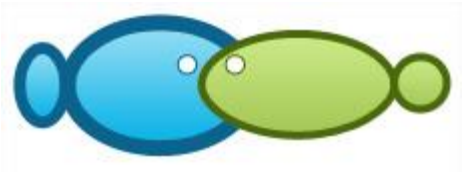

Screening of flavonoids from *Muntingia calabura* aqueous leaf extract and its potential influence on different metabolic enzymes in *Danio rerio*

[1,2]John S. Nas

[1] Department of Biology, College of Arts and Sciences, University of the Philippines Manila, Manila, Philippines; [2] Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Manila, Philippine. Corresponding author: J. S. Nas, jbnas@up.edu.ph

Abstract. Healthy nutrition in aquaculture enhances the growth and health of fish. This improvement marks high fish quality. However, expensive commercial feeds and nutrients may be a problem for small-scale fish farms. In this study, the potential of *Muntingia calabura* aqueous leaf extract (MCALE) was assessed as an alternative supplement to enhance fish growth and health. The flavonoids present in MCALE were screened,based on their Absorption, Distribution, Metabolism, Excretion, and Transport (ADMET) properties, solubility (log S) and distribution (log D) coefficient, and binding affinity on various metabolic enzymes in *Danio rerio* to determine which compounds have the most favorable ADMET properties. The binding interactions of the candidate flavonoids with the target enzyme were assessed as well, to hypothesize the possible mode of action of the flavonoid. 5 out of the 40 flavonoids found in MCALE have desirable ADMET, log S, and log D properties. These compounds were 2′,4′-dihydroxychalcone, 2′,4′-dihydroxydihydrochalcone, 5-hydroxy-7-methoxyflavone, 2′,4′-dihydroxy-3′-methoxychalcone, and 2′,3′-dihydroxy-4′-methoxychalcone. These compounds have a high binding affinity to Vitamin D3 A receptor (VD3RA), which is a vital enzyme in the endocrine system of *D. rerio*. The mixture of hydrophobic interactions and H bonds in these flavonoids with VD3RA supplements their high binding affinity. These findings suggest that particular flavonoids in MCALE may potentially affect the mineral homeostasis, immune response, and neural integrity in *D. rerio*. Further investigations are needed.
Key Words: fish metabolism, fish nutrition, molecular docking, natural product.

Introduction. In aquaculture, the feed of the fish is pivotal in its growth and development (Millamena et al 2002; Craig et al 2017). However, low-scale fish farms may not be able to afford high-quality feeds to optimize the growth of fish. This problem encourages the use of alternative feeds. Several studies paved the way for different fish feeds (Sargent & Tacon 1999; Durham 2010; Turchini et al 2010). *Muntingia calabura*, which is traditionally used as fish feed (Figueiredo et al 2008) and has antiparasitic properties (Nurhuda et al 2018) receives less attention. Despite its traditional usage, studies on *M. calabura* are scarce. Nonetheless, *M. calabura* extracts exhibit promising medicinal properties in mammals (Zakaria et al 2007; Nivethetha et al 2009).

Numerous studies use *D. rerio* as the model organism for drug development in humans. Interestingly, some extracts found effective in *D. rerio* may not be valid for humans. However, researchers rarely apply these extracts to fish. In this study, *D. rerio* was the model organism for other freshwater fish to study the effects of flavonoids from *M. calabura* aqueous leaf extract (MCALE). The accessibility of the information about the different enzymes in *D. rerio* makes it a desirable model organism.

The binding affinity of the 5 flavonoids with 6 metabolic enzymes in *D. rerio* was investigated, such as cellular retinol-binding protein II(CRBPII), vitamin D3 receptor A (VD3RA), fatty acid-binding protein 10-A (FABP10A), prostaglandin I2 synthase (CYP8A1), polo-like kinase 1 (PLK1), and fatty acid-binding protein 6 ileal (gastrotropin). These enzymes are essential in the integrity of *D. rerio*. For example, CRBPII is involved in egg production and organogenesis (Liu et al 2005), VD3RA in basic endocrine functionalities (Craig et al 2008), and FABP10A for transport and synthesis of fatty acids (Denovan-Wright

et al 2000). Other enzymes are essential in the growth and development of *D. rerio*. CYP8A1 is essential during its sexual maturation (Lister & Van Der Kraak 2009), PLK1 regulates cellular division (Xu et al 2013), and gastrotropin plays a role in bile regulation and transport (Capaldi et al 2009).

To identify which flavonoids have favorable ADMET properties, different flavonoids were screened using various physicochemical properties and binding affinity. To understand the potential mechanisms of action of the top binding flavonoids, the crystal structures of the ligand docked to the enzyme were characterized for all possible non-covalent interactions.

Material and Method

M. calabura aqueous extraction and phytochemical screening. The *M. calabura* leaves used in this study were collected from Diliman, Quezon City, Philippines. The experiment was conducted in January 2020. The leaves were sun dried for 3 days and pulverized using a grinder. The powdered leaves were soaked in distilled water (100 g L^{-1}) overnight. The aqueous extract underwent filtration using Whatman filter paper. The storage temperature of the filtrate before usage was 4°C.

The phytochemical screening followed the work of a previous study (Nas et al 2020). 1 mL of 10 mg mL^{-1} of the extract underwent phytochemical screening to determine the presence of phenols and flavonoids.

Assessment of the ADMET properties of the flavonoids. A list of all structurally distinct flavonoids extracted from MCALE from various literature was compiled (Yusof et al 2013; Krishnaveni & Dhanalakshi 2014; Pereira et al 2018). The compounds were assessed for ADMET properties. Information about these compounds was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). For ADMET properties, the molecular weight, log P, number of H-bond acceptors, number of H-bond donors, number of rotatable bonds, and polar surface area were investigated (Long et al 2009). The compounds within the acceptable values of the different parameters were chosen. The acceptable values of 700 different compounds used to treat *D. rerio* used by Long et al (2009) were also used in this study. The compounds with violations for the next screening were not considered.

Solubility and distribution of flavonoids. The compounds with desired ADMET properties underwent further screening for the log S and log D. Compounds were considered to be active when their log S value was between -7–2.3 (Long et al 2009). Meanwhile, the log D value of the active compounds should fall within 2.83-3.81, at a pH of 7.5 (Brugman 2016). The compounds that did not meet the desired log S and log D values were no longer applicable for the next screening.

Virtual docking of the flavonoids. The crystal structures of the following enzymes CRBPII (1kqw), VD3RA (2hbh), FABP10A (2qo5), CYP8A1 (3b99), PLK1 (3d5w), gastrotropin (3elz) were retrieved from the Protein Data Bank (https://www.rcsb.org/). The enzymes were prepared for the docking experiment through Autodock tools version 4.2.6 (The Scripps Research Institute, CA, USA) and Mcule (Mcule Inc., CA, USA). The docking score of each flavonoid was ranked from the most negative to the least negative. A more negative kcal mol^{-1} means higher binding affinity (Nas 2020).

Visualization of the ligand-enzyme interaction. The visualization of the compounds bound to the enzyme with the highest binding affinity was performed with PLIP (BIOTEC TU Dresden, Germany). The amino acid residues from the enzyme of interest that interacted with the candidate flavonoids were identified. These binding interactions were compared with the binding interactions of the known enzyme inhibitor. The generated crystal structure of the redocked known inhibitory ligand was validated by superimposing the redocked crystal structure with the original crystal structure through Superpose v.1.0 (Wishartlab University of Alberta, Canada). The root-mean-square deviation (RMSD) value of the superimposed structures should be lower than 1.2 Å to be considered similar (Kufareva & Abagyan 2012).

Results and Discussion. Based on the phytochemical screening, the MCALE tested positive for phenols and flavonoids.

Assessment of the ADMET properties of the different flavonoids. The 40 distinct flavonoids found in *M. calabura*, based on scientific literature were the following: Delphinidin-3-O-glucoside (CID: 443650), Cyanidin-3-O-glucoside (CID: 197081), Gallocatechin (CID: 65084), Epigallocatechin (CID: 72277), Catechin (CID: 73160), Epicatechin (CID: 72276), Naringenin (CID: 439246), Rutin (CID: 5280805), Quercetin (CID: 5280343), Protocatechuic acid (CID: 72), 4-Hydroxybenzoic acid (CID: 135), Vanillic acid (CID: 8468), Gentisic acid (CID: 3469), Gallic acid (CID: 370), Chlorogenic acid (CID: 1794427), Caffeic acid (CID: 689043), p-Coumaric acid (CID: 637542), Sinapic acid (CID: 637775), Ferulic acid (CID: 445858), 2′,4′-dihydroxy-3′-methoxydihydrochalcone (CID: 14157883), 3'-Formyl-2',6',beta-trihydroxy-4'-methoxy-5'-methylchalcone (CID: 5319471), 5′-hydroxy-7,3′,4′-trimethoxyflavanone (CID: 5272653), 8-hydroxy-10-methoxy-5H-isochromeno[4,3-b]chromen-7-one (CID: 139030740), 7-hydroxyflavanone (CID: 1890), 2′,4′-dihydroxychalcone (CID: 5376979), 2′,4′-dihydroxydihydrochalcone (CID: 586491), 5,7-dimethoxy-4-hydroxyflavone (CID: 161172), 3,5-dihydroxy-6,7-dimethoxyflavone (CID: 13291608), 5-hydroxy-7-methoxyflavone (CID: 4101463), 3,7-dimethoxy-5-hydroxyflavone (CID: 5748697), 3,5-dihydroxy-7-methoxyflavone (CID: 5318691), 5,7-dihydroxy-3,8-dimethoxyflavone (CID: 9972910), galangin (CID: 5281616), chrysin (CID: 5281607), 7-hydroxy-8-methoxyflavanone (CID: 71762131), 4′-hydroxy-7-methoxyflavanone (CID: 676307), 2′,4′-dihydroxy-3′-methoxychalcone (CID: 10612087), 5-hydroxy-3,7,8-trimethoxyflavone (CID: 21632881), 2′,3′-dihydroxy-4′-methoxychalcone (CID: 5743235), and 7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone (CID: 5403474). Compounds were selected based on the desired molecular weight, octanol-water partition coefficient (log P), number of H-bond acceptor, number of H-bond donor, number of rotatable bonds, and polar surface area (PSA). Out of the 40 flavonoids, only 23 compounds fall within the acceptable range of the different parameters used for the screening (Table 1). Most of the violations were regarding the molecular weight and number of H-bond acceptors.

Solubility and distribution of the different flavonoids. After selecting the flavonoids with the desired ADMET properties, the researcher screened the 20 compounds which qualified from the previous screening on their solubility coefficient (log S) and distribution coefficient (log D) at pH 7.5. Out of the 20 compounds, only five flavonoids abide by the acceptable range of the log S and log D, as shown in Table 2. These compounds were 2′,4′-dihydroxychalcone, 2′,4′-dihydroxydihydrochalcone, 5-hydroxy-7-methoxyflavone, 2′,4′-dihydroxy-3′-methoxychalcone, and 2′,3′-dihydroxy-4′-methoxychalcone. All of the 20 compounds follow the acceptable range of the log S, but only five did not violate the acceptable range of log D at pH 7.5.

Virtual docking of the flavonoids. Through *in silico* docking, the docking scores of 2′,4′-dihydroxychalcone, 2′,4′-dihydroxydihydrochalcone, 5-hydroxy-7-methoxyflavone, 2′,4′-dihydroxy-3′-methoxychalcone, and 2′,3′-dihydroxy-4′-methoxychalcone were ranked on different metabolic enzymes in *D. rerio*, such as CRBPII, VD3RA, FABP10A, CYP8A1, PLK1, and gastrotropin. 5-hydroxy-7-methoxyflavone ranks first on most of the enzymes, particularly on FABP10A, CYP8A1, PLK1, and gastrotropin (Table 3). Both 2′,3′-dihydroxy-4′-methoxychalcone and 2′,4′-dihydroxydihydrochalcone rank first in VD3RA, but differently on CRBPII and PLK1, respectively. Most flavonoids have a high binding affinity to VD3RA and a low one to FAB10A.

Table 1

Assessment of the ADMET properties of the different flavonoids

Flavonoids	*Molecular weight*	*Log P*	*H-bond acceptor*	*H-bond donor*	*No of rotatable bonds*	*Polar surface area*	*No of violations*
Acceptable values	*206-489*	*1.3-5.3*	*1-7*	*0-3*	*0-9*	*20.2-123.5*	*0*
Delphinidin-3-O-glucoside	465.3	0.09	12	9	4	214	4
Cyanidin-3-O-glucoside	484.8	-2.61	11	8	4	193.44	4
Gallocatechin	306.2665	1.25	7	6	1	130.61	3
Epigallocatechin	306.2665	1.25	7	6	1	130.61	3
Catechin	290.2671	1.55	6	5	1	110.38	1
Epicatechin	290.2671	1.55	6	5	1	110.38	1
Naringenin	272.2517	2.51	5	3	1	86.99	0
Rutin	610.5159	-1.69	16	10	6	269.43	5
Quercetin	302.2346	1.99	7	5	1	131.36	2
Protocatechuic acid	154.1197	0.80	4	3	1	77.76	2
4-Hydroxybenzoic acid	138.1203	1.09	3	2	1	57.53	2
Vanillic acid	168.1462	1.10	4	2	2	66.76	2
Gentisic acid	154.1197	0.80	4	3	1	77.76	2
Gallic acid	170.1191	0.50	5	4	1	97.99	2
Chlorogenic acid	354.3078	-0.65	9	6	5	164.75	4
Caffeic acid	180.1568	1.20	4	3	2	77.76	2
p-Coumaric acid	164.1574	1.49	3	2	2	57.53	1
Sinapic acid	224.2093	1.51	5	2	4	75.99	0
Ferulic acid	194.1834	1.50	4	2	3	66.76	1
2′,4′-dihydroxy-3′-methoxydihydrochalcone	272.2948	2.92	4	2	5	66.76	0
3'-Formyl-2',6',beta-trihydroxy-4'-methoxy-5'-methylchalcone	328.3148	3.01	6	3	5	104.06	0
5′-hydroxy-7,3′,4′-trimethoxyflavanone	328.3148	3.19	6	1	4	78.13	0
8-hydroxy-10-methoxy-5H-isochromeno[4,3-b]chromen-7-one	326.2989	3.08	6	1	2	78.13	0
7-hydroxyflavanone	240.2529	3.10	3	1	1	46.53	0
2′,4′-dihydroxychalcone	240.2529	2.99	3	2	3	57.53	0
2′,4′-dihydroxydihydrochalcone	242.2689	2.91	3	2	4	57.53	0
5,7-dimethoxy-4-hydroxyflavone	298.2889	3.18	5	1	3	68.9	0
3,5-dihydroxy-6,7-dimethoxyflavone	314.2883	2.89	6	2	3	89.13	0
5-hydroxy-7-methoxyflavone	270.2789	3.11	4	1	2	55.76	0
3,7-dimethoxy-5-hydroxyflavone	298.2889	3.18	5	1	3	68.9	0
3,5-dihydroxy-7-methoxyflavone	284.2623	2.88	5	2	2	79.9	0
5,7-dihydroxy-3,8-dimethoxyflavone	314.2883	2.89	6	2	3	89.13	0
galangin	270.2358	2.58	5	3	1	90.9	0

Table 1

Assessment of the ADMET properties of the different flavonoids (continuation)

Flavonoids	*Molecular weight*	*Log P*	*H-bond acceptor*	*H-bond donor*	*No of rotatable bonds*	*Polar surface area*	*No of violations*
Acceptable values	*206-489*	*1.3-5.3*	*1-7*	*0-3*	*0-9*	*20.2-123.5*	*0*
chrysin	254.2364	2.87	4	2	1	70.67	0
7-hydroxy-8-methoxyflavanone	268.2629	3.17	4	1	2	59.67	0
4′-hydroxy-7-methoxyflavanone	268.2629	3.17	4	1	2	59.67	0
2′,4′-dihydroxy-3′-methoxychalcone	270.2789	3.00	4	2	4	66.76	0
5-hydroxy-3,7,8-trimethoxyflavone	328.3148	3.19	6	1	4	78.13	0
2′,3′-dihydroxy-4′-methoxychalcone	270.2789	3.00	4	2	4	66.76	0
7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone	344.3584	4.74	4	1	4	59.67	0

Table 2

Assessment of the solubility and distribution coefficient of the flavonoids

Flavonoids	*Log S*	*Log D pH 7.5*	*Violations*
Acceptable values	*-7.1–2.3*	*2.83–3.81*	*0*
Naringenin	-3.11	2.61	1
Sinapic acid	-2.55	-1.865	1
2′,4′-dihydroxy-3′-methoxydihydrochalcone	-3.94	2.79	1
3'-Formyl-2',6',beta-trihydroxy-4'-methoxy-5'-methylchalcone	-4.05	1.9	1
5′-hydroxy-7,3′,4′-trimethoxyflavanone	-4.18	2.41	1
8-hydroxy-10-methoxy-5H-isochromeno[4,3-b]chromen-7-one	-3.82	2.065	1
7-hydroxyflavanone	-3.33	2.555	1
2′,4′-dihydroxychalcone	-3.27	3.325	0
2′,4′-dihydroxydihydrochalcone	-3.27	3.64	0
5,7-dimethoxy-4-hydroxyflavone	-3.96	2.305	1
3,5-dihydroxy-6,7-dimethoxyflavone	-3.6	2.47	1
5-hydroxy-7-methoxyflavone	-3.44	3.24	0
3,7-dimethoxy-5-hydroxyflavone	-3.76	2.465	1
3,5-dihydroxy-7-methoxyflavone	-3.58	2.345	1
5,7-dihydroxy-3,8-dimethoxyflavone	-3.7	1.945	1
galangin	-3.36	1.48	1
chrysin	-3.38	1.935	1
7-hydroxy-8-methoxyflavanone	-3.71	1.9	1
4′-hydroxy-7-methoxyflavanone	-3.86	2.465	1
2′,4′-dihydroxy-3′-methoxychalcone	-3.92	3.165	0
5-hydroxy-3,7,8-trimethoxyflavone	-4.08	2.575	1
2′,3′-dihydroxy-4′-methoxychalcone	-3.95	3.24	0
7-(benzyloxy)-6,8-dimethoxy-5-hydroxyflavone	-4.67	4.44	1

Table 3

Ranking of the top binding ligands in the different metabolic enzymes in *Danio rerio*

CRBP II	*VD3RA*	*FABP10A*	*CYP8A1*	*PLK1*	*Gastrotropin*
2′,4′-dihydroxy-3′-methoxychalcone -8.5	2′,4′-dihydroxydihydrochalcone -8.9	5-hydroxy-7-methoxyflavone -7.9	5-hydroxy-7-methoxyflavone -8.5	5-hydroxy-7-methoxyflavone -8.1	5-hydroxy-7-methoxyflavone -8.8
2′,3′-dihydroxy-4′-methoxychalcone -8.5	2′,3′-dihydroxy-4′-methoxychalcone -8.9	2′,3′-dihydroxy-4′-methoxychalcone -7.6	2′,4′-dihydroxydihydrochalcone -8.4	2′,4′-dihydroxydihydrochalcone -8.1	2′,3′-dihydroxy-4′-methoxychalcone -8.0
2′,4′-dihydroxychalcone -8.4	2′,4′-dihydroxy-3′-methoxychalcone -8.7	2′,4′-dihydroxychalcone -7.6	2′,3′-dihydroxy-4′-methoxychalcone -8.1	2′,4′-dihydroxy-3′-methoxychalcone -7.6	2′,4′-dihydroxychalcone -7.8
2′,4′-dihydroxydihydrochalcone -8.1	2′,4′-dihydroxychalcone -8.6	2′,4′-dihydroxy-3′-methoxychalcone -7.5	2′,4′-dihydroxychalcone -8.0	2′,4′-dihydroxychalcone -7.5	2′,4′-dihydroxydihydrochalcone -7.8
5-hydroxy-7-methoxyflavone -7.9	5-hydroxy-7-methoxyflavone -8.0	2′,4′-dihydroxydihydrochalcone -7.0	2′,4′-dihydroxy-3′-methoxychalcone -7.9	2′,3′-dihydroxy-4′-methoxychalcone -7.1	2′,4′-dihydroxy-3′-methoxychalcone -7.4

Visualization of the ligand-enzyme interaction. The interactions of flavonoids with the enzymes were visualized. The binding interaction of 2′,4′-dihydroxychalcone, 2′,4′-dihydroxydihydrochalcone, 2′,4′-dihydroxy-3′-methoxychalcone, and 2′,3′-dihydroxy-4′-methoxychalcone are presented in Figure 1. These compounds were compared with a known VD3RA inhibitor, XE4. In XE4, there were 11 hydrophobic interactions with phe29, leu42, leu48, val49, trp101, tyr110, val115, leu128, tyr214, val231, and phe235. 2′,4′-dihydroxychalcone has hydrophobic interactions with tyr22, tyr26, phe29, leu48, tyr51, ad tyr110. It also forms H bonding with asp23, ser52, arg89, and ser93. The 2′,4′-dihydroxychalcone also has pi-stacking in tro101 and pi-cation interactions with arg89. In 2′,4′-dihydroxydihydrochalcone there were 6 hydrophobic interactions found in leu45, leu48, ile86, trp101, tyr110, and val115. There were also H bonding found in ser52, arg89, ser90, and ser93. In 2′,4′-dihydroxy-3′-methoxychalcone there were 8 hydrophobic interactions tyr22, tyr26, leu45, leu48, trp101, tyr110, and val115. There was also H bonding in tyr26, arg89, ser93, and pi-stacking in phe29. The hydrophobic interactions in 2′,3′-dihydroxy-4′-methoxychalcone were phe29, leu48, trp101, and leu128. It was also able to form H bond with tyr22, ser52, and arg89.

The binding interactions of CHD, an inhibitory ligand, and 5-hydroxy-7-methoxyflavone in gastrotropin were compared. CHD had hydrophobic interactions in trp49, val83, ile92, and thr101. It also had H bonding with trp49, gln51, val74, leu90, tyr97, and gln99. Contrastingly, the hydrophobic interactions of -hydroxy-7-methoxyflavone were found in trp51, asn63, phe65, val85, and leu92. There were also H bonding and pi-stacking in gln53 and gln101, and trp51, respectively.

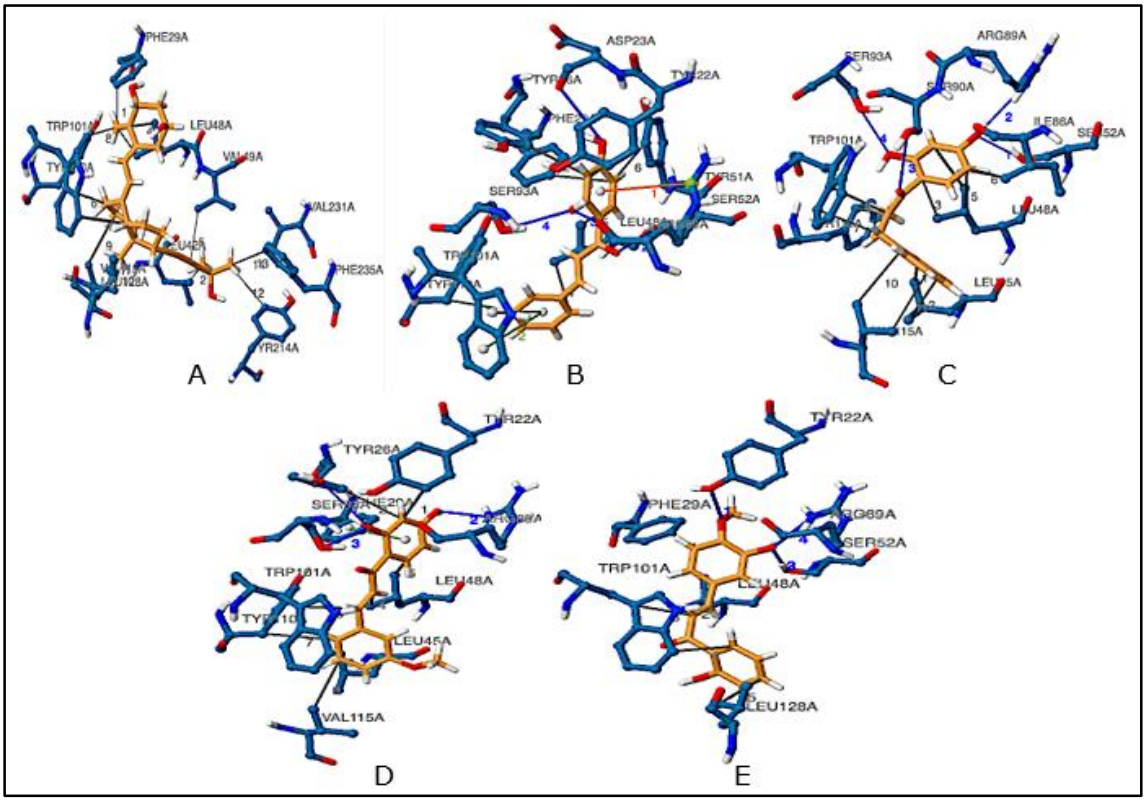

Figure 1. The ligand dock-pose of the flavonoids with the highest binding affinity in the amino acid residues VD3RA. A - XE4 docked to VD3RA through the amino acid residues (phe29, leu42, leu48, val49, trp101, tyr110, val115, leu128, tyr214, val231, and phe235); B - 2′,4′-dihydroxychalcone docked to VD3RA through the amino acid residues (tyr22, tyr26, phe29, leu48, tyr51, tyr110, asp23, ser52, arg89, ser93, trp101, and arg89). C **-** 2′,4′-dihydroxydihydrochalcone docked to VD3RA through the amino acid residues (leu45, leu48, ile86, trp101, tyr110, val115ser52, arg89, ser90, and ser93); D - 2′,4′-dihydroxy-3′-methoxychalcone docked to VD3RA through the amino acid residues (tyr22, tyr26, leu45, leu48, trp101, tyr110, val115, tyr26, arg89, ser93, and phe29); E - 2′,3′-dihydroxy-4′-methoxychalcone docked to VD3RA through the amino acid residues (phe29, leu48, trp101, leu128, tyr22, ser52, and arg89).

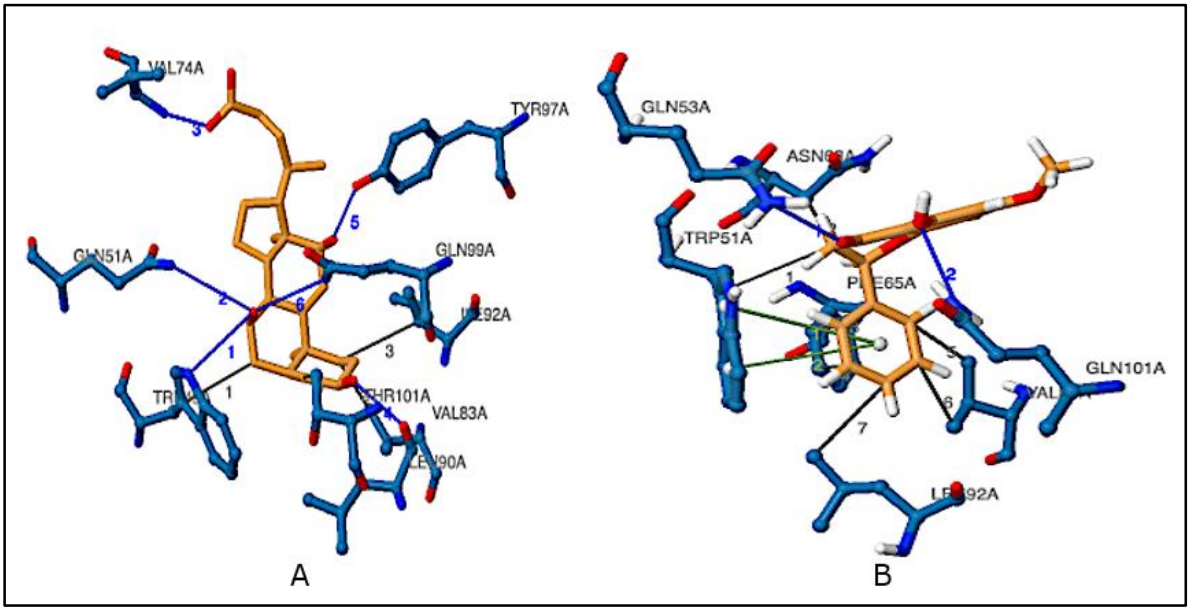

Figure 2. Ligand binding interactions with gastrotropin. A - CHD, a known enzyme inhibitor, docked in gastrotropin through the amino acid residues (trp49, val83, ile92, thr101, gln51, val74, leu90, tyr97, and gln99); B - 5-hydroxy-7-methoxyflavone docked to gastrotropin through the amino acid residues (trp51, asn63, phe65, val85, leu92, gln53, gln101, and trp51).

Several studies show that *M. calabura* extract contains sugar, sterols, steroids, saponin, glycosides, amino acid, oils, and flavonoids (Yusof et al 2013; Krishnaveni & Dhanalakshi 2014; Pereira et al 2018). However, in this study, the screening was only for phenols and flavonoids. The interest was only in these two metabolites to ensure that MCALE contains flavonoids.

Other studies associate ADMET properties with the drug-likeness of a compound (Lipinski 2004). In this study, the ADMET properties were investigated to gain an idea of the permeability of the compound to *D. rerio* (Long et al 2009). The study of Long et al (2009), which evaluated the ADMET properties of 700 active compounds in *D. rerio*, serves as the reference for the acceptable values in this study. The compounds with undesired ADMET properties mostly fall outside the desired range of the molecular weight and the number of H-bond acceptors. Compounds with high molecular weight may have a hard time crossing cell membranes (Keller et al 2006). Additionally, the H-bond acceptor indicates whether the protein surface interaction is favorable or not (Desaphy et al 2012).

To differentiate flavonoids, the ligand dock poses were examined and the amino acid residues in the enzyme that interacts mainly with the compounds were identified. The ligand dock pose of the enzyme with the highest binding affinity with flavonoids was considered. In VD3RA, the non-covalent interactions identified in XE4 were stronger than the flavonoids. The hydrophobic interactions greatly affect the binding affinity of the compounds, which is stronger than the H bonding (Chang 2005). Flavonoids like 2′,4′-dihydroxychalcone, 2′,4′-dihydroxydihydrochalcone, 2′,4′-dihydroxy-3′-methoxychalcone, and 2′,3′-dihydroxy-4′-methoxychalcone have a lower number of hydrophobic interactions compared to XE4. The H bonding and pi-stacking in these compounds may have helped to increase their binding affinity with VD3RA (Chen et al 2016). Meanwhile, in gastrotropin, even though 5-hydroxy-7-methoxyflavone have high number of hydrophobic interactions than CHD, the high number of H bond may have compensated to its higher binding affinity in contrast with the low number of H bonding and pi-stacking in 5-hydroxy-7-methoxyflavone.

Conclusions. Only 5 of the 40 different flavonoids present in MCALE may potentially be active in *D. rerio*. These putative active flavonoids may affect the vitamin D3 A receptor in

D. rerio, which may influence various metabolic pathways involved in the endocrine, immune, and nervous system of the fish.

Acknowledgements. The researcher is grateful to the help of Gab Enriquez and Anton Villa-Ignacio for the extraction and phytochemical screening, and to Dr. Salunga for the collected leaves.

References

Brugman S., 2016 The zebrafish as a model to study intestinal inflammation. Developmental & Comparative Immunology 64:82-92.

Capaldi S., Saccomani G., Fessas D., Signorelli M., Perduca M., Monaco H. L., 2009 The X-ray structure of zebrafish (*Danio rerio*) ileal bile acid-binding protein reveals the presence of binding sites on the surface of the protein molecule. Journal of Molecular biology 385(1):99-116.

Chang R., 2005 Physical chemistry for the biosciences. 1st Edition. University Science Books, 678 p.

Chen D., Oezguen N., Urvil P., Ferguson C., Dann S. M., Savidge T. C., 2016 Regulation of protein-ligand binding affinity by hydrogen bond pairing. Science advances 2(3):e1501240, 17 p.

Craig S., Helfrich L. A., Kuhn D., Schwarz M. H., 2017 Understanding fish nutrition, feeds, and feeding. Virginia Cooperative Extension, Virginia Tech, Virginia State University, Publication 420-256, 6 p.

Craig T. A., Sommer S., Sussman C. R., Grande J. P., Kumar R., 2008 Expression and regulation of the vitamin D receptor in the zebrafish, *Danio rerio*. Journal of Bone and Mineral Research 23(9):1486-1496.

Denovan-Wright E. M., Pierce M., Wright J. M., 2000 Nucleotide sequence of cDNA clones coding for a brain-type fatty acid binding protein and its tissue-specific expression in adult zebrafish (*Danio rerio*). Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1492(1):221-226.

Desaphy J., Azdimousa K., Kellenberger E., Rognan D., 2012 Comparison and druggability prediction of protein–ligand binding sites from pharmacophore-annotated cavity shapes. Journal of Chemical Information and Modeling 52(8):2287-2299.

Durham S., 2010 Finding alternative fish feeds for aquaculture. Agricultural Research 58(9):8-11.

Figueiredo R. A. D., Oliveira A. A. D., Zacharias M. A., Barbosa S. M., Pereira F. F., Cazela G. N., Viana J. P., Camargo R. A. D., 2008 Reproductive ecology of the exotic tree *Muntingia calabura* L. (Muntingiaceae) in southeastern Brazil. Revista Árvore 32(6):993-999.

Keller T. H., Pichota A., Yin Z., 2006 A practical view of 'druggability'. Current Opinion in Chemical Biology 10(4):357-361.

Krishnaveni M., Dhanalakshi R., 2014 Qualitative and quantitative study of phytochemicals in *Muntingia calabura* L. leaf and fruit. World Journal of Pharmaceutical Research 3(6):1687-1696.

Kufareva I., Abagyan R., 2012 Methods of protein structure comparison. Methods in Molecular Biology 857:231-257.

Lipinski C. A., 2004 Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies 1(4):337-341.

Lister A. L., Van Der Kraak G. J., 2009 Regulation of prostaglandin synthesis in ovaries of sexually mature zebrafish (*Danio rerio*). Molecular Reproduction and Development: Incorporating Gamete Research 76(11):1064-1075.

Liu R. Z., Sun Q., Thisse C., Thisse B., Wright J. M., Denovan-Wright E. M., 2005 The cellular retinol-binding protein genes are duplicated and differentially transcribed in the developing and adult zebrafish (*Danio rerio*). Molecular Biology and Evolution 22(3):469-477.

Long K., Kostman S. J., Fernandez C., Burnett J. C., Huryn D. M., 2019 Do zebrafish obey Lipinski rules? ACS Medicinal Chemistry Letters 10(6):1002-1006.

Millamena O. M., Coloso R. M., Piedad-Pascual F., 2002 Nutrition in tropical aquaculture: essentials of fish nutrition, feeds, and feeding of tropical aquatic species. Aquaculture Department, Southeast Asian Fisheries Development Center, 221 p.
Nas J. S. B., 2020 Exploring the binding affinity and non-covalent interactions of anthocyanins with aging-related enzymes through molecular docking. Philippine Journal of Health Research and Development 24(3):9-19.
Nas J. S. B., Dangeros S. E., Chen P. D., Dimapilis R. C., Gonzales D. J., Hamja F. J., Ramos C. J., Villanueva A. D., 2020 Evaluation of anticancer potential of *Eleusine indica* methanolic leaf extract through Ras-and Wnt-related pathways using transgenic *Caenorhabditis elegans* strains. Journal of Pharmaceutical Negative Results 11(1):42-46.
Nivethetha M., Jayasri J., Brindha P., 2009 Effects of *Muntingia calabura* L. on isoproterenol-induced myocardial infarction. Singapore Medical Journal 50(3):300-302.
Nurhuda M., Kholista M. A., Ismi Y., Maulidiya N., Hariyadi H., Hakim R. R., 2018 Effectiveness of cherry leaf extract (*Muntingia calabura*) with different levels as treatment of seeds of Sangkuriang catfish (*Clarias gariepinus*) infected by *Trichodina* sp. Indonesian Journal of Tropical Aquatic 1(1):41-49.
Pereira G. A., Arruda H. S., de Morais D. R., Eberlin M. N., Pastore G. M., 2018 Carbohydrates, volatile and phenolic compounds composition, and antioxidant activity of calabura (*Muntingia calabura* L.) fruit. Food Research International 108:264-273.
Sargent J. R., Tacon A. G. J., 1999 Development of farmed fish: a nutritionally necessary alternative to meat. Proceedings of the Nutrition Society 58(2):377-383.
Turchini G. M., Ng W. K., Tocher D. R., 2010 Fish oil replacement and alternative lipid sources in aquaculture feeds. 1st Edition. CRC Press, Boca Raton, 551 p.
Xu J., Shen C., Wang T., Quan J., 2013 Structural basis for the inhibition of Polo-like kinase 1. Nature Structural & Molecular biology 20(9):1047-1053.
Yusof M., Izwan M., Salleh M., Kek T. L., Ahmat N., Azmin N. F. N., Zakaria Z. A., 2013 Activity-guided isolation of bioactive constituents with antinociceptive activity from *Muntingia calabura* L. leaves using the formalin test. Evidence-Based Complementary and Alternative Medicine 2013:715074, 9 p.
Zakaria Z. A., Hazalin N. M., Zaid S. M., Ghani M. A., Hassan M. H., Gopalan H. K., Sulaiman M. R., 2007 Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. Journal of Natural Medicines 61(4):443-448.
***https://pubchem.ncbi.nlm.nih.gov/
***https://www.rcsb.org/

Received: 04 August 2020. Accepted: 15 September 2020. Published online: 30 October 2020.
Authors:
John Sylvester Nas, Department of Biology, College of Arts and Sciences, University of the Philippines Manila, 1000 Manila, Philippines; Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, 1000 Manila, Philippines, e-mail: jbnas@up.edu.ph

How to cite this article:
Nas J. S., 2020 Screening of flavonoids from *Muntingia calabura* aqueous leaf extract and its potential influence on different metabolic enzymes in *Danio rerio*. AACL Bioflux 13(5):3046-3055.