Trop J Nat Prod Res, April 2024; 8(4):6957-6962 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Possible Potential Features of Phytoestrogens of Ambones Banana Blossom (*Musa Acuminata Colla*) as Nutraceuticals for Menopausal Vaginal Elasticity: An *In Silico* Study

Surmiasih[1]*, Adi Prayitno[2], Brian Wasita[3], Ida Nurwati[4]

[1]*Doctoral Program of Medical Sciences, Faculty of Medicine, Sebelas Maret University, Indonesia*
[2]*Department of Dentistry, Faculty of Medicine, Sebelas Maret University Surakarta, Indonesia*
[3]*Department of Anatomical Pathology Faculty of Medicine, Sebelas Maret University Surakarta, Indonesia*
[4]*Department of Biomedical Sciences, Faculty of Health, Sebelas Maret University Surakarta, Indonesia*

ARTICLE INFO

Article history:
Received 24 January 2024
Revised 16 April 2024
Accepted 18 April 2024
Published online 01 May 2024

Copyright: © 2024 Surmiasih *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Menopause is the period when the reproductive system stops producing estrogen and progesterone. This condition can cause various changes, including vaginal atrophy. Structural changes can occur because cytoskeletal proteins (actin) and junction cells (claudin-1) that regulate permeability undergo changes that cause thinning of the epithelium in the vagina which will lead to reduced vaginal elasticity. This study aimed to predict the activity of anthocyanin and genistein compounds with claudin-1 and Actin receptors in silico. This research was conducted using Autodock Vina software supported by the Vega ZZ, Pymol, and BIOVIA Discovery Studio programs to create visual profiles of Claudin-1 and Actin ligand proteins used as comparisons with *Anthocyanin* and *Genistein* test compounds, as well as pharmacokinetic predictions using pkCSM. The results of the post-docking analysis in the form of binding affinity for amino acid interactions and pharmacokinetics using *anthocyanin* and *genistein* compounds showed the largest binding affinity for *Anthocyanin* compounds with a Gbinding score of -6.81 kcal / mol (72.60%) close to estrogen's Gbinding score of -9.33 kcal/mol. The RMSD value of claudin-1 was 1.43 Å. This means that the claudin-1 protein has the right interaction between the original ligand and the test ligand. The results of the study have the potential to recommend nutraceuticals for drug discovery using natural ingredients containing *anthocyanin* and *genistein* compounds which can overcome complaints of vaginal elasticity in menopause. Further research can be done *in vitro* and *in vivo* to investigate these compounds clinically..

***Keywords*:** Phytoestrogen, nutraceutical, vaginal elasticity, menopausal, molecular docking

Introduction

Menopause is a period of permanent cessation of menstruation due to the loss of ovarian function in producing reproductive hormones. Reduced production of estrogen and progesterone hormones is the reason for various complaints, such as hot flashes, night sweats, urogenital symptoms, and psychogenic symptoms including, anxiety, depression, sleep disorders, loss of concentration, and self-confidence.[1] Urogenital symptoms are mostly felt during menopause. Vaginal atrophy caused by low estrogen levels during menopause leads to this condition. Complaints are commonly in the forms of burning, irritation, pain during sexual intercourse (dyspareunia), and susceptibility to infection. [2,3]

Hormone therapy is becoming one of the most effective treatments for menopausal symptoms. Hormone replacement therapy is effective in replacing the hormone estrogen and relieving menopausal symptoms. However, synthetic hormone therapy is expensive, and its long-term use increases the risk of breast cancer, endometrial cancer, cardiovascular disease, and other side effects.[4,5]

***Corresponding author. E mail: surmiasih@student.uns.ac.id
Tel: +6285279135462

Citation: Surmiasih, Prayitno A, Wasita B, Nurwati I. Possible Potential Features of Phytoestrogens of Ambones Banana Blossom (*Musa Acuminata Colla*) as Nutraceuticals for Menopausal Vaginal Elasticity: An *In Silico* Study. Trop J Nat Prod Res. 2024; 8(4):6957-6952. https://doi.org/10.26538/tjnpr/v8i4.29

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Alternative therapies that are currently being developed aim to address menopausal symptoms by using plants that contain *flavonoids. Flavonoids* contain polyphenol group compounds that have many benefits as nutritional modulators of disease control and health supplements. *Flavonoid* compounds can ward off free radicals, and a decrease in estrogen levels due to menopause. *Flavonoid* compounds can exert phytoestrogenic activity by binding to estrogen receptors.[6,7] *Anthocyanins* and *genistein* are one of the *flavonoid* classes that have a structure similar to estrogen and have the activity as phytoestrogens. Phytoestrogen compounds can exert their activity by binding to α estrogen receptors (ER-α) and β estrogen receptors (ER-β).[8, 9 ,10] In addition, these two bioactive compounds have antioxidant, anti-inflammatory, and antineoplastic properties. For example, the blossom of the banana which is underutilized and considered waste, contains minerals, fatty acids, vitamins A, C, and E, potassium, and active compounds such as *alkaloids, flavonoids, saponins*, and *tannins* as a source of antioxidants, as well as many phytochemicals to improve health. [11,12]

The hypoestrogen condition produces changes in cell morphology and extracellular matrix in the vaginal wall in the form of atrophy, dystrophy of the mucosa, vulva, and urogenital tract.[13] Globular actin resides in the cytoplasm as the cytoskeleton shows in the form of parallel filaments or filamentous tissue. Globular actin determines cell rigidity in response to a stimulus and allows cell exchanges for the maintenance of cell bonds. Tight junction networks of transmembrane proteins such as occludin, claudin, and adhesion junction molecules contribute to regulating tight connection permeability.[14,15] Estrogen plays a role in modulating epithelial permeability in the vagina by loosening the junctions. In addition, estrogen can increase protein

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

regulation at tight junctions. Thus, the administration of estrogen in hypoestrogenic conditions can help increase elasticity in the vagina.[16]
The extensive development of nutraceuticals suggests the use of natural ingredients as treatment and hormone replacement therapy for menopause. This serves as the basis for the development of nutritional products to overcome the discomfort of menopause. The development of this treatment could lead to hormone replacement using safer and more affordable natural ingredients.
Research for drug discovery of herbal compounds and their potential applications can be done through computational modeling. Molecular docking is a computational method that predicts the binding of ligands to receptors for the discovery and development of drugs from natural materials.[17] Computational modeling can increase efficiency, reduce errors, and save material and time. Computational approaches are also accelerating drug discovery with modern scientific methodology.[18,19,20] Using computational approaches, this study, therefore, was conducted to investigate the potential interaction of bioactive compounds found in banana blossom extract, namely anthocyanin, genistein, claudin-1, and actin through an in silico approach for menopause therapy.

Materials and Methods

This study used Asus i5 laptop and AutoDock4 Tools (v1.5.6) from Molecular Graphics Laboratory, The Scripps Research Institute for receptor preparation, and docking validation. Banana bracts' active compounds and their ligand structures were tested using the Vega ZZ (version vega 3.0, Istituto di Chimica Farmaceutica e Tossicologica "Pietro Pratesi", Università degli Studi di Milano) application which was downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Datasets of claudin-1 target protein structure PDB ID: 5M3D, https://doi.org/10.2210/pdb5M3D/pdb, and Actin PD protein ID: 2PBD, PDB DOI: https://doi.org/10.2210/pdb2PBD were downloaded from www.rcsb.org. The docked results combined with claudin-1 and actin proteins were then visualized using the BIOVIA Discovery Studio. The swissADME program was accessed via (http://www.swissadme.ch.Specialist). Last but not least, the toxicity prediction was done using web pkCSM (http://structure.bioc.cam.ac.uk/pkcsm).

Preparation of Protein and Ligands
Proteins (Claudin 1 and actin) were visualized using the Biovia DS Visualizer app. The production of claudin-1 and actin proteins involves the separation of proteins from initial ligands and receptor residues, followed by the storage of all files. The preparation was carried out by optimizing the 3-dimensional structure of anthocyanin and genistein compounds using the Vega ZZ application by entering SMILES. Any compound obtained from the PubChem (http://pubchem.ncbi.nlm.nih.gov) database was stored in PDB format and converted to pdbqt using the Autodock tool.

Docking Parameter Validation
AutoDock Tools software (v1.5.6) was used to validate this study. The validation process involves redocking the original ligands of claudin-1 protein (PDB ID:5M3D) and actin protein (PDB:2PDB). The procedure resulted in grid parameters and RMSD (Root Mean Square Deviation) values. RMSD is a measure of the average distance value that relates to the size of the docking zone. The smaller the RMSD value (< 2 Å) obtained, the closer the docking pose to the crystallographic ligand pose. The grid box arrangement of the docking process could determine the binding of the ligand's chamber. Grid box calculations were run based on ligands attached to protein macromolecules during the download process. [21]

Molecular Docking
The molecular tethering process was carried out using AutoDock Vina 1.5.6 software.[22] Appropriately prepared receptor and ligand files were saved in PDBQT format and the same folder, in particular, the vina folder located inside folder C. The molecular tethering process started using "command prompt" (CMD) software, and the government process was executed in the program located in the folder. To derive the docking score and root-mean-square deviation (RMSD) metric from the docking process, the next thing to do was go to the vina .exe-config conf command into Vina's executable configuration file. Then, run the command 'txt-log log.txt' inside the Command Prompt. All documents to be used in the docking process were placed in a common directory.
The claudin-1 and actin proteins in pdbqt format were then transcribed into a new file titled conf.txt, while the ligands were described as ligands. In the given grid box, the given values represent the x, y, and z coordinates and the x, y, and z axis dimensions in pdbqt format, respectively. Root mean square deviation (RMSD) was obtained from the molecular docking procedure by executing the vina command. To proceed with the executable configuration file, run the 'txt-log log' command in the command prompt. The root mean square deviation (RMSD) value for each confirmation was shown < 2 Å. The docking method was considered valid if it had an RMSD value of < 2 Å. If the RMSD value was > 2 Å, the method used was invalid.[23]

The Visualization of Molecular Docking Data
The Biovia Discovery Studio Visualizer (v20.1) application was utilized to visually represent molecular docking data. To assess the outcomes of linking test compounds to proteins, the molecular docking data were visualized using the 3D Pymol software. This involved examining the bond formed between ligand and protein, thereby enabling the observation of the surface area occupied by the test ligands within the protein. The visualization outcomes were utilized to establish the interactions between compounds and to generate images depicting the docking of proteins with each examined ligand.

Analysis of Pharmacokinetic and Toxicity
SwissADME web and pkCSM were used to predict the pharmacokinetic properties and toxicity of anthocyanin and genistein. The results obtained as ADME attributes were described as characteristics of absorption, distribution, metabolism, and excretion. The pharmacokinetic profile of the ligand was predicted based on Lipinsk's rule.[24]

Results and Discussion

Validation of the molecular tethering method was carried out through grid box arrangement in a grid box. This validation process determines the interaction between ligands and amino acids at the specified target protein binding site. The grid box was adjusted to the size of the original ligand and the test compound to ensure that the original ligand and test compound were included in the grid box.[25] The grid box settings included the grid size and grid center. The grid box values in this study were x: 5.655, y: -21.952, z: -2.122 with a box size (40x 40x40x). The grid box value was determined based on the position of the standard ligand compound on the claudin-1 protein contained in the Protein Data Bank (PDB) file. In silico research was conducted to determine the binding interaction between *anthocyanin* compounds, *genistein* with claudin-1, and actin receptors. The study began with protein and ligand preparation using the BIOVIA DS Visualizer application. The preparation of ligands was carried out by optimizing the 3D structure of *anthocyanin* and *genistein* compounds using the Vega ZZ application.
Molecular tethering applications for designing drugs and their affinity to proteins and pkCSM models for pharmacokinetic prediction were used. The result of molecular tethering reflected the strength of affinity binding referred to as the docking score. Based on the results of molecular tethering and interaction of the original ligand, the G-binding scores obtained were -5.78 kcal/ mol (296.41%) from claudin-1 with *anthocyanin* test ligands and -3.35 kcal/mol (171.79%) from *genistein* with the test ligand. Results of molecular tethering showed actin test ligands with *anthocyanin* test ligands had a value of -6.81 kcal / mol (72.60%), and *genistein* with *anthocyanin* test ligands had a value of -4.53 kcal/mol (48.29%). The affinity binding score of *anthocyanin* compounds was close to the estrogen Gbinding score of -6.81 kcal/mol (72.60%) (Table 1). The score indicated that *anthocyanin* compounds had a binding affinity with estrogen. *Anthocyanins* belong to the group of *flavonoids* that have a structure and biosynthetic pathway similar to other groups of *flavonoids*, namely *isoflavones*, and *flavanones*. The

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

flavonoid group has a structure similar to estrogen 17β estradiol which can increase the activity of the hormone estrogen. Phytoestrogen compounds can exert their activity by binding to estrogen receptors α (ER-α) and estrogen receptors β (ER-β). Phytoestrogen are potentially effective for reduce complaints of diseases that arise due to estrogen deficiency.[26]

The result of redocking was indicated by the RMSD value. RMSD values were used to see differences in crystallographic binding orientation on the active side of proteins of several poses using the same ligand. The RMSD value was valid if it was < 2 Å.[27] In this study, the RMSD value of claudin-1 was 1.43 Å, and that of actin was 2.19 Å. This means that the protein claudin-1 has the right interaction between the original ligand and the test ligand. RMSD values of > 2 indicate large deviations and greater prediction errors of ligand-protein interactions. Thus, ligand-receptor interactions cannot be continued as a reference in silico.[28] This study found that the molecular docking estrogen as a standard drug compound showed an RMSD value of 0.001 Å (< 2 Å).

Molecular docking results were visualized to identify interactions between test ligands and target proteins. The interaction of *anthocyanin* with claudin-1 proteins formed amino acid residues on hydrogen bonds (Table 2) namely Asn172, Cysl175, and non-hydrogen bonds Asn173, Pro176, Ser179, Gly148, Ser177, and Lys148. The interaction of *genistein* compounds with claudin-1 protein formed amino acid residues on Asn172, Cys175 hydrogen bonds and Lys148, Asn173, Pro176, Ser179, Gly178, and Ser177 hydrogen bonds. While the interaction between actin proteins and *anthocyanin* compounds formed amino acid residues on hydrogen bonds Ser14, Gly158, and Vall59, and non-hydrogen bonds formed Gly74, Vall59, and Ser14. On the other hand, the interaction of *genistein* and actin compounds formed amino acid residues on Ser14, Gly158 hydrogen bonds, and Gly74, Sers14 non-hydrogen bonds.

Table 1: Results of Molecular Docking between Ambones Banana Bracts and Estrogen and Claudin-1 and Actin Ligand Proteins

Ligand	Protein	Compound	Gbinding Score (kcal/mol)	Percentage	RMSD
Banana Ambones Bracts		Original ligand			
	Claudin-1	Original ligand	-1.95	100%	1.43 Å
	Anthocyanin	Test ligand	-5.78	296.41%	
	Genistein	Test ligand	-3.35	171.79%	
	Actin	Original ligand	-9.38	100%	2.19 Å
	Anthocyanin	Test ligand	-6.81	72.60%	
	Genistein	Test ligand	-4.53	48.29%	
	Estrogen	Original ligand	-9.33	100%	0.001 Å

Anthocyanins have a higher Gbinding score than *Genistein* of -6.81 kcal/mol, close to the estrogen standard of -9.33 kcal/mol, and RMSD values of <2 Å

Based on binding affinity and RMSD values of <2, claudin-1 has potential phytoestrogen effects comparable to standard therapy (estrogen). According to Lipinski's rule, good compound candidates for drugs must have a molecular weight of < 500 g/mol, log P smaller than 5, 5 hydrogen donors, and less than 10 hydrogen acceptor bonds.[29,30] Based on Lipinski's rule, the prediction results in Table 3 show that *Genistein* did not meet Lipinski's rule. It is proved that the molecular weight, hydrogen bond donor, hydrogen bond acceptor, and Log P values are 594.52 g/mol, 15.9, and 1.83, respectively. So, in this study, it can be said that *Genistein* has poor chemical bonds and is not included in the active compound compared to *anthocyanin* compounds that meet Lipinski's rule. Therefore, this compound is predicted to be classified as a drug candidate for dealing with complaints due to estrogen deficiency.

Based on the prediction of the pharmacokinetic profiles of substance absorption using pkCSM, *anthocyanin* compounds had good absorption ability (high) with a log Kp value of -5.07. It is predicted that this compound can be considered for new drug development. Predicting the distribution of *anthocyanin* compounds yields a blood-brain barrier (BBB) result (Yes), suggesting that this compound will readily cross the brain barrier. The blood-brain barrier plays an important role in controlling the entry and exit of substances important for the metabolic activities of the brain and nerves. Therefore, BBB integrity and function are important for maintaining brain microenvironment homeostasis.[31]

The metabolic reactions of a compound involve an oxidation process. The processes involve cytochrome P450 which is a detoxification enzyme found in the liver. This enzyme works by oxidizing incoming foreign bodies, including drugs.[32] The metabolism of *anthocyanin* compounds was predicted to be CYP1A2 inhibitors and CYP2D6 inhibitors. In addition, the excretion of *anthocyanin* test compound indicated good skin permeability as the log Kp value was >-2.5 cm.

In silico testing which predicts the chemical activity and mutagenicity was carried out using AMES.[33] The results of toxicity prediction in banana bracts ethanol extract using AMES (Table 5) showed the possibility of potential mutagen compounds with results (No), hepatotoxicity (No), and skin allergies (No). The toxicity of compounds was carried out with LD_{50} parameters. Acute oral toxicity prediction in rats (LD_{50}) aims to define acute toxicity standards to assess the relative toxicity of a compound. LD_{50} is a lump sum dose of the compound that caused the deaths of 50% of the test animal group. Table 5 shows the toxicity prediction results. It describes an LD_{50} value of 1.848 mol/kg from *anthocyanin* compounds, while 2.76 mol/kg from *genistein* toxicity prediction is the highest dose value that technically can still be given to test animals. The maximum tolerant dose in humans is 0.609 mg/kg/day (Table 5).

Conclusion

The compounds from the ethanol extract of the Ambones banana (*Musa Acuminta Colla*) blossom have a docking score close to estrogen (-9.33 kcal/mol). Results of molecular tethering and interaction of original ligands showed claudin-1's Gbinding score (kcal/mol) was -1.95 kcal/mol (100%), *anthocyanin* test ligands' score was -5.78 kcal/mol (296.41%), and *genistein's* score was -3.35 kcal/mol (171.79%). However, from the molecular tethering and interaction, the G-binding score (kcal/mol) was -9.38 kcal/mol (100%) for actin's original ligand, -6.81 kcal/mol (72.60%) for *anthocyanin* test ligands, and -4.53 kcal/mol (48.29%) for *genistein*. RMSD values were 1.43 Å for claudin-1 protein and 2.19 Å for action. *Anthocyanin* and *genistein* compounds from the ethanol extract of Ambones banana blossom were not toxic. The current research results can be helpful for drug discovery using natural ingredients containing *anthocyanin* and *genistein* compounds. Further research can be done *in vitro* and *in vivo* to explore these compounds clinically.

Conflict of Interest

The authors declare no conflict of interest.

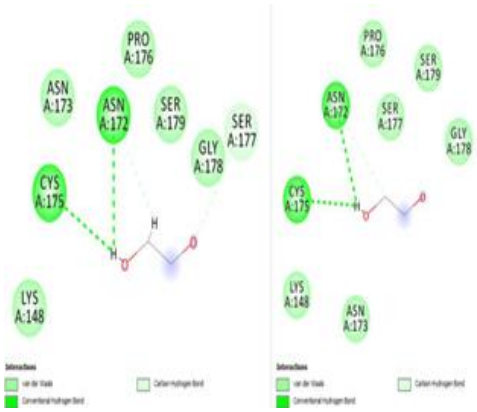
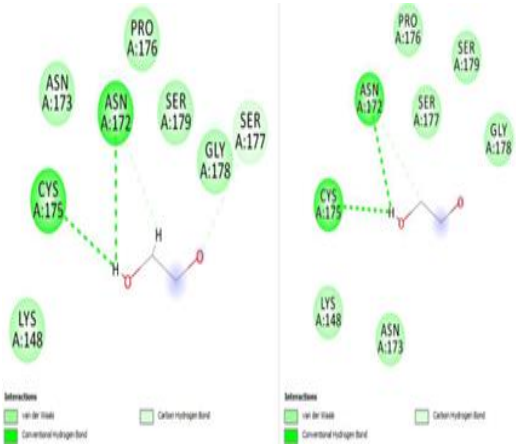
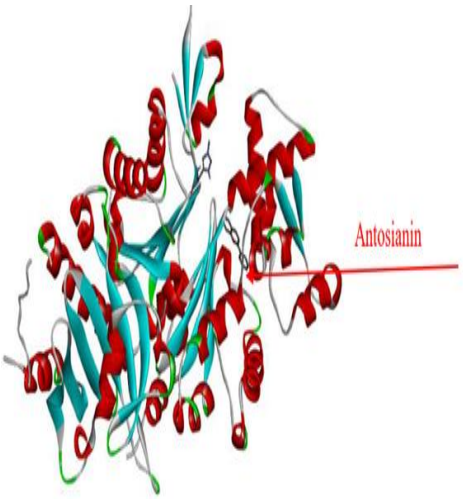
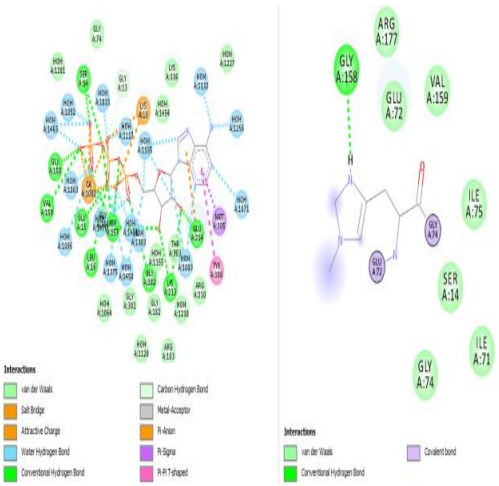
Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Visualizations of Molecular Docking between Ligands in 3D

Claudin-1	Visualise 3D	Docking of Molecules and Ligands
Anthocyanin		
Genistein		
Actin		
Anthocyanin		

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Genistein

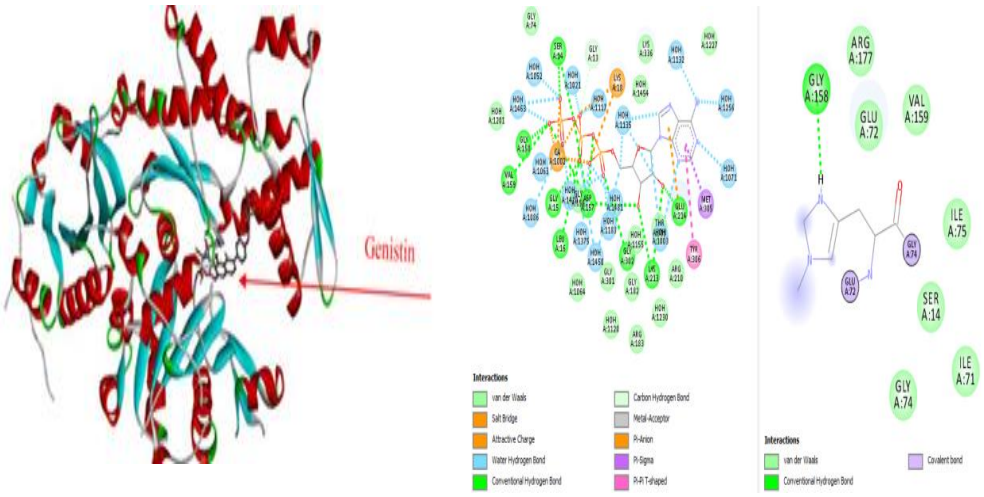


Table 3: Molecular Properties of pkCSM

Compound	Formula	Molecular Weight (g/mol)	H-Bond Acceptor < 10	H-Bond Donors < 5	Log P < 5
Anthocyanin	$C_{15}H_{11}O+$	207.25	1	1	-0.76
Genistein	$C_{27}H_{30}O_{15}$	594.52	15	9	1.83

Note: Anthocyanins have relative atomic mass value < 500 Da; = Log P value < 5; C = Donor bond H < 5

Table 4: Prediction of Pharmacokinetic Characteristics (Absorption, Distribution, Metabolism, and Excretion)

Banana Bracts		
A: Absorption	GI Absorption	High
	TPSA	13.14 $Å^2$
	Bioavailability	0.55
D: Distribution	BBB permeant	Yes
	P-gp substrate	Yes
M: Metabolism	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	Yes
	CYP3A4 inhibitor	No
E: Excretion	Log *Kp*	-5.07 cm/s

Prediction of ADME compounds having a high GIA facilitates the absorption process in the digestive tract. Compounds penetrate the brain barrier and are hydrophobic (P-gp substrate), so they can affect the distribution process and bioavailability.

Table 5: Toxicity Prediction Results

Model's Name	Anthocyanin	Genistein
AMES toxicity	No	No
Max tolerated doses (human)	0.44 mg/kg	0.609 mg/kg
hERG I inhibitor	No	No
hERG II inhibitor	No	Yes
Acute oral toxicity (LD_{50}) in rats	1.848 mol/kg	2.76 mol/kg
Chronic oral toxicity in rats	1.118 log mg/kg/bb	5.68 log mg/kg/bb
Hepatotoxicity	No	No
Skin sensitisation	No	No
Minnow toxicity	0.172 mM	5.166 log mM

Anthocyanin vs *Genistein* does not cause toxicity, safe doses in humans are lower, do not inhibit hERG1, oral and renal rat doses Toxicity is higher, does not cause allergies, and hepatotoxicity

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Acknowledgements

The authors would like to thank the Aisyah Lampung Foundation for its financial support of our research project. The authors also appreciate the Head of the Laboratory Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia for endorsing the research permits and facilities to support the research.

References

1. Talaulikar V. Menopause transition: Physiology and symptoms. Best Pract Res Clin Obstet Gynaecol. 2022;81(5):3–7.
2. Marino JM. Genitourinary Syndrome of Menopause. J Midwifery Women's Heal. 2021;1(11):1-11.
3. Wańczyk-Baszak J, Woźniak S, Milejski B, Paszkowski T. Genitourinary syndrome of menopause treatment using lasers and temperature-controlled radiofrequency. Prz Menopauzalny. 2018;17(4):180-184.
4. Cagnacci A, Venier M. The controversial history of hormone replacement therapy. Med. 2019;55(602):1-11.
5. Mehta J, Kling JM, Manson JAE. Risks, Benefits, and Treatment Modalities of Menopausal Hormone Therapy: Current Concepts. Front Endocrinol (Lausanne). 2021;12(564781):1-14.
6. Canivenc-Lavier MC, Bennetau-Pelissero C. Phytoestrogens and Health Effects. Nutrients. 2023;15(317):1–44.
7. Panche AN, Diwan AD, Chandra SR. *Flavonoids*: An overview. J Nutr Sci. 2016;5(47):1–15.
8. Nanashima N, Horie K, Maeda H. Phytoestrogenic activity of *blackcurrant anthocyanins* is partially mediated through estrogen receptor beta. Molecules. 2018;23(74):1–11.
9. Kiyama R. Estrogenic flavonoids and their molecular mechanisms of action. J Nutr Biochem. 2023;114(109250):1–50.
10. Fithri AN, Yueniwati Y, Arsana IW, Khotimah H, Nurwidyaningtyas W. The Potential Health Benefit of *Cowpea* Estrogen-like Activity to Restore Vaginal Epithelial Cells Thinning Due to Menopausal Syndrome. Trop J Nat Prod Res. 2024;8(3):6624-6630.
11. Chiang SH, Yang KM, Lai YC, Chen CW. Evaluation of the in vitro biological activities of *Banana flower* and bract extracts and their bioactive compounds. Int J Food Prop. 2021;24(1):1–16.
12. Thagunna B, Kandel K. *Banana Blossom*: Nutritional Value, Health Benefits and It's Utilization. Rev Food Agric (RFNA). 2023;4(2):62-66.
13. Musbahi E, Kamp E, Ashraf M, DeGiovanni C. Menopause, skin and common dermatosis. Part 3: genital disorders. Clin Exp Dermatol. 2022;47(12):2123-2129.
14. Garcia MA, Nelson WJ, Chavez N. Cell–Cell Junctions Organize Structural and Signaling Networks. Cold Spring Harb Perspect Biol. 2018;10(4):1–27.
15. Otani T, Furuse M. Tight Junction Structure and Function Revisited. Trends Cell Biol. 2020;30(10):805-817.
16. Kyung, Lee HS, Ahn K, Park K. Estrogen Modulates Expression of Tight Junction Proteins in Rat Vagina. Biomed Res Int. 2016;(2016):1–6.
17. Carpio LE, Sanz Y, Gozalbes R, Barigye SJ. Computational strategies for the discovery of biological functions of health foods, nutraceuticals and cosmeceuticals: a review. Mol Divers. 2021;25(3):1425-1438.
18. Rohman MS, Sulistomo HW, Kusumastuty I, Nugroho DA, Nugraheni NIP. In Silico Discovery of *Green Tea* and *Green Coffee* Bioactive Compounds Against. Trop J Nat Prod Res. 2024;8(3):6594-6603.
19. Asiamah I, Obiri SA, Tamekloe W, Armah FA, Borquaye LS. Applications of molecular docking in natural products-based drug discovery. Sci African. 2023;20(8):1–8.
20. Sadybekov A V., Katritch V. Computational approaches streamlining drug discovery. Nature. 2023;616(7958):673-685.
21. Yuliantini A, Ocktavyanie S, Febrina E, Asnawi A. Virtual Screening Using a Ligand-based Pharmacophore Model from *Ashitaba* (*Angelica keiskei K.*) Isolates and Molecular Docking to Obtained New Candidates as α-Glucosidase Inhibitors. Trop J Nat Prod Res. 2024;8(1):5811-5819.
22. Hosseini M, Chen W, Xiao D, Wang C. Computational molecular docking and virtual screening revealed promising SARS-CoV-2 drugs. Precis Clin Med. 2021;4(1):1–16.
23. Maruyama Y, Igarashi R, Ushiku Y, Mitsutake A. Analysis of Protein Folding Simulation with Moving Root Mean Square Deviation. J Chem Inf Model. 2023;63(5):1529-1541.
24. Karami TK, Hailu S, Feng S, Graham R, Gukasyan HJ. Eyes on Lipinski's Rule of Five: A New "Rule of Thumb" for Physicochemical Design Space of Ophthalmic Drugs. J Ocul Pharmacol Ther. 2022;38(1):43–55.
25. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. J Chem Inf Model. 2021;61(8):3891-3898.
26. Lecomte S, Demay F, Ferri F, Pakdel F. Phytochemicals Targeting Estrogen Receptors : Beneficial Rather Than Adverse Effects. Mol Sci. 2017;18(1381):1–19.
27. Inayati I, Arifin NH, Febriansah R, Indarto D, Suryawati B, Hartono H. Biofilm-forming Klebsiella pneumoniae : An In Silico Study. Trop J Nat Prod Res. 2023;7(10):4249-4255.
28. Makeneni S, David F. Thieker and RJW. Applying Pose Clustering and MD Simulations To Eliminate False Positives in Molecular Docking. J Chem Inf Model. 2018;58(3):605-614.
29. Chen X, Li H, Tian L, Li Q, Luo J, Zhang Y. Analysis of the Physicochemical Properties of Acaricides Based on Lipinski's Rule of Five. J Comput Biol. 2020;27(9):1-10.
30. Kralj S, Jukič M, Bren U. Molecular Filters in Medicinal Chemistry. Encyclopedia. 2023;3(2):501-511.
31. Kadry H, Noorani B, Cucullo L. A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids Barriers CNS. 2020;17(69):1–24.
32. Peter Guengerich F. Cytochrome P450 research and The Journal of Biological Chemistry. J Biol Chem. 2019;294(5):1671-1680.
33. Honma M. An assessment of mutagenicity of chemical substances by (quantitative) structure-activity relationship. Genes Environ. 2020;42(23):1–13.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License