Research Article

Insights on Anthocyanidins' Binding Affinity and Molecular Interactions with Zika Virus Protein Targets

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ABSTRACT

Neonatal microencephaly and some neurological disorders have been associated with Zika virus (ZikV) infection. In recent years, the pathophysiology of ZikV was well elucidated; hence, different drug targets have been proposed to inhibit its activities. However, there has been no approved drug against ZikV infection to date. This paper examined the binding affinity and non-covalent binding interactions of the different classes of anthocyanidins against ZikV drug targets. Anthocyanidins are plant pigments, where their bio-functionalities are reasonably well studied. Anthocyanidins and their derivatives have shown antiviral properties; however, their mechanism against ZikV remains elusive. Through *in silico* molecular docking, this paper illuminates the understanding of these compounds' binding interactions and binding energies with the different ZikV protein drug targets, namely NS3 helicase, NS2B-NS3 protease, NS5 methyltransferase, NS5 polymerase, and Axl kinase. Results have shown that anthocyanidins generally have a higher binding affinity with NS5 methyltransferase compared to the others. Also, the top-binding anthocyanidin differs in each protein. This paper hypothesized that the inhibitory potential of the different classes of anthocyanidins might differ due to the contrasting binding interactions with the various ZikV protein drug targets.

Key words: Anthocyanidins, Anthocyanin, Zika virus, molecular docking, *in silico*.

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INTRODUCTION

Currently, no particular drug is approved against Zika virus (ZikV) infection.[1] This virus is typically transmitted to the human host from the bite of an *Aedes* species mosquito carrying the virus. Other means of transmission may be through sex, blood transfusion, and pregnant woman to the fetus.[2] ZikV infection poses a detrimental effect on the development of the fetus, which may lead to brain defects, miscarriage, and even stillbirth.[3]

In the past few years, we learned a lot from the pathogenesis of this flavivirus, particularly the potential drug targets for its inhibition. The ZikV is a single-stranded RNA with only one open reading frame, which, when translated, leads to the expression of three structural proteins and seven non-structural proteins.[4] The three structural proteins are the capsid, envelope, and precursor membrane proteins, whereas the seven non-structural proteins are the NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. This study examined four non-structural proteins, namely NS3 helicase, NS2B-NS3 protease, NS5 methyltransferase, and NS5 polymerase. These proteins are essential in the lifecycle and replication of ZikV. Since ZikV contains a positive-strand RNA, the NS5 protein must create a negative strand for its replication.[2] The replication process of ZikV would not be completed without the complex formation of NS2B-NS3, which leads to NS2B-NS3 protease.[4]

Moreover, the Axl receptor tyrosine kinase attracts attention for it is a critical target against cancer and the entry point of the virus to the human cells.[5] Studies

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have shown that the envelope protein of ZikV attaches to the Axl kinase receptor.[4,5] This instance activates the clathrin-mediated endocytic pathway causing the envelope protein to undergo a conformational change.[4] This conformational change is advantageous to the virus as it allows the fusion of the envelope protein with the host cell membrane receptor.[4,5]

Several lead compounds have been identified as potential inhibitors to these non-structural proteins of ZikV. Among the lead compounds examined were flavonoids. Studies have shown that flavonoids have antioxidant, anticancer, and antiviral properties.[6] One particular group of flavonoids is anthocyanins, which are widely represented in various fruits and vegetables. There are six classes of anthocyanidins, the aglycone form of anthocyanins, investigated in this paper. These anthocyanidins were cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, as shown in Figure 1.

The 3D structure of the anthocyanidin compounds underwent molecular docking to understand their binding interactions with the different drug targets in ZikV. This study aims to gain insight into the possible mechanism of anthocyanidins against ZikV infection.

MATERIALS AND METHODS

Retrieval of Anthocyanidin 3D structures

The 3D structure of the following anthocyanidins was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/): cyanidin (CID: 128861), delphinidin (CID: 68245), malvidin (CID: 159287), pelargonidin (CID: 440832), peonidin (CID: 441773), and petunidin (CID: 441774).

Preparation of ZikV non-structural proteins

The PDB crystal structures of the following non-structural proteins were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org): NS3 helicase (PDB ID: 5JRZ), NS2B-NS3 protease (PDB ID: 5H4I), NS5 methyltransferase (PDB ID: 5KQR), NS5 polymerase (PDB ID: 5M2Z), and Axl kinase (PDB ID: 5U6B). Before docking, these proteins were pre-processed by adding hydrogens and Gasteiger charges; merging charges; and removing non-polar hydrogens, lone-pairs, water molecules, and non-standard residues.[7]

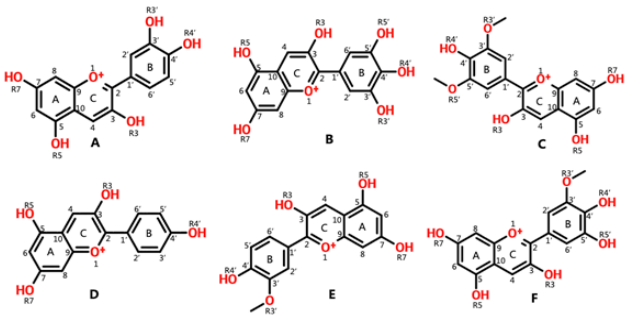

Figure 1: Structures of the different classes of anthocyanidin. A: cyanidin, B: delphinidin, C: malvidin, D: pelargonidin, E: peonidin, F: petunidin.

Molecular Docking

Ligands in the original crystal structures were re-docked in each protein molecule to validate the docking.[7] The ligands used were (hydroxy-oxido-phosphoryl) hydrogen phosphate (POP) for NS3 helicase, benzimidazol-1-ylmethanol (7HQ) for NS2B-NS3 protease, S-Adenosylmethionine (SAM) for NS5 methyltransferase, S-Adenosyl-L-Homocysteine (SAH) for NS5 polymerase, and (10R)-7-amino-11-chloro-12-fluoro-1-(2-hydroxyethyl)-3,10,16-trimethyl-16,17-dihydro-1H-8,4-(azeno)pyrazolo[4,3-h][2,5,11] benzoxadiazacyclotetradecin-15(10H)-one (7YS) for Axl kinase. Superimposed crystal structures with a root mean square deviation (RMSD) value of <2.0 Å were considered in the study.[7] The 3D structure of the different anthocyanidins was docked in the binding center of the respective protein crystal structure using mcule (Mcule Inc., USA). The structures with the lowest docking score were considered the best-docked pose.

Post-Dock Analysis

The crystal structures of the best-docked pose were downloaded and visualized using PyMol format. The non-covalent binding interactions between the ligand and amino acid residues were noted.

RESULTS

Binding affinity of the anthocyanidins with ZikV proteins

The anthocyanidins underwent *in silico* docking with ZikV proteins to hypothesize the possible target enzyme of the compounds. Results showed that the anthocyanidins have the highest binding affinity to NS5 methyltransferase, ranging from -7.4 to -8.5, as shown in Table 1. Delphinidin, in particular, has the highest binding affinity with the enzyme of interest, -8.5. The enzymes following NS5 transferase with the highest docking are the NS3 helicase and NS5 polymerase, with a binding score ranging from -7.5 to -7.8 and -7.2 to -7.7, respectively. Lastly, the enzymes with the lowest binding score with the anthocyanidins were NS2B-NS3 protease and Axl kinase, which have a docking score ranging from -7.0 to -7.4 and -7.0 to -7.3, respectively.

Overall, the docking scores of the anthocyanidins with ZikV are comparable.

Non-covalent interactions of anthocyanidins with NS3 helicase

The NS3 helicase inhibitory ligand, POP, and the other anthocyanidins cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin were docked at the chain A of NS3 helicase, where all of these ligands form H bonds with the amino acid residues of the enzyme, as shown in Figure 2, only in POP where water bridges and salt bridges were evident. In contrast, there were hydrophobic interactions and pi-cation interactions distinct in the different anthocyanidins.

Table 1: Ranking of the Docking Scores (kcal/mol) of the Ligand-Protein Binding.

NS3 helicase	NS2B-NS3 protease	NS5 methyltransferase	NS5 polymerase	Axl kinase
Cyanidin -7.8	Pelargonidin -7.4	Delphinidin -8.5	Delphinidin -7.7	Petunidin -7.3
Malvidin -7.8	Peonidin -7.4	Cyanidin -8.1	Pelargonidin -7.6	Malvidin -7.2
Delphinidin -7.6	Petunidin -7.4	Petunidin -8.1	Petunidin -7.6	Peonidin -7.2
Peonidin -7.6	Cyanidin -7.3	Peonidin -7.9	Cyanidin -7.5	Delphinidin -7.1
Petunidin -7.5	Delphinidin -7.2	Malvidin -7.6	Peonidin -7.5	Cyanidin -7.1
Pelargonidin -7.5	Malvidin -7.0	Pelargonidin -7.4	Malvidin -7.2	Pelargonidin -7.0

POP forms 2 H bonds with gly197 and a single H bond with ala198, gly199, lys200, thr201, and asn417. There are three water bridges formed with arg459 and only one with gln455 (Figure 2A). Lastly, two salt bridges with lys200 and three salt bridges with arg462 were also formed. In cyanidin, the hydrophobic interaction of C2' interacts with thr201 (Figure 2B). Meanwhile, the H bonds in cyanidin were formed with R7 (gly197, ala198, gly199, lys200), R5 (gln455, lys200), O1 (thr201), R3 (gly415, glu231), and R3' (glu231). It also has pi-cation interaction lys200 and arg462 (2). In delphinidin, its C2' has hydrophobic interaction with thr201. Delphinidin forms H bonds with the enzyme through R7 (gly197, ala198, gly199, lys200), R5 (lys200, gln455), R3 (glu231, gly415) (Figure 2C). Its pi-cation interactions were with lys200 and arg462 (2). In malvidin, the hydrophobic interactions of its C2' were observed with thr201 (Figure 2D). It also forms H bonds with NS3 helicase through R7 (his195, gly197, ala198, gly199, lys200), R5 (lys200, gln455), R3 (gly415), R5' (asn417), and O1

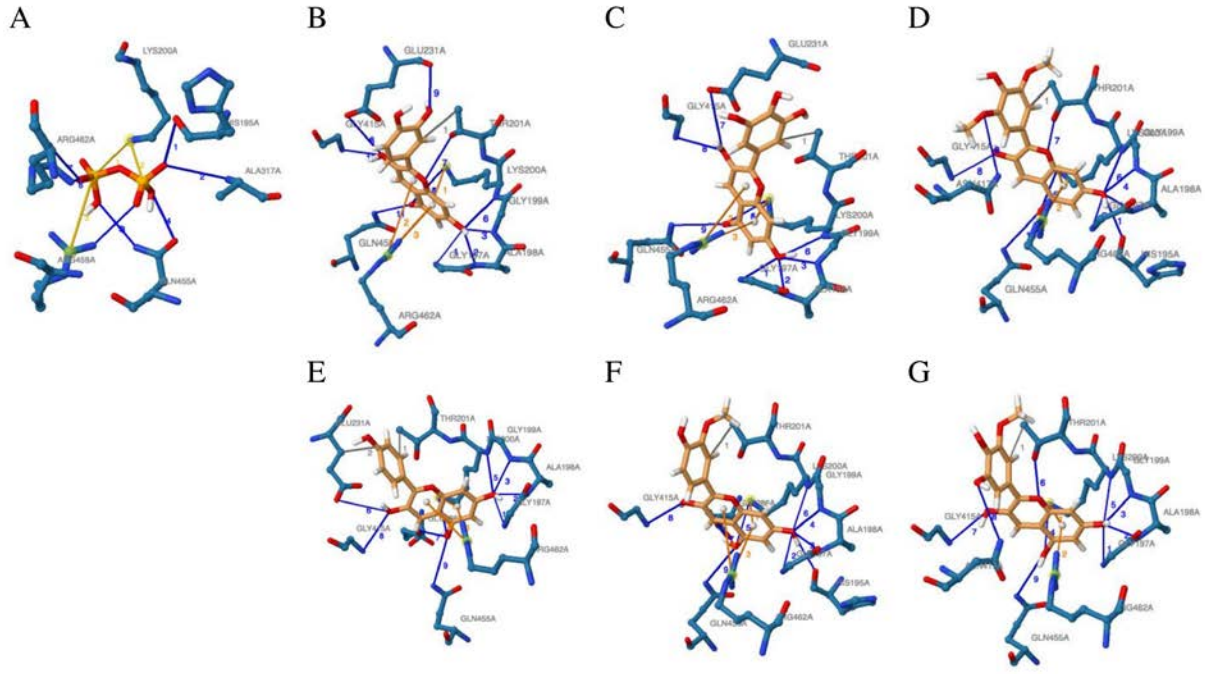

Figure 2: Docked crystal structures of the different classes of anthocyanidin with NS3 helicase. A: POP, B: cyanidin, C: delphinidin, D: malvidin, E: pelargonidin, F: peonidin, G: petunidin.

(thr201). Its pi-cation interactions were with lys200 and arg462. In pelargonidin, the hydrophobic interactions were observed in C2' and C3', which interacts with thr201 and glu231, respectively. Pelargonidin forms H bonds with the enzyme through R7 (gly197, ala198, gly199, lys200), R5 (lys200, glu286, gln455), and R3 (glu231, gly415) (Figure 2E). Its pi-cation interactions were with lys200 and arg462 (2). In peonidin, the hydrophobic interaction was observed in C2' where it interacts with thr201 (Figure 2F). Peonidin forms H bonds with the enzyme through R7 (his195, gly197, ala198, gly199, lys200), R5 (lys200, glu286, gln455), and R3 (gly415). Its pi-cation interactions were with lys200 and arg462 (2). Lastly, the hydrophobic interactions in petunidin are evident in C2', where it interacts with thr201 (Figure 2G). Petunidin forms H bonds with the enzyme through R7 (gly197, ala198, gly199, lys200), R5 (lys200, gln455), R3 (gly415), O1 (thr201), and R5' (asn417). Its pi-cation interactions were with lys200 and arg462.

The POP binding site of NS3 helicase, gly197, ala198, gly199, and lys200 are essential amino acid residues for forming an H bond. Interestingly, the R7 substructure of the anthocyanidin typically forms an H bond with these amino acids. Similar to POP, the anthocyanidins form pi-cation interaction with lys200 and arg462.

Non-covalent interactions of anthocyanidins with NS2B-NS3 protease

The NS2B-NS3 protease was docked with 7HQ, its inhibitory ligand, and the anthocyanidins cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin, as shown in Figure 3. These ligands were docked at the chain B of NS3 helicase, where they formed hydrophobic interactions, H bonds, and pi-stacking with the amino acid residues of the enzyme. 7HQ forms hydrophobic interaction with tyr161, H bonds with thr134, and pi-stacking with tyr161 (Figure 3A). In cyanidin, C2' forms hydrophobic interacts with tyr161 (Figure 3B). Meanwhile, the H bonds in cyanidin were formed with R5 (tyr130), R3 (gly151), and R3' (asn152 and gly153). It also has pi-stacking with tyr161. In delphinidin, the hydrophobic interaction is evident in C2' interacting with tyr161, whereas C8 with val155 (Figure 3C). Meanwhile, the H bonds in delphinidin were formed with R3 (ser135), R4' (asn152), R5' (his51), and 2 H bonds in R3' (gly153). It also has pi-stacking with tyr161. On the other hand, malvidin forms hydrophobic interaction through C6 with his51 (Figure 3D). Meanwhile, the H bonds in malvidin were formed with R7 (lys54), R5 (asp83), and R3 (asn152, gly153, and tyr161). It also has pi-stacking with his51 (2) and tyr161. In pelargonidin, the hydrophobic interaction of C5' is evident with tyr150 (Figure 3E). Meanwhile, the H bonds in pelargonidin were formed with O (tyr161), 2 H bonds R3 (ser135), and 2 H bonds R4' (tyr130). It also has pi-stacking with tyr161. In peonidin, H bonds were formed with O1 (tyr161), R5 (his51),

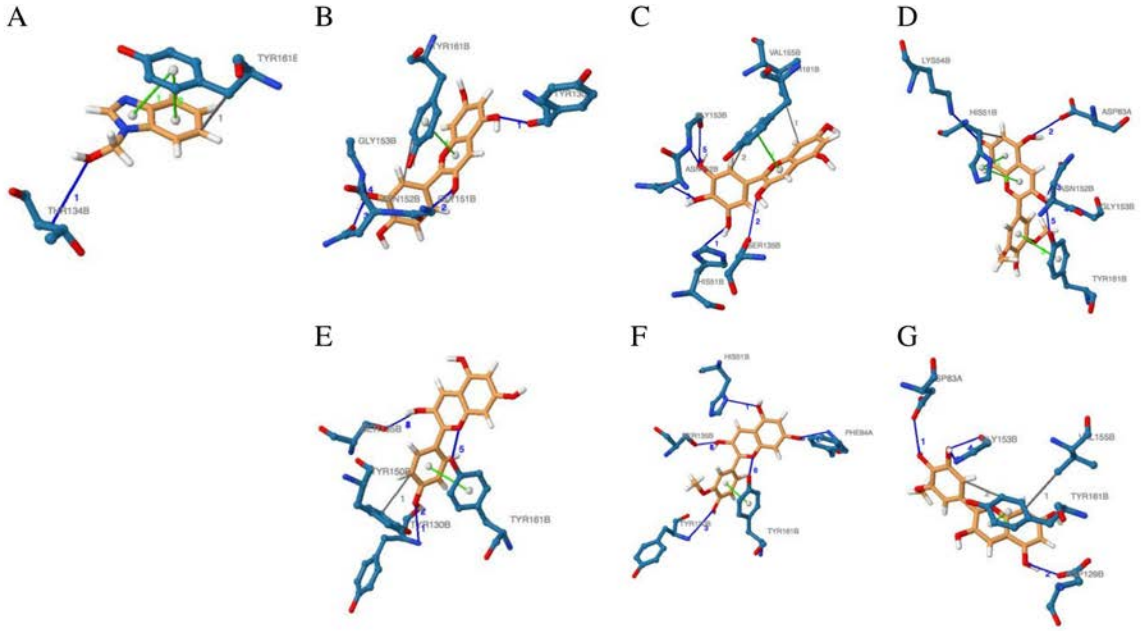

Figure 3: Docked crystal structures of the different classes of anthocyanidin with NS2B-NS3 protease. A: 7HQ, B: cyanidin, C: delphinidin, D: malvidin, E: pelargonidin, F: peonidin, G: petunidin.

2 H bonds R7 (phe84), R4' (tyr130), and 2 H bonds R3 (ser135) (Figure 3F). It also has pi-stacking with tyr161. In petunidin, the hydrophobic interactions are in C8 and R2', which interact with val155 and tyr161 (Figure 3G). Meanwhile, the H bonds in petunidin were formed with R4' (asp83), 2 H bonds in R5 (asp129), and 2 H bonds R3' (gly153). It also has pi-stacking with tyr161.

Non-covalent interactions of anthocyanidins with NS5 methyltransferase

The NS5 methyltransferase was docked with SAM, its inhibitory ligand, and the anthocyanidins cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin, as shown in Figure 4. These ligands were docked at the chain A of NS5 methyltransferase, where they formed H bonds and salt bridges with the amino acid residues of the enzyme. Only in SAM where water bridges are observable, whereas there are hydrophobic interactions in the anthocyanidins.

SAM forms a single H bond with ser56, cys82, gly85, gly86, trp87, thr104, lys105, val132, and 2 H bonds in glu111 and asp131 (Figure 4A). Also, SAM forms water bridges with gly81, cys82, arg84, and ser88. Meanwhile, it forms a salt bridge with glu111 and asp146. In cyanidin, the C8 forms hydrophobic interaction with arg57 (Figure 4B). It also forms H bonds in cyanidin were formed with R4' (ser56, cys82, arg84, gly85, and gly86), R7 (arg57), R3' (trp87), and R3 (asp146). Meanwhile, cyanidin forms a salt bridge with lys61. In delphinidin, its C8 forms hydrophobic interaction with arg57 (Figure 4C). It forms H bonds in delphinidin were formed with R4' (ser56, gly85, and gly86), R3' (ser56, trp87), R5' (targ84, glu111), R3 (asp146), R5 (glu218), and R7 (arg57). Meanwhile, delphinidin forms a salt bridge with lys61. In malvidin, H bonds were formed with R4' (ser56, gly86, and trp87), R3' (arg84), and R7 (lys105, gly106) (Figure 4D). The C8 of pelargonidin forms hydrophobic interaction with arg57 (Figure 4E). H bonds in pelargonidin were also observed with R4' (ser56, cys82, arg84, gly85, and gly86), R3 (asp146), R5 (lys182), and R7 (arg57). Meanwhile, it forms pi-cation interaction with lys61. The C8 of peonidin formed hydrophobic interactions with arg57 (Figure 4F). It also forms H with R4' (ser56, cys82, arg84, gly85, and gly86), R3' (trp87), and R7 (arg57), whereas pi-cation interaction is observed with lys61. In petunidin, the hydrophobic interaction with arg57 is observed in C8 (Figure 4G). It also forms H bonds with R4' (ser56, gly85, and gly86), R3' (ser56, trp87), R5' (arg84 (2), glu111), R5 (glu218), and R7 (arg57). Meanwhile, it forms pi-cation interaction with lys61.

Non-covalent interactions of anthocyanidins with NS5 polymerase

The NS5 polymerase was docked with SAH, its inhibitory ligand, and the anthocyanidins cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin, as shown in Figure 5. These ligands were docked at the chain E of NS5 polymerase, where they formed H bonds with the amino acid residues of the enzyme, whereas only the anthocyanidins have hydrophobic interactions.

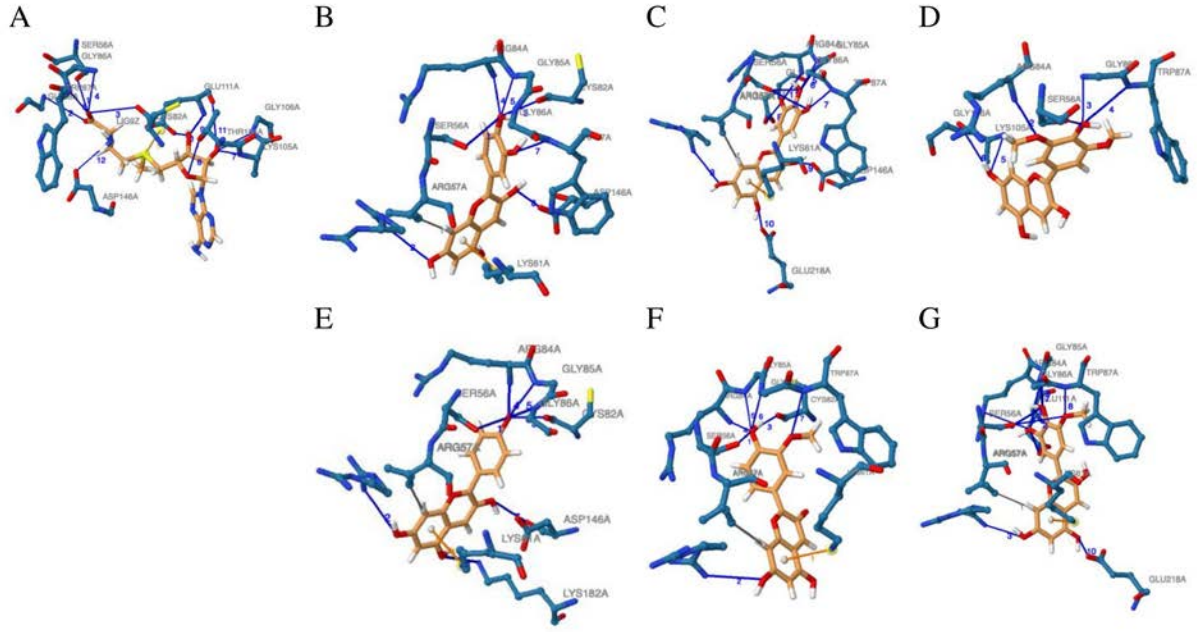

Figure 4: Docked crystal structures of the different classes of anthocyanidin with NS5 methyltransferase. A: SAM, B: cyanidin, C: delphinidin, D: malvidin, E: pelargonidin, F: peonidin, G: petunidin.

SAH forms a single H bond with the different amino acids in NS5 polymerases, such as ser56, gly86, trp87, thr104, lys105, and 2 H bonds with asp146 (Figure 5A). In cyanidin, the C6 and C8 form hydrophobic interactions with lys105, whereas C4 and C10 with ile147 (Figure 5B). The H bonds in cyanidin were formed with R3 (gly148), O1 (thr104), and R7 (val132). In delphinidin, the C6' forms hydrophobic interactions with lys105 (Figure 5C). The H bonds in delphinidin were formed with R3 (lys105, gly106), R7 (gly148, lys182 (2)), R5' (val130, asp131) and R4' (asp131). In malvidin, the C8 forms hydrophobic interactions with lys105, whereas C6, C10, and C4 with ile147 (Figure 5D). The H bonds in malvidin were formed with O1 (thr104), R3 (gly148), and R7 (val132). In pelargonidin, the C2' and C6' form hydrophobic interactions with lys105, while C5' with val132 (Figure 5E). The H bonds in pelargonidin were formed with R3 (lys105, gly106), R7 (gly148 (2), lys182), and R4' (val132, gly148). In peonidin, the C2' and C6' form hydrophobic interactions with lys105, while C5' with val132 (Figure 5F). The H bonds in peonidin were formed with R3 (lys105, gly106), R7 (gly148 (2), lys182), and R4' (val132, gly148). The C8 and C6 of petunidin form hydrophobic interactions with lys105, while C4 and C10 with ile147 (Figure 5G). The H bonds in petunidin were formed with O1 (thr104), R3 (gly148), R7 (val130, val132).

Non-covalent interactions of anthocyanidins with Axl kinase

The Axl kinase was docked with SAM, its inhibitory ligand, and the anthocyanidins cyanidin, delphinidin, malvidin, peonidin, pelargonidin, and petunidin, as shown in Figure 6. These ligands were docked at chain A of Axl kinase forming H bonds and hydrophobic interactions with the amino acid residues of the enzyme. The hydrophobic interactions of 7YS forms are evident in val550, lys567, leu620, and phe622 of Axl kinase (Figure 6A). Single H bonds were observed in pro621 and met623, while 2 H bonds with asp627 and ser630. In cyanidin, the C6, C4, C5', and C6' form hydrophobic interactions with phe622, ala565, lys567, and val550 (Figure 6B), respectively. The H bonds in cyanidin were formed with R5 (met623), R3' (arg676), and R4' (asp677, asp690). In delphinidin, the C4 and C6' of delphinidin form hydrophobic interactions with ala565 and val550, respectively, whereas C6 with phe622 and leu542 (Figure 6C). Two H bonds formed with R5 (met623) and R4' (asp690). Meanwhile, in malvidin, the C4, C6, C6' form hydrophobic interactions with ala565, phe622, and val550 (Figure 6D), respectively. The H bonds were formed with R5 (met623) and R3' (asp690). In pelargonidin, the C4 and C8 form hydrophobic interactions with leu542 and ala565, respectively, whereas two amino acids, lys567 and leu620, form hydrophobic interaction with C3' (Figure 6E). One H bond is formed with R5 (met623) and 2 H bonds with

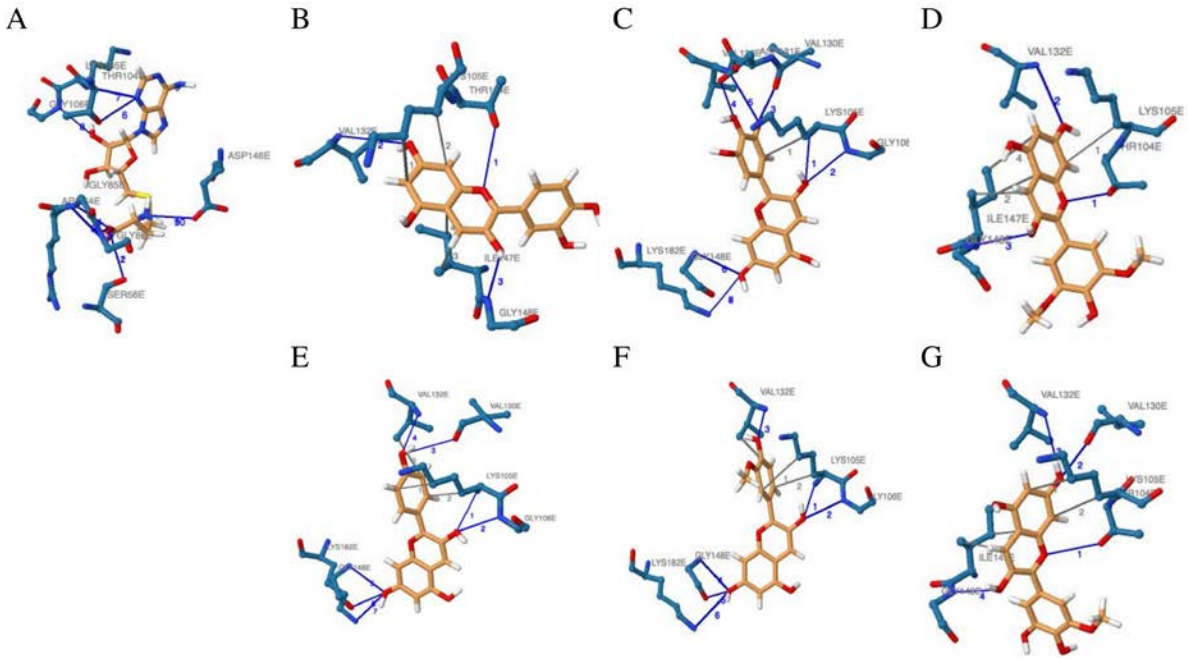

Figure 5: Docked crystal structures of the different classes of anthocyanidin with NS5 polymerase. A: SAH, B: cyanidin, C: delphinidin, D: malvidin, E: pelargonidin, F: peonidin, G: petunidin.

R4' (asp690). Meanwhile, peonidin's C6', C4, C5', and C6 form hydrophobic interactions with val550, ala565, lys567, and phe622 (Figure 6F), respectively. One H bond is formed with R4' (asp690) and 2 H bonds with R5 (met623). Lastly, in petunidin, the C6' and C4 of petunidin form hydrophobic interactions with val550 and ala565 (Figure 6G), respectively. The H bonds in petunidin are formed with R5 (met623) and R3 (asp690).

DISCUSSION

The virtual docking reveals that anthocyanidins have the highest binding affinity with NS5 methyltransferase compared with the other ZikV enzymes. Recent studies show that anthocyanidin-3,5-diglucoside has potential inhibitory activity when bound to the SAH-binding site of the dengue virus (DENV).[8] Several studies also investigated other flavonoids for antiviral properties, and their findings seem to have promising results. In Figure 7, the hypothesized mechanism of action of the different anthocyanidins against ZikV is summarized.

Inhibiting NS3 helicase disrupts viral replication, particularly the unwinding of viral RNA after NTP hydrolysis.[9] Cyanidin and malvidin were the top-binding anthocyanidins with NS3 helicase. Structurally, the difference between the two compounds is the presence of methoxy groups in the R3' and R5' prime of malvidin, whereas there is only a hydroxy group in the R3' of cyanidin. Apparently, the high number of H bonds on the hydroxy groups of cyanidin and malvidin and the amino acid residue of NS3 helicase may be the reason for the low docking score. Similarly, studies show that flavones like 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone (5DP), 5-hydroxy-3,6,7,8,3',4'-hexamethoxyflavone (5HH), and myricetin-3-O-rhamnoside (M3OR) displayed a high binding affinity with ZikV NS3 helicase.[10]

Similar to NS3 helicase, NS2B-NS3 protease is pivotal in viral replication. Inhibition of this protein impedes the viral polyprotein processing into various individual proteins.[11] Interestingly, there were three top-binding anthocyanidins in the NS3 helicase crystal structure: pelargonidin, peonidin, and petunidin. The similarity in the structure between petunidin and peonidin is closer than pelargonidin. They only differ in the presence of the hydroxy group in petunidin's R5' group. Meanwhile, pelargonidin does not contain a methoxy group in its R3'. However, both pelargonidin and peonidin's hydroxy group demonstrated an H bond formation in their R4' with similar amino acid residue compared to petunidin's R4' hydroxy group. Moreover, the R3' methoxy group of petunidin seems advantageous in forming an H bond compared to peonidin's. Despite these disparities in their intermolecular binding interactions, all of them were predicted to have comparable binding energies with NS2B-NS3 protease. Similarly, flavonoids like epigallocatechin gallate, isoquercetin, rutin, and sanggenon O also demonstrated high binding affinity with ZikV NS2B-NS3 protease through binding in the catalytic triad his51, asp75, and ser135.[12]

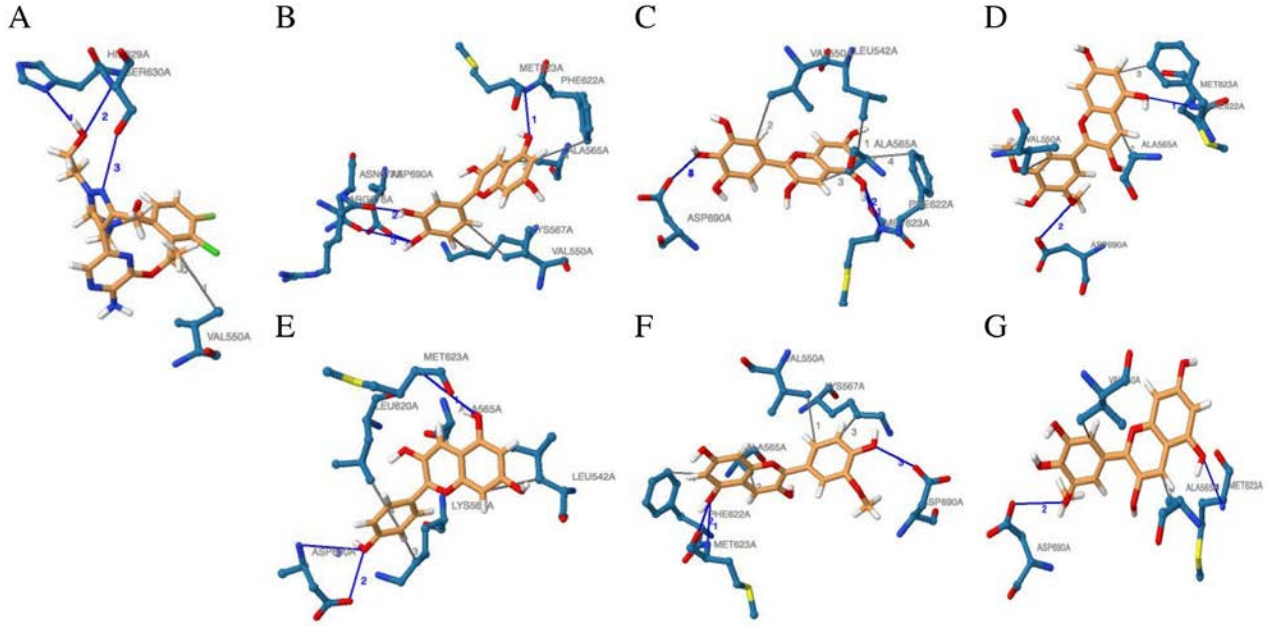

Figure 6: Docked crystal structures of the different classes of anthocyanidin with Axl kinase. A: SAM, B: cyanidin, C: delphinidin, D: malvidin, E: pelargonidin, F: peonidin, G: petunidin.

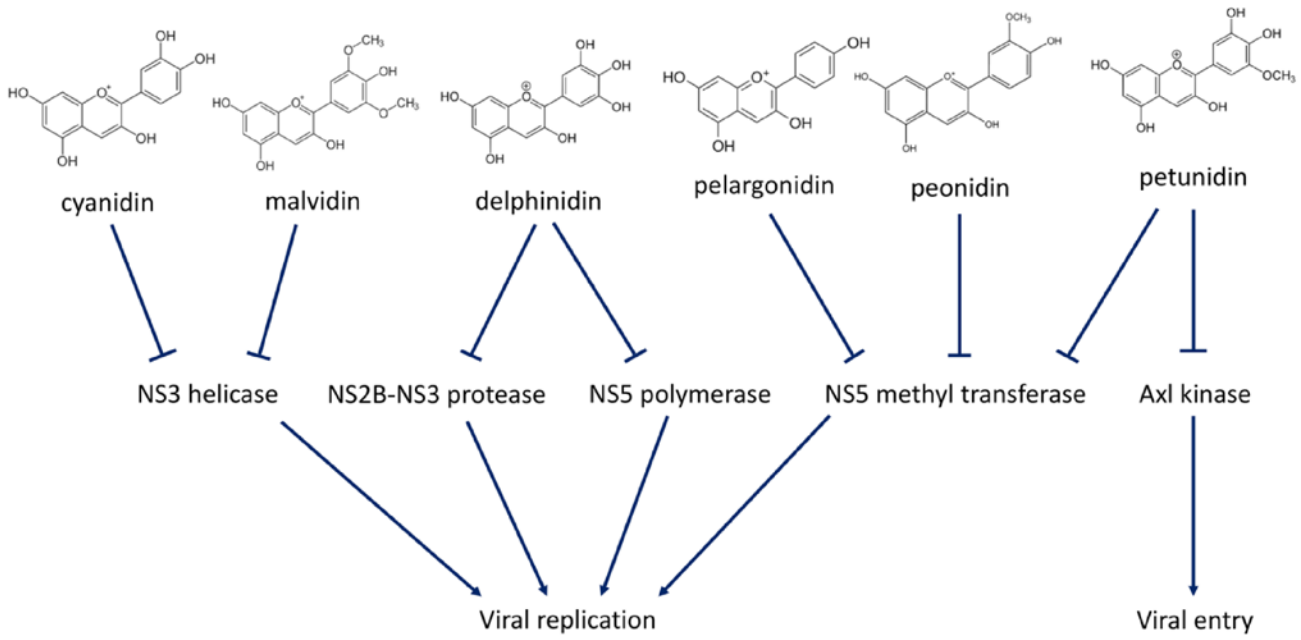

Figure 7: The hypothesized mechanism of anthocyanidins against ZikV infection.

NS5 methyltransferase is claimed to be a potent drug target due to its inhibition disabling the methylation of the RNA cap, which suppresses the formation of N-7-methyl-guanosine and 2'-O-methyl-adenosine.[13] The high binding affinity of delphinidin to NS5 methyltransferase may be attributed to the H bond formed with the following substructures: R4', R3', R5', R3, R5, and R7. Not all of these substructures present in other anthocyanidin displayed H bonding with the amino acid residues of NS5 methyltransferase. Consistent with other studies done on DENV, cyanidin (ChEBI ID: 72705) and delphinidin (ChEBI ID: 80430) have a high binding affinity with NS5 methyltransferase.[14] On the other hand, the citrus polymethoxyflavone, tangeretin, demonstrated a competitive inhibition on NS5 methyltransferase.[15] Another polyphenol, theaflavin, has also displayed NS5 methyltransferase inhibition.[13]

Another viral replication protein, NS5 polymerase, is a potential target against ZikV. Lead compounds that inhibit this protein can downregulate viral RNA synthesis.[16] Anthocyanidins Similar to NS5 methyltransferase, delphinidin appears to have a high binding affinity with NS5 polymerase. This high binding affinity may be due to the high H bond formation with delphinidin and NS5 polymerase compared to the other anthocyanidins. Notably, the hydroxy group in R3 and R7 seems an essential substructure in this interaction. Another study reported that baicalein and baicalin, both flavonoid analogs, were found to suppress ZikV NS5 RNA polymerase.[17]

Among the protein targets observed, Axl kinase is the only protein that does not directly affect viral replication. This protein was elucidated to be the entry point of ZikV, which also acts as an immune modulator.[5] The anthocyanidins have a generally lower binding affinity to this protein compared to the other proteins tested. However, petunidin appeared to be the best binder. A study on isoquercitrin and quercetin demonstrated that the sugar moiety bound to a flavonoid impacts the antiviral activity against ZikV.[4] It appears that the aglycone form, quercetin, fails to share a similar Axl kinase-related antiviral mechanism with the isoquercitrin.[4] This instance may explain the low binding affinity of the anthocyanidins, the aglycone form of anthocyanins, with Axl kinase.

CONCLUSION

In the present study, the docking revealed that the different classes of anthocyanidins have varying binding affinity and binding interactions with ZikV drug targets. The top-binders for each enzyme are as follows: NS3 helicase (cyanidin and malvidin), NS2B-NS3 protease (delphinidin), NS5 methyltransferase (pelargonidin, peonidin, and petunidin), NS5 polymerase (delphinidin), and Axl kinase (petunidin). Whether or not the anthocyanidins such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin exert antiviral activities *in vitro* and *in vivo* remains to be further examined. It is worth considering that anthocyanidins may be an appealing antiviral compound to investigate

due to their well understood biological fate in humans and their availability in several fruits and vegetables.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ZikV: Zika vius; **RMSD:** root mean square deviation.

SUMMARY

The top-binding anthocyanidins on ZikV protein drug target varies: NS3 helicase (cyanidin and malvidin), NS2B-NS3 protease (delphinidin), NS5 methyltransferase (pelargonidin, peonidin, and petunidin), NS5 polymerase (delphinidin), and Axl kinase (petunidin).

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