Computational Study of Natural Compounds in Melon Fruit (*Cucumis melo* L. 'GMP') as Inhibitor of Epidermal Growth Factor Receptor Protein

Wiko Arif Wibowo[1] Teuku Nanda Saifullah Sulaiman[2] Supriyadi Supriyadi[3]

Budi Setiadi Daryono[4,*]

[1]*Doctoral Program, of Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Sekip Utara, Sleman, Yogyakarta, Indonesia*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Sleman, Yogyakarta, Indonesia*
[3]*Department of Food and Agriculture Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta, Indonesia*
[4]*Departmen of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Sekip Utara, Sleman, Yogyakarta, Indonesia*
[*]*Corresponding author. Email: bs_daryono@mail.ugm.ac.id*

ABSTRACT
'GMP' melon is a breeding cultivar that has a bitter taste and fragrant aroma. The bitter taste character indicates the presence of potential natural compounds that can be used as anti-cancer. This study aims to reveal the natural compounds of 'GMP' melon and its use as an anti-cancer computationally. Experimenting with the content of natural compounds in 'GMP' melon with fruit extraction at medium maturity level and analyzed using Liquid Chromatography-Mass Spectrometry. The probable potential of natural compounds was predicted by PASS online. Analysis computationally of natural compounds potency using specific docking of EGFR protein with AutoDock Vina program from PyRx software. Visualization of docking results using Biovia Discovery Studio and comparing binding affinity values. The screening results showed 'GMP' melon had 42 potential natural compounds that passed RO5 screening out of 63 detected metabolites. Based on PASS online, the antioxidant compound group is predicted to have direct activity as a kinase inhibitor, while the cucurbitacin compound group is expected to have antineoplastic activity associated with lung cancer. The docking results show 9 potential natural compound candidates that have a lower binding affinity value than the native ligand (17.7 kcal/mol), namely Quercetin (-8.9 kcal/mol), Cucurbitacin B (-8.5 kcal/mol), Kaempferol (-8.5 kcal/mol), Naringenin (-8.5 kcal/mol), Isorhamnetin (-8.4 kcal/mol), Cucurbitacin I (-8.4 kcal/mol), Catechin (-8.3 kcal/mol), Isocucurbitacin R (-8.3 kcal/mol)), and Cucurbitacin R (-8.2 kcal/mol).

Keywords: *Anti-cancer, EGFR protein, GMP melon, Molecular docking, Natural compounds.*

1. INTRODUCTION

Melon 'GMP' (Gama Melon Parfum) is one of the melons produced by breeding from Universitas Gadjah Mada, Indonesia. The 'GMP' melon has phenotypic characters, consists of small fruit size (± 150 gr), oblate, no net, the pattern on the skin of the fruit, white flesh, orange skin when ripe [1]. Melon 'GMP' has a unique character, specifically, a turbine structure in the basal part of the fruit, a strong fragrant aroma, and a bitter taste in the fruit flesh [2]. This character causes 'GMP' melons to belong to the bitter melon group and is not edible.

The profile of volatile compounds influences the sweet aroma of GMP melon, include 3 alcohols, 3 esters, 1 ketone, and 1 hydrocarbon, with key aroma characteristics influenced by 3-penten-2-ol, hexyl acetate, and 3-hydroxy 2-butanone [3]. Meanwhile, the compounds that correlated to the bitter taste of 'GMP' melon were calcium minerals, bitter amino acids, and total phenol [4]. Based on research [5], it was successfully revealed the presence of cucurbitacin B compounds in 'GMP' melons which are associated with a bitter taste in the Cucurbitaceae plant group.

The strong fragrant aroma and bitter taste indicate the presence of high concentrations of natural compounds in the phytochemical profile of 'GMP' melon. Therefore the use of 'GMP' melons is directed to the cosmetic and health industries. Studies [6] and [7] stated that 'GMP' melon extract is effective as a larvicide and mosquito repellent due to the ability of flavonoid, terpenoid, and saponin compounds. However, the specific phytochemical profile of 'GMP' melon is unknown. Furthermore, the use of 'GMP' melons in the health sector has not been explored in depth.

One potential utilization of plant extracts in the health sector is to inhibit the abnormal growth of cells known as cancer [8]. Cancer can be found in various organs and caused by errors in varied metabolic pathways; an example is the deregulation activity of the EGFR protein (Epidermal Growth Factor Receptor) [9]. EGFR protein is a transmembrane protein that acts as a receptor [10]. EGFR protein is composed of 3 domains, consists of the extracellular domain, transmembrane domain, and kinase domain [11]. EGFR protein deregulation activity through increased phosphorylation activity in the kinase domain; therefore alternative to inhibit abnormal growth in cells is to block the binding between the kinase domain and ATP toward repressing the phosphorylation process [12].

This study aims to determine the phytochemical profile of 'GMP' melons and reveal the potential of natural compounds from 'GMP' melons as inhibitors of EGFR protein in silico through molecular docking. The computational experiment is an alternative for preliminary studies of cancer research and medical research. The results of in silico study can be used as a basis for further research and development in vitro and in vivo with a more specific target scope.

2. METHODS

2.1. Plants Material

The cultivation of GMP melons was executed in the research greenhouse of the Gama Melon Research Team in Madurejo village, Sleman regency. The 'GMP' melons were harvested at 51 DAP (medium stage). Fruit extraction is done by crushing the fruit into a liquid extract with a water ratio of 1:1. The extract was dried for 6 days and ground into powder. The powder was macerated using 96% methanol for 3 days at a ratio of 1:5. The maceration solution was allowed to stand for 72 hours at room temperature. Furthermore, the solvent was deposited using a vacuum rotary evaporator at a temperature of 40 ^{o}C to obtain a paste extract.

2.2. Liquid Chromatography Mass Spectrometry

The extract was injected with a volume of 1 µL into Shimadzu LCMS – 8040 LC/MS with a *column* Shimadzu Shim Pack FC-ODS 2 mm x 150 mm, 3 µm. The capillary voltage used is 3.0 kV with a column temperature of 35 ^{o}C. The flow gradient settings are 0/0 at 0 minutes, 15/85 at 5 minutes, 20/80 at 20 minutes, and 90/10 at 24 minutes with a flow rate of 0.5 mL/minute. The MS ion mode to be used is Io type [M]+ with Collison energy 5.0 V. The selected desolvation gas flow is 60 mL/day with a desolvation temperature of 350 ^{o}C. Fragmentation method in the form of Low energy CID. Ionization using ESI with scanning 0.6 sec/scan (mz: 10-1000) at a source temperature of 100 ^{o}C for 60 minutes.

2.3. Ligands and Protein Preparation

Ligands derived from natural compounds are contained in GMP melons. All compounds were screened using Lipinski's Rule of Five (RO5) [13]. The results of compound screening were followed by the prediction of biological activity using PASS online (http://www.way2drug.com/passonline/). The selected compounds were downloaded in SDF format on PubChem (https://pubchem.ncbi.nlm.nih.gov). The ligand sample was minimized and converted the ligand format to PDB format using PyRx software. Meanwhile, the EGFR protein was collected from Uniprot (https://www.uniprot.org), which was sourced from the Homo sapiens organism. EGFR protein was downloaded in PDB format and sterilized. Sterilization was conducted against water, ligands, and other proteins using PyMol software.

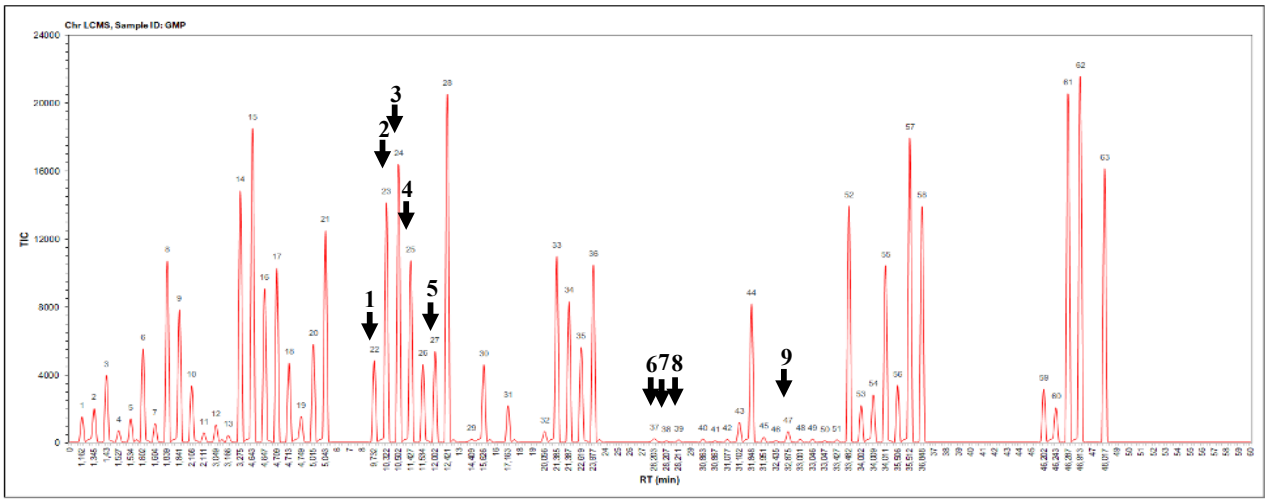
2.4. Molecular Docking and Visualization

Molecular docking was analyzed with the AutoDock Vina program from PyRx software [14]. The minimized ligands were inserted with the "Add Ligand" tool, and the pure protein was inserted with the "Add Macromolecule" tool. The grid box was designed on Center X: 0.9132 Y: -53.050 Z: -25.1396 and Dimension X: 14.8530 Y: 16.4084 Z: 19.2041 to perform specific docking on the active site of the EGFR protein and continued with Run Vina. The results obtained are screened with the lowest Binding Affinity value to be visualized.

3. RESULTS AND DISCUSSION

Sixty-three natural compounds were detected in the 'GMP' melon (Fig. 1). There are several compounds with higher quantitative values indicated by the peak area (area > 10,000), specifically p-coumaric acid, esculetin, caffeic acid, ferulic acid, kaempferol, catechin, quercetin, chlorogenic acid, hirsutrin, isoorientin-2”- O-glucopyranoside, isovitexin-2”-O-(6’’’ -(E)-p-coumaroyl) glucoside, isovitexin 2”-O-(6’’’-feruloyl) glucoside, isoscoparin-2” -(6-(E)-p-coumaroylglucoside).

All data found on natural compounds were filtered using RO5 consisting of molecular weight (MW), high lipophilicity (mLOGP), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and violation calculation (Violation) [13]. The results obtained were 21 compounds not appropriate and only 42 compounds that could be used as ligands for molecular docking.



➔ Compounds that have a lower binding affinity than ATP

Figure 1 Chromatogram peak of phytochemical profile ‘GMP’ melons fruit in medium stage

The compounds screened out violated more than 1 Lipinski regulation [15]. The failed compounds were 19-Norlanosta-5,24-dien-11-one; isoorientin; Hirsutrin, ε-Carotene; β-Carotene; Lutein; Zeaxhantin; Apigenin-7-(6"-p-coumarylglucoside); Saponarins; Meloside A; Vicenin 2; Meloside L; Isoorientin-2"-O-Glucopyranoside; Isoscoparin-4'-O-Glucoside; Arvenin III, Arvenin I, Isovitexin-2"-O-(6'"-(E)-p-coumaroyl) Glucoside; Isovitexin2"-O-(6'"-feruloyl) Glucoside; Isoscoparin-2"- (6-(E)-pcoumaroylglucoside). Meanwhile, there were 2 compounds not sufficient due to incomplete atomic structure in the PubChem database, namely β-Sitosterol and Vitexin.

Based on the prediction of biological activity using PASS online, several compounds showed biological activity associated with specific kinase and antineoplastic domains in lung cancer. These compounds are thought to be associated with the EGFR protein, which has a kinase domain related to the EGFR protein’s function as a transmembrane receptor protein tyrosine kinase. EGFR protein also has an important role in the development of NSCLC (non-small-cell lung cancer). A group of compounds related to antineoplastic (lung cancer) is also thought to act as an inhibitor of EGFR protein.

Table 1. Prediction of biological activity of potential compounds related to anticancer

Compounds	Pa	Pi	Activity
Naringenin	0,838	0,004	Kinase inhibitor
Kaempferol	0,959	0,001	Kinase inhibitor
Catechin	0,743	0,009	Kinase inhibitor
Quercetin	0,809	0,005	Kinase inhibitor
Isorhamnetin	0,945	0,002	Kinase inhibitor
Cucurbitacin I	0,925	0,003	Antineoplastic (lung cancer)
Cucurbitacin R	0,833	0,004	Antineoplastic (lung cancer)
Isocucurbitacin R	0,777	0,004	Antineoplastic (lung cancer)
Cucurbitacin B	0,883	0,03	Antineoplastic (lung cancer)

Table 2. Candidates of natural compounds contained 'GMP' melon fruit as an inhibitor of EGFR protein

Compounds	RT	Curve Area	Composition (%)	Lipinski's Rules					Binding Affinity
				MW	mLogP	HBA	HBD	Violation	
Naringenin	9,732	4854,39503	1,28425	272,25	0,71	5	3	0	-8,5*
Kaempferol	10,322	14142,51803	3,74146	286,24	-0,03	6	4	0	-8,5*
Catechin	10,502	16379,74742	4,33333	290,27	0,24	6	5	0	-8,3
Quercetin	11,427	10732,15044	2,83923	302,24	-0,56	7	5	0	-8,9*
Isorhamnetin	12,002	5386,77041	1,22471	316,26	-0,31	7	4	0	-8,4
Cucurbitacin I	28,203	253,31003	0,04881	514,65	1,36	7	4	1	-8,4
Cucurbitacin R	28,207	110,46006	0,06068	518,68	1,53	7	4	1	-8,2
Isocucurbitacin R	28,211	184,51582	0,02919	518,68	1,53	7	4	1	-8,3
Cucurbitacin B	32,875	676,77486	0,02972	558,70	1,76	8	3	1	-8,5*
Native ligand (ATP)									-7,7

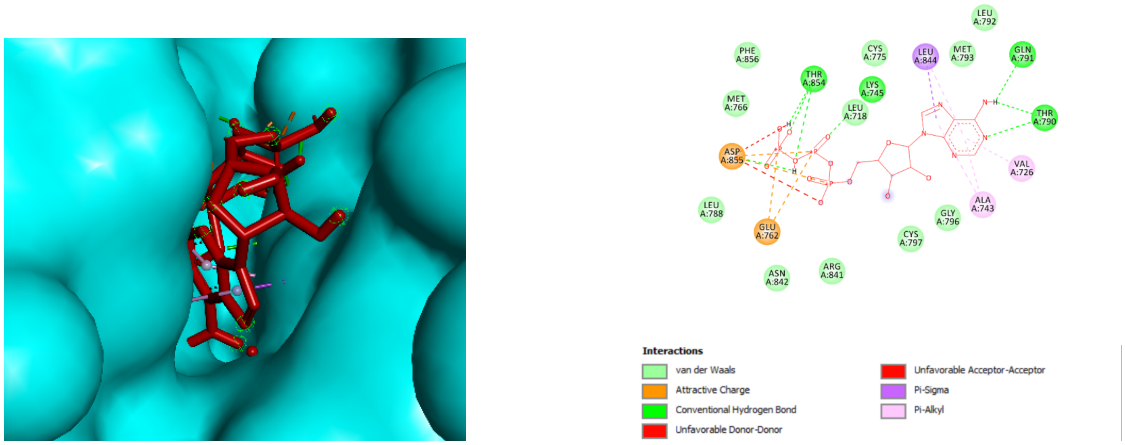
*top 4 compounds with the best scoring power

The EGFR protein has nucleotide binding at positions 718-726 and 790-791 as ATP binding sites [16][17], so the preparation is not only water sterilization but also done by designing a grid box for specific docking of nucleotide-binding. Based on [14], the specific EGFR protein nucleotide-binding site on the amino acids Leu718, Val726, Gly745, Leu788, Gly796, Cys797, Leu844, Asp855. Meanwhile, the preparation of the ligand consists of energy minimization and conversion of the ligand to the pdbqt format [18].

The results of the docking analysis refer to the scoring power metric of the binding affinity value [19]. Binding affinity indicates a prediction of the stability of the bond formed; the lower the value of binding affinity, the more stable the bond formed. The binding affinity value of ATP on nucleotide-binding is -7.7 kcal/mol.

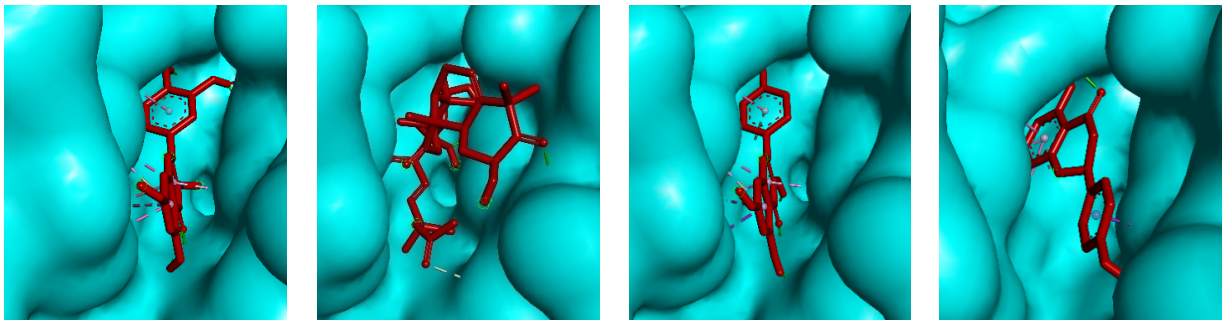
Natural compounds with a lower binding affinity value than ATP are thought to inhibit the binding of ATP to the EGFR protein receptor and inhibit the phosphorylation process [20]. Potential natural compounds with the lowest binding affinity values were Quercetin (-8.9 kcal/mol), Cucurbitacin B (-8.5 kcal/mol), Kaempferol (-8.5 kcal/mol), and Naringenin (-8.5 kcal/mol) (Table 1).

Evaluation of scores on docking performance can be determined into 3 metrics, namely "docking power", "ranking power", "scoring power", and "screening power" [19]. Scoring power is a metric based on identification from binding affinity values. This Study's visualization of the results was based on the top 4 compounds with the best scoring power, specifically quercetin, cucurbitacin B, kaempferol, and naringenin (Fig. 3).



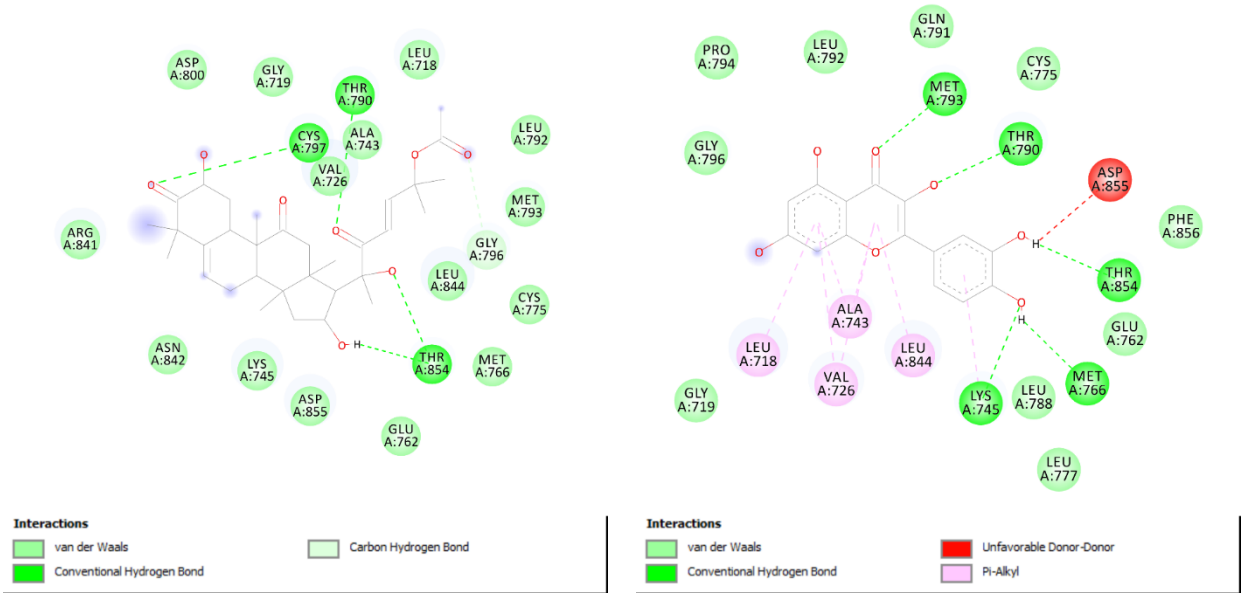
(a) (b)

Figure 2. Visualization of cavity binding (a); and interaction from ATP as native ligand (b)



(a) (b) (c) (d)

Figure 3. Cavity site of (a) Quercetin; (b) Cucurbitacin B; (c) Kaempferol; and (d) Naringenin in the active site of EGFR protein



(a) (b)

Figure 4. Comparison of interactions between cucurbitacin B (a) and quercetin (b) binding cavity against EGFR protein

The similar coverage of amino acid residues due to of the interaction between the ligand and the receptor also strengthens the prediction that a ligand can inhibit the activity of the native ligand [14]. ATP as the native ligand has 18 amino acid residues that are shared in van der Waals bond interactions (MET A: 766; PHE A: 856; LEU A: 718; CYS A: 775; MET A: 793; LEU A: 792; GLY A: 796; CYS A: 797; ARG A: 841; ASN A: 842; LEU A: 788), attractively charged bonds (ASP A: 855; GLU A: 762), hydrogen bonds (THR A: 854; LYS A: 745; GLN A: 791; THR A: 790), Pi-Sigma bonds (LEU A: 844), and Pi-Alkyl bonds (ALA A: 743; VAL A: 726). Cucurbitacin B, a specific compound in melon plants, has a range of amino acid residues that almost covers all amino acid residues from ATP binding, particularly a total of 15 amino acid residues of the identical (LEU A: 718; LYS A: 745; GLU A: 762; MET A: 766; CYS A: 775; THR A: 790; LEU A: 792; MET A: 793; GLY A: 796; CYS A: 797; ARG A: 841; ASN A: 842; LEU A: 844; THR A: 854; ASP A: 855) (Fig. 4).

Quercetin is a flavonoid compound widely distributed in various plant groups as an antioxidant in food. Quercetin is widely used as an anticancer drug. Furthermore, quercetin has many pharmacological effects in protection from various diseases, such as osteoporosis, tumors, pulmonary and cardiovascular disorders [21]. Quercetin has also been widely reported in multiple computational studies as a potential natural compound in cancer treatment [22].

Cucurbitacin B is a terpenoid secondary metabolite compound. Cucurbitacin compounds specifically belong to the triterpene group and have many variations of side chains; therefore, the compounds are classified alphabetically [23]. Studies on the utilization of cucurbitacin B compounds as anti-cancer have been executed from extracts of various plants [24]. In melon plants, it is known that the specific cucurbitacin compound is cucurbitacin B [25]. Additionally to acting as an anti-cancer, cucurbitacin B also has cellular

activity as hepatoprotective, anti-inflammatory, and anti-microbial [26].

Kaempferol is a natural compound that belongs to the flavonoid group. Kaempferol is widely found in various food plants and traditional medicinal plants [27]. Flavonoids are a group of compounds commonly known to have antioxidant and anti-inflammatory activities. Kaempferol specifically also has bioactive abilities in various types of cancer, such as breast cancer, prostate cancer, bladder cancer, cervical cancer, colon cancer, liver cancer, lung cancer, uterine cancer, leukemia, etc. [28].

Naringenin is a flavonone compound that is tasteless and colorless and belongs to the flavonoid group. Naringenin is commonly found in grapes, bergamot, citrus fruits, cherries, tomatoes, etc. Naringenin has positive activity against Alzheimer's sufferers; besides that, it has the potential as anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, antioxidant, and anti-cancer [29][30].

Sixty-three compounds were detected in the melon 'GMP' in the medium maturity phase. Some of these compounds have bioactive abilities. Based on a computational approach, 9 compounds were thought to have potential as inhibitors of deregulation of the phosphorylation process in the EGFR protein. The best compounds based on scoring power were quercetin, cucurbitacin B, kaempferol, and naringenin. This is based on the lower binding affinity value than ATP as a native ligand.

AUTHORS' CONTRIBUTIONS

WAW conducted the research, data analysist and wrote the manuscript, TNSS, S, and BSD supervised the research

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