from large library consist of random sequences, or through method called Systematic Evolution of Ligands by Exponential Enrichment (SELEX). However, to be precise there is no aptamer studied that can bind against tricetin specifically.

The current method of identifying, isolating, and extracting aptamer are limited, and can be laborious and expensive to perform (Feng et al., 2017). Such method is column chromatography, by using different absorbent such as silica gel, and polyamide, and High- Performance Liquid Chromatography (HPLC). Due to the potential of aptamer to bind broad spectrum of ligands, makes it the best candidate to bind against tricetin. By determining the aptamer that can bind to tricetin, we can improve the current method of extraction. However the ssDNA aptamer that specifically binds to tricetin is still unavailable in any accessible database. Finding and constructing the ssDNA aptamer allows widen the potential of future study in understanding the potential application of aptamer. The only available ssDNA aptamer is LUT#3, constructed from SELEX in a study conducted by Tunam Sabah et al (2018) that specifically binds toward luteolin. However, both luteolin and tricetin is in the same group of flavonoids which is the flavone. Investigating the important residue from both aptamer and ligand is important to further evaluate the binding of the two. Hence, LUT#3 ssDNA aptamer is chosen to study and understand the interaction between the receptor and the ligand.

Materials and methods

The LUT#3 aptamer nucleotide sequence and 2D structure has been reported in research conducted by Tuma Sabah et al. (2018). The LUT#3 aptamer 3D structure can be constructed using the available nucleotide sequence through RNAComposer web service. Due to software only accept RNA nucleotide base, the thymine base in the ssDNA LUT#3 aptamer can be changed into uracil base. The result exported in PDB file, which can be open through Pymol, and mutate the uracil base back to thymine base by using Biovia DS visualizer. The 2D and 3D structure for tricetin can be retrieve from available database such as FlavoDb, and PubChem. From PubChem, the structure can be saved in SDF file, which later can be viewed using either using Pymol or Discovery Studio Visualizer.

Next, both structure LUT#3 and the tricetin undergo energy minimization step using YASARA Web server, prior to molecular docking. The molecular docking is performed using Autodock Vina, and further docking evaluation is performed using Biovia DS Visualizer for the nucleotide ligand interaction between the aptamer and the tricetin. The LUT#3-Tricetin complex again is selected for molecular docking against a reporter, fluorescein.

Next, truncation of LUT#3 ssDNA aptamer performed using the RNAcomposer, with addition step of eliminating the nucleotide bases 1 to 20, and 38 to 48, to evaluate the effect of shortening nucleotides from the original aptamer sequence producing shorter LUT#3 ssDNA aptamer sequence.

Molecular docking of aptamer against tricetin conducted using available docking tools such as AutoDock Vina. For Binding assessment, the most suitable tool to carried out is Discovery Studio Visualizer, and Pymol.

Results and discussion

Using the aptamer LUT#3 primary sequence from previous studies conducted by Tuma Sabah et al, the aptamer 2D structure were constructed using DNA Folding Form from mFold. The primary sequence for the LUT#3 aptamer retrieved from Tuma Sabah et al, is as follow; 5′- CGT ACG GAA

TTC GCT AGC ATT TTC CCC CTT AAT AAA ACA GCC CGG CGC CCT TCC TTA GGG GGG ATC CGA GCT CCA CGT G- 3 ′.

From mFold, the delta value retrieved were -7.98 kcal/mol at 37 °C, which indicated that the aptamer is stable. The structure predicted were identical to the reported studies by Tuma Sabah et al (2018). Structural validation for the aptamer LUT#3 were performed using MolProbity MolProbity nucleic acid geometry indicated that the aptamer LUT#3 consist of 0 bad bond and 0.72% bad angles

For prediction of LUT#3 aptamer 3D structure, RNAComposer were used. The primary sequence of LUT#3 aptamer was modified firsthand, due to the feature of RNAComposer that only accept RNA sequences. The nucleotide base of the ssDNA LUT#3 aptamer was changed, from thymine to uracil, to produce the structure in .pdb file format. The file later viewed using Biovia DS visualizer, and the nucleotide base are changed from uracil to thymine through mutation.

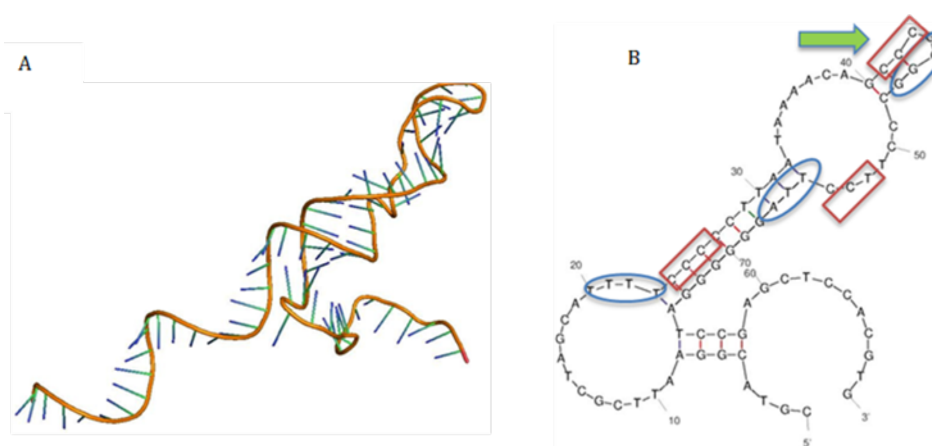

Figure 1 LUT#3 3D structure predicted using RNAComposer and viewed using Pymol. Each nucleotide (adenosine, thymine, guanine, cytosine) designated with different colors (orange, red, yellow, green). B; 2D structure of LUT#3 with blue circle representing the conserved sequence, and the red rectangle represent the consensus motifs.

Based on the figure 1 B, the 2D structure of LUT#3 showed that the structure are consist of stem and bulges. The bulges resulted from the presences of mismatched nucleotides in consecutive manner disrupting the formation of hydrogen bonds and the successful paired nucleotide formed the stem. According to Tuma Sabah et al (2018), the stem and the loop motifs (red rectangle) is responsible in ligand recognition and binding. The author stated that the stem responsible in stabilization, and the loop motif supports the binding (the green arrow). However, the presence of conserved and consensus sequence on several location indicate that the possibility of the different recognition and binding sites. The consensus sequence CCC, and which are found localized at the stem and the loop, signifying it to be an essential structural element in ligand recognition and stabilization of aptamer-ligand complex

For the targeted ligand, tricetin were screened from the existing flavonoids database, FlavoDb. The structure was provided in .sdf file format and viewed using Pymol as shown in Figure 2.

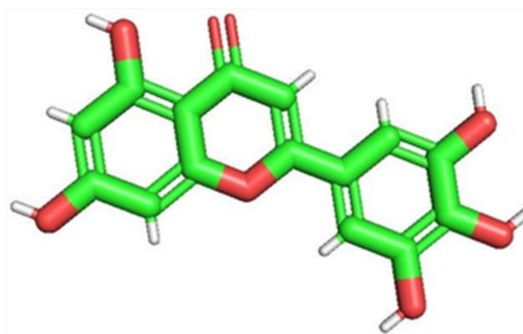

Figure 2 Tricetin 3D structure retrieved from FlavoDb and viewed using Pymol.

Energy minimization perform to reduce overall potential energy of the molecules (receptor) and the ligand, prior to molecular docking, molecular simulation, or interaction prediction. The energy minimization for ssDNA aptamer and tricetin were perform using YASARA Energy minimization Server. In table 1, the initial potential energy for ssDNA aptamer before energy minimization is -370,287.9 kj/mol and the potential energy after energy minimization significantly reduced to -396,102.4 kj/mol. Approximate 25,000 kj/mol of potential energy were minimized prior to energy minimization. As for the ligand, tricetin, Yasara able to minimize the initial potential energy from -302.1 kj/mol. To -316.8 kj/mol. The minimized ssDNA aptamer and tricetin are used for molecular docking simulation using AutoDock Vina.

The enenergy minimized structures is selected for molecular docking using AutoDock Vina. For the molecular docking of LUT#3 ssDNA aptamer against Tricetin, the result shown the highest binding affinity were -4.9, with three conformations. The first conformation is best pose from the docking with
0.000 for both rmsd l.b and rmsd u.b, while the second and the third has 1.038, 2.059, and 3.329, 5.195 for rmsd l.b and rmsd u.b respectively. In docking simulation, RMSD value is used to compare the docked conformation with the reference conformation or with other docked conformation. The conformation with the lowest RMSD value is chosen for further analysis. Hence the first conformation is chosen for further socking simulation against a reporter. For the molecular docking of the LUT#3-Tricetin complex against a reporter fluorescein, the highest binding is affinity value of -8.5.

Next, when performing docking, the tricetin binds at the nucleotides C24, C25, C26, C27, within the stem. Hence, the ssDNA aptamer are truncated, eliminating the nucleotide base 1 to 20, and 38 to 48, to evaluate the effect of shortening nucleotides particularly the binding affinity of tricetin when performing molecular docking simulation. RNAcomposer were used to construct the the truncated ssDNA aptamer 3D structure, by removing the targeted nucleotide base from the original sequence. AutoDock Vina was used to perform molecular docking of truncated ssDNA and energy-minimized tricetin. The docking result from the docking simulation shows highest binding affinity is -5.1, slightly improved compared to the original sequence. The stem length and the structural size ratio may contribute in increasing stabilization

Table 1: The comparison of binding affinity between truncated LUT#3 aptamer and the original LUT#3 aptamer.

ssDNA aptamer	Number of nucleotide bases	Binding Affinity
Truncated LUT#3 aptamer	49	-5.1
Original LUT#3 aptamer	79	-4.9

An extensive evaluation of molecular docking analysis is performed using Biovia DS Visualizer for each previous docking simulation results (Aptamer-Tricetin complex, Aptamer-Tricetin-Repoter complex, and Truncated Aptamer-Tricetin complex). The parameter that were highlighted during the evaluation is the nucleotide-ligand interaction between the LUT#3 ssDNA aptamer against tricetin and were successfully tabulated.

Table 2: The nucleotide ligand interaction between LUT#3 aptamer and Tricetin.

Interacting residue	Category	Type of bond	Distance
DC24:H42 - :TRIC:O4	Hydrogen Bond	Conventional Hydrogen Bond	2.60841
DC25:H41 - :TRIC:O4	Hydrogen Bond	Conventional Hydrogen Bond	2.71122
DC25:H42 - :TRIC:O4	Hydrogen Bond	Conventional Hydrogen Bond	2.84102
DC25:H42 - :TRIC:O2	Hydrogen Bond	Conventional Hydrogen Bond	2.27711
DC26:H42 - :TRIC:O5	Hydrogen Bond	Conventional Hydrogen Bond	2.20198
DC26:H42 - :TRIC:O3	Hydrogen Bond	Conventional Hydrogen Bond	2.57231
DC27:H41 - :TRIC:O3	Hydrogen Bond	Conventional Hydrogen Bond	2.58777
DC27:H42 - :TRIC:O3	Hydrogen Bond	Conventional Hydrogen Bond	2.51436

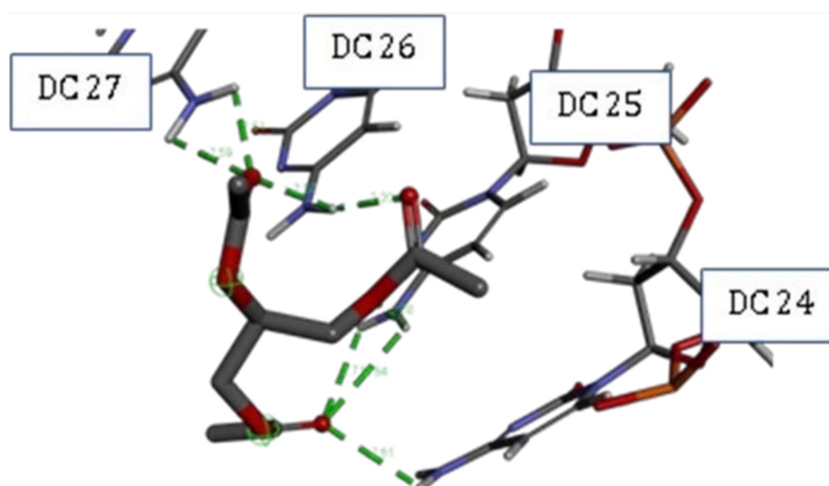

Figure 3 The nucleotide ligand interaction between LUT#3 aptamer and Tricetin

Table 3: The overall nucleotide ligand interaction between LUT#3 aptamer Tricetin complex against reporter extracted from Biovia DS visualizer

Interacting residue	Category	Type of bond	Distance
DG61:H22 -:FLU:O	Hydrogen Bond	Conventional Hydrogen Bond	2.02145
DC27:C1*-:FLU:O	Hydrogen Bond	Carbon Hydrogen Bond	3.61878
DG61:C4*-:FLU:O	Hydrogen Bond	Carbon Hydrogen Bond	3.4225
DC67 - :FLU	Hydrophobic	Pi-Pi Stacked	4.01164
DG68 - :FLU	Hydrophobic	Pi-Pi Stacked	5.77876
DG68 - :FLU	Hydrophobic	Pi-Pi Stacked	4.5763
DG61 - :FLU	Hydrophobic	Pi-Pi T-shaped	5.34887

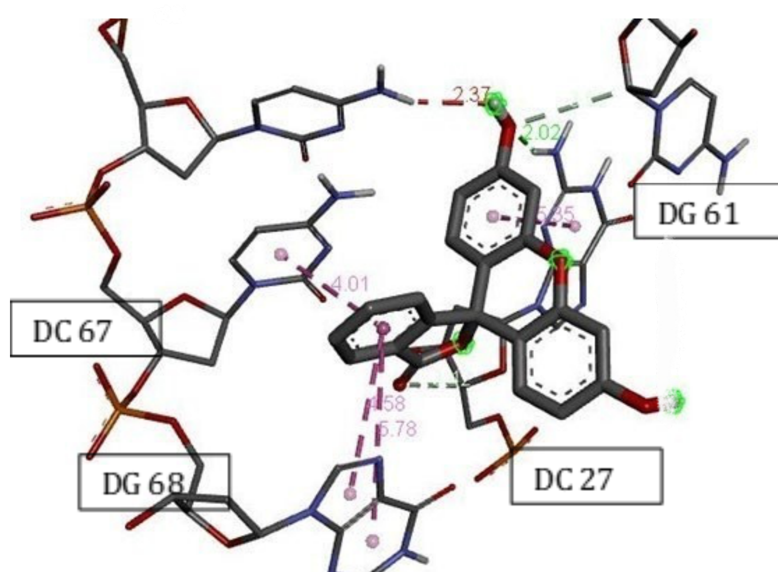

Figure 4 The nucleotide ligand interaction between LUT#3 aptamer Tricetin complex against reporter.

Table 4: Nucleotide-ligand interactions between Truncated LUT#3 aptamertricetin extracted from Biovia DS Visualizer

Interacting residue	Category	Type of bond	Distance
DA14:H61-:TRIC:O4	Hydrogen Bond	Conventional Hydrogen Bond	2.32232
DA14:H62-:TRIC:O1	Hydrogen Bond	Conventional Hydrogen Bond	2.64572
DA15:H62-:TRIC:O4	Hydrogen Bond	Conventional Hydrogen Bond	2.42319
DC23:H41-:TRIC:O3	Hydrogen Bond	Conventional Hydrogen Bond	2.60957
DC23:H42-:TRIC:O3	Hydrogen Bond	Conventional Hydrogen Bond	2.91132
TRIC:C1-A-:DT21:OP2	Hydrogen Bond	Carbon Hydrogen Bond	3.62642
TRIC:C8 - A:DA11	Hydrophobic	Pi-Sigma	3.87798

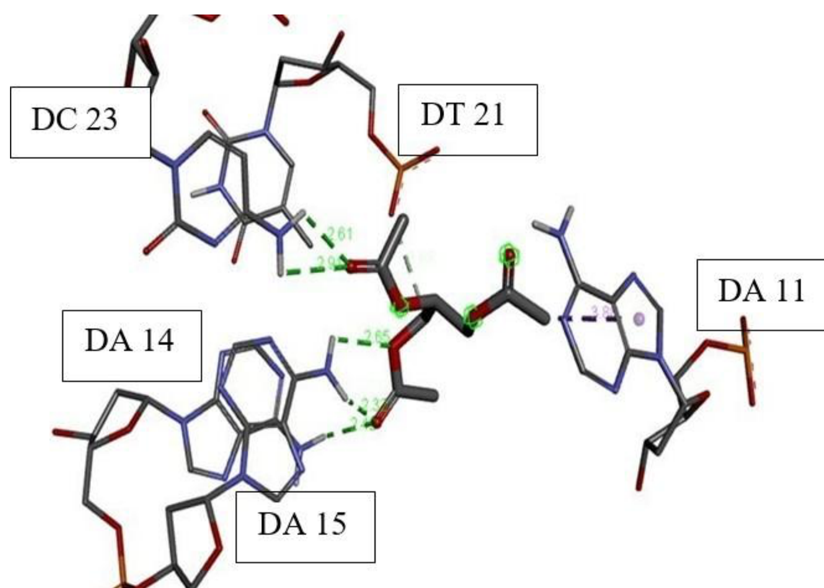

Figure 5 Nucleotide ligand interaction between truncated LUT#3 aptamer against Tricetin

Conclusion

In conclusion, the LUT#3 ssDNA aptamer against tricetin were successfully evaluated via *in silico*. Further analysis must perform such as molecular dynamic simulation to simulate the natural motion of the molecular system to further understands the behavioral of the aptamer and the ligand.

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References

Cheng, A. X., Han, X. J., Wu, Y. F., & Lou, H. X. (2014). The Function and Catalysis of 2 Oxoglutarate-Dependent Oxygenases Involved in Plant Flavonoid Biosynthesis. *International Journal of Molecular Sciences,* 15(1), 1080–1095.

Feng, W., Hao, Z., & Li, M. (2017). Isolation and Structure Identification of Flavonoids. Flavonoids From Biosynthesis to Human Health.

Hsu, Y. L., Uen, Y. H., Chen, Y., Liang, H. L., & Kuo, P. L. (2009). Tricetin, a Dietary Flavonoid, Inhibits Proliferation of Human Breast Adenocarcinoma MCF-7 Cells by Blocking Cell Cycle Progression and Inducing Apoptosis. *Journal of Agricultural and Food Chemistry*, 57(18), 8688–8695.

Kalra, P., Dhiman, A., Cho, W. C., Bruno, J. G., & Sharma, T. K. (2018). Simple Methods and Rational Design for Enhancing Aptamer Sensitivity and Specificity. *Frontiers in Molecular Biosciences*, 5.

Kopustinskiene, D. M., Jakstas, V., Savickas, A., & Bernatoniene, J. (2020). Flavonoids as Anticancer Agents. *Nutrients*, 12(2), 457.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. J*ournal of Nutritional Science*, 5.

Pietta, P. G. (2000). Flavonoids as Antioxidants. Journal of Natural Products, 63(7), 1035–1042.

Radom, F., Jurek, P. M., Mazurek, M. P., Otlewski, J., & Jeleń, F. (2013). Aptamers: Molecules of great potential. *Biotechnology Advances,* 31(8), 1260–1274.

Ren, J., Yuan, L., Wang, W., Zhang, M., Wang, Q., Li, S., Zhang, L., & Hu, K. (2019). Tricetin protects against 6-OHDA-induced neurotoxicity in Parkinson's disease model by activating Nrf2/HO-1 signaling pathway and preventing mitochondria-dependent apoptosis pathway. *Toxicology and Applied Pharmacology*, 378, 114617.

Smit, S., Rother, K., Heringa, J., & Knight, R. (2008). From knotted to nested RNA structures: a variety of computational methods for pseudoknot removal. *RNA 14*(3): 410-416.

Shirdel, S. A., & Khalifeh, K. (2019). Thermodynamics of protein folding: methodology, data analysis and interpretation of data. European Biophysics Journal, 48(4), 305–316.

Song, K. M., Lee, S., & Ban, C. (2012). Aptamers and Their Biological Applications. *Sensors*, 12(1), 612–631.

Tuma Sabah, J., Zulkifli, R. M., Shahir, S., Ahmed, F., Abdul Kadir, M. R., & Zakaria, Z. (2018). In vitro selection and characterization of single-stranded DNA aptamers for luteolin: A possible recognition tool. *Analytical Biochemistry*, 549, 72–79.

Wu, J., Zhu, Y., Xue, F., Mei, Z., Yao, L., Wang, X., Zheng, L., Liu, J., Liu, G., Peng, C., & Chen, W. (2014). Recent trends in SELEX technique and its application to food safety monitoring. *Microchimica Acta*, 181(5–6),