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Estrogenic effect, druglikeness, molecular docking, and pathway analysis of active compounds from fruit flour extract *Rhizophora mucronata*

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ABSTRACT

Treatment of estrogen through hormone replacement therapy to cause side effects. *Rhizophora mucronata* extract has potential phytoestrogens from natural ingredients. This study aims to analyze estrogenic activity, druglikeness, and molecular docking as an alternative to estrogen hormone therapy. Estrogenic activity analysis using Way2drug Pass online revealed that the five compounds tested had a Pa value (probability of being active) ≥ 0.3. Druglikeness analysis using the SwissAdme program revealed the potential as medicinal compounds for oral administration meet the provisions of the rule of five. The results of the molecular docking study show that the five compounds interact with the estrogen receptor protein through the formation of hydrogen bonds and alkyl bonds. Zearalenone produces the strongest interaction with the estrogen receptor protein with a binding affinity of –9.23 Kcal/mol and the formation of three hydrogen bonds to the amino acid residues, Glu353, Leu387, and Arg394. Reinforce by data from pathway analysis results, which show that the estrogenic potential of zearalenone is through activation of the estrogen receptor (ESR1), nuclear receptor subfamily 1 (NR112), and androgen receptor with an activation score of 0.795, a binding strength of 0.234, and an inhibition power of 0.318.

INTRODUCTION

Estrogen is a hormone that has a selective function in female reproduction, as well as physiological functions in almost all tissues in the body [1]. Estrogen deficiency can occur in women who experience menopause or have an oophorectomy [2]. Treatment of estrogen deficiency can be done with the aromatase enzyme, estrogen receptor antagonists, aryl hydrocarbon receptors, phytoestrogens, or hormone replacement therapy (HRT) [3,4].

In recent years, HRT has apparently caused side effects in patients, namely, the appearance of spots and itching on the skin, pain in the breasts, and weight gain [2,5]. Several studies also state that HRT can also increase the risk of cancer [6–9], coronary heart disease [10–12], stroke [13], and osteoporosis and endometriosis [1,14–16]. Therefore, treatment of estrogen deficiency is recommended through alternative therapy, such as the use of phytoestrogens [2,17,18].

The characteristic feature of phytoestrogens is the presence of phenolic rings, which are a prerequisite for binding

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to estrogen receptors [18]. Phytoestrogens are divided into four groups, namely, chalcones, flavonoids (flavones, flavonols, flavanones, and isoflavonoids), lignans, and stilbenoids [19]. Phytoestrogens can bind to estrogen receptors, so they can carry out the same function as estrogen in the body [20]. Apart from that, this compound is also reported to have estrogenic and antiestrogenic effects, which depend on the body's estrogen levels [21]. Phytoestrogens can be found in vegetables, fruits, and grains [22,23].

In previous research, we succeeded in identifying five compounds from the ethyl acetate and ethanol fractions of R mucronate fruit flour. The five compounds are aromadendrin, cianidanol (flavonoid group), zearalenone, ethylestrenol, and pinoresinol. Ethylestrenol has similarities to the molecular structure of 17β estradiol and ethynyl estradiol in mammals, which act as estrogen receptors α and β. In this research, studies were carried out on the estrogenic potential, druglikeness, molecular docking, and pathway analysis of these five compounds. The molecular structures of the test and control compounds (ethynyl estradiol and 17β estradiol). It is hoped that this research can become a reference in exploring the potential of R mucronata fruit flour as an alternative therapy to replace the hormone estrogen.

MATERIAL AND METHODS

Estrogenic activity study

Estrogenic activity was analyzed for potency using Way2drug Pass online (https://www.way2drug.com/passonline/). Tracing the Pa value (probability to be active) is used to describe the potential of a compound being tested. If the Pa value is more than 0.7, it means that the compound is predicted to have high estrogenic potential by computing and laboratory tests. If the Pa value is more than 0.3 but less than 0.7, it means that the compound has computationally estrogenic capabilities but has not been proven in laboratory tests or has little potential. The control compounds used were ethynyl estradiol and 17β estradiol, which were used as comparisons because they have the potential to act as estrogen agonists [24]. The molecular structures of the test and control compounds (ethynyl estradiol and 17β estradiol) are presented in Figure 1.

Druglikeness analysis

The compounds were analyzed with SwissAdme software to determine their ability as medicinal compounds. Compounds were selected based on the rule of five (RO5) requirements [25]. Next, the binding strength with the estrogen protein as a receptor was analyzed to obtain the best binding model with the lowest binding affinity.

Molecular docking study

Receptor determination

The material used for molecular docking studies is a protein with the code NF-κB.

Receptor preparation

The material used for the molecular docking study was the NF-κB protein (pdb id: 4IDV), which was downloaded from https://www.rcsb.org/structure/4IDV. The 3D structure

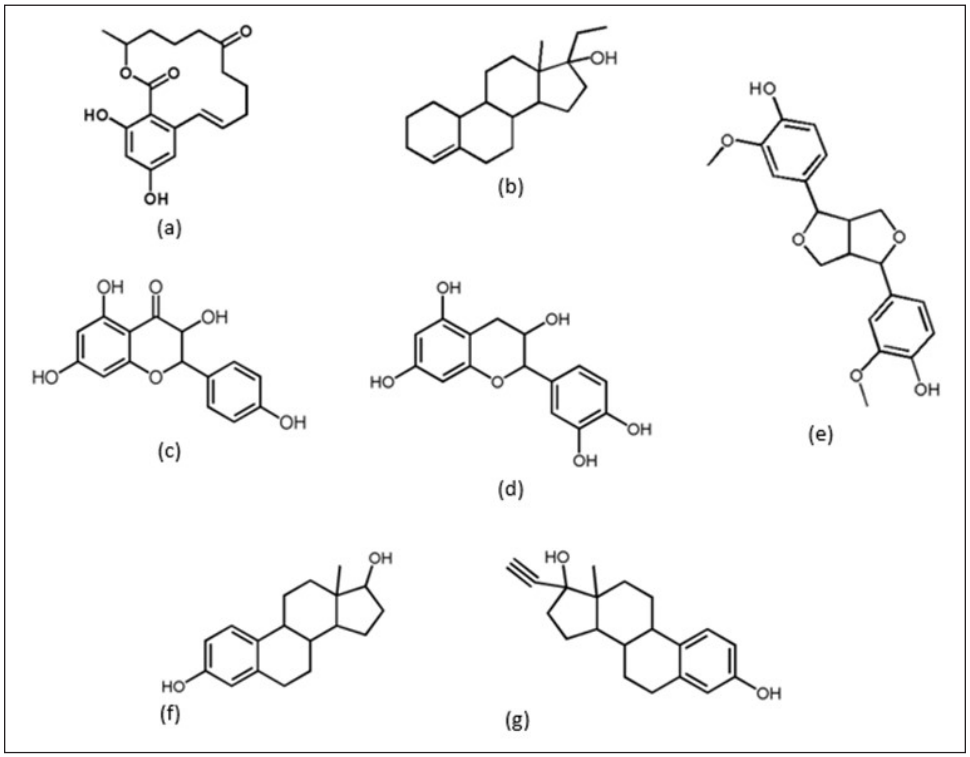

Figure 1. Molecular structure of test and control compounds: (a) zearalenone, (b) ethylestrenol, (c) aromadendrin, (d) cianidanol, (e) pinoresinol, (f) 17β estradiol/control-1 dan, and (g) ethynil estradiol/control-2.

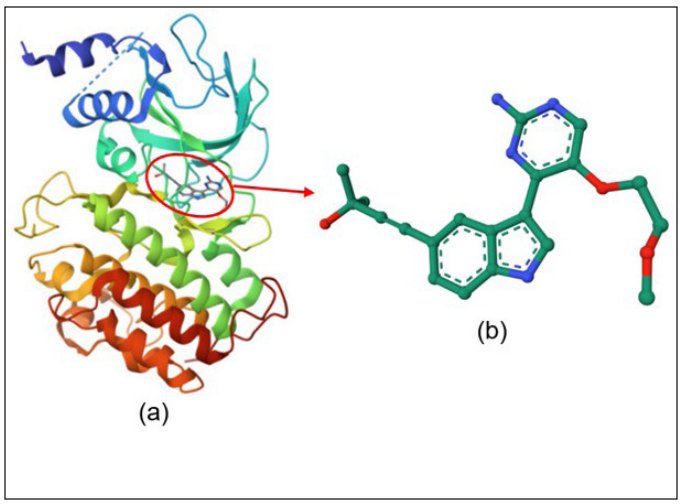

Figure 2. Structure 3D (a) protein NF-κB and (b) ligan standard (13V).

of the NF-κB protein binding the ligand 4-{3-[2-amino-5-(2-methoxyethoxy) pyrimidin-4-yl]-1H-indol-5-yl}-2-methylbut-3-yn -2-ol (13V) is presented in Figure 2. This research uses a computer with Intel® CoreTM i7 processor specifications, 8550U @ 1.80GHz 1.99GHz, 8.00 GB RAM. Meanwhile, the software used includes HyperChem 8, Chimera 1.10.1, Autodock 4.2, and Discovery studio® 3.1. (Accelerys, San Diego, USA).

Ligand preparation

The 3D structure of the ligand was taken from the PubChem website. The ligand file is downloaded and then saved. Ligand files in the sdf format were then converted into the pdb format using PyMol version 2.4.1. The file is then converted to the pdbqt format using AV.

Docking receptor ligan

NF-κB protein preparation begins by selecting the active form of the protein that binds to the original ligand (13V). Next, the NF-κB protein and native ligand were separated using the Chimera 1.10.1 program to provide space (pockets/cavities) so that the pocket shape and pocket coordinates were known as docking material.

Validation of the molecular docking method

Validation of the molecular docking method was carried out by docking the native ligand back to the target protein, which had the native ligand removed using the Autodock 4.2 program. The method is said to be valid if the root mean square deviation (RMSD) value obtained is ≤3 Å so that the test compound can be docked with the target protein [26]. An RMSD value ≤3 Å indicates that the native position of the ligand is not much different before and after redocking.

Docking data analysis

The docking analysis stage was carried out using the Discovery studio® 4.5 visualizer program. Data analysis is carried out based on the binding energy and interactions formed between the ligand and the receptor protein. The smaller the binding energy, the more stable the bond between the receptor protein and the ligand. Apart from that, docking data analysis was also carried out by looking at the type, number, and distance of bonds formed between the ligand and the NF-κB protein.

RESULT AND DISCUSSION

Estrogenic activity study

Based on studies of estrogenic activity using Way2drug Pass online, it is known that the five compounds tested have a Pa value (probability to be active) ≥0.3. Pa values above 0.3 indicate that these five compounds have the potential to have estrogenic activity. The estrogenic activity of cianidanol, pinoresinol, and aromadendrin was predicted via estrogen beta receptor agonist and estrogen agonist. The estrogenic activity of zearalenone is via estrogen agonist, while ethylestrenol is via estrogen agonist, estradiol 17β dehydrogenase stimulant, and estrone sulfotransferase simultaneously [27–29]. The Pa values of the five compounds compared with estradiol and ethynylestradiol (as control) are presented in Figure 3.

Druglikeness analysis

Based on a druglikeness study using the SwissAdme program, it is known that four test compounds (ethylestrenol, aromadendrin, pinoresinol, and zearalenone) have the potential as drug compounds for oral administration because they meet the provisions of the RO5. Cianidanol cannot be given orally because it has a number of hydrogen bond donors of 5. Lipinski *et al.* [25] explain that the drug compound can be given orally if it has a molecular weight (<500Da) and high lipophilicity (LogP < 5), hydrogen bond donors are less than 5, hydrogen bond acceptors are less than 10, and molar refractivity is between 40 and 130. Data from the druglikeness analysis of the five test and control compounds are presented in Table 1.

Molecular docking study

Validation of the molecular docking method through standard ligand redocking (13V) obtained an RMSD value of 0.51 Å and a binding energy of –5.56 kcal/mol. This value indicates that the standard ligand conformation before and after readdition is not much different. A comparison of the conformations of the standard 13V ligand before and after redocking is presented in Figure 4.

The conformations and grid boxes resulting from the redocking of standard 13V ligands were then used as a reference for molecular docking studies of the compounds: zearalenone, ethylestrenol, aromadendrin, cianidanol, and pinoresinol. These compounds are compounds contained in the ethyl acetate and ethanol fractions of mangrove fruit based on the results of our previous research. Ethynil estradiol and estradiol were used as controls. Data on the molecular formula, molecular weight, PubChem CID, and canonical smiles for each compound are presented in Table 2. Meanwhile, the 3D molecular structure resulting from geometric optimization of the seven compounds is presented in Figure 5.

The results of molecular docking analysis show that the zeralenone compound forms hydrogen bonds with

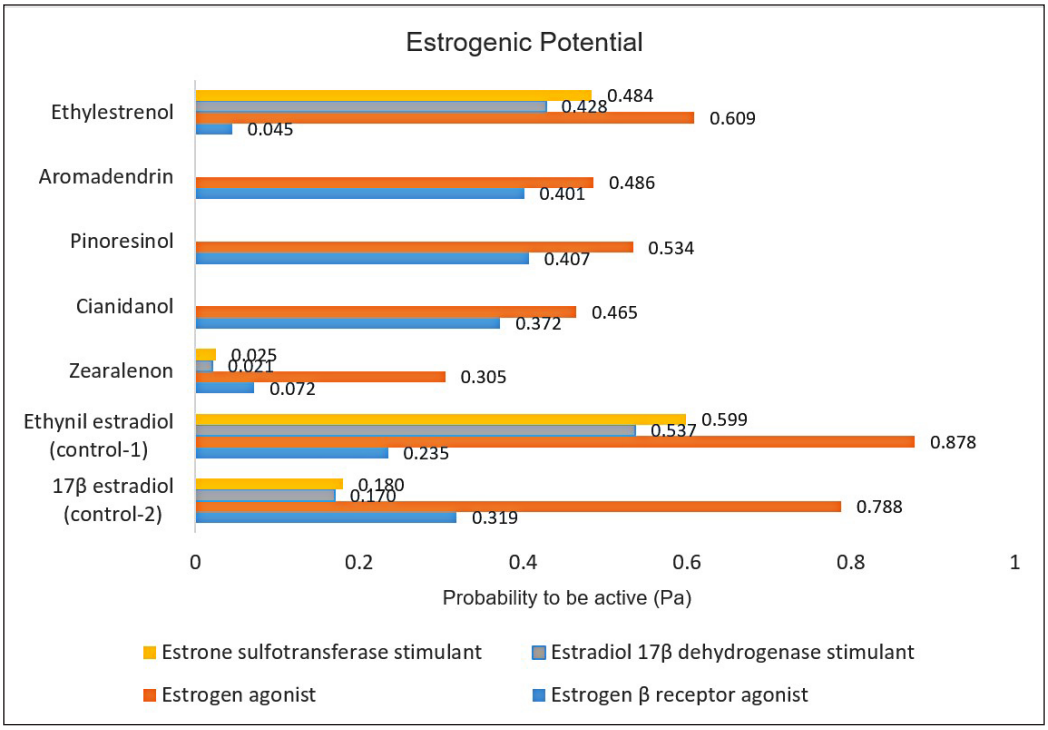

Figure 3. Probability to be active (Pa) values for test and control compounds.

Table 1. Data from druglikeness analysis of test and control compounds.

No.	Compound	Molekular Weight <500 Dalton (g/mol)	High Lipophilicity (expressed LogP <5)	Hydrogen donor bonds <5	hydrogen acceptor bonds <10	Refractifity molar 40–130
1	Ethylestrenol	288.47	4.86	1	1	90.26
2	Cianidanol	290.27	0.24	5*	6	74.33
3	Aromadendrin	288.25	-0.10	4	6	72.73
4	Zearalenone	318.36	2.06	2	5	88.40
5	Pinoresinol	358.39	1.17	2	6	94.90
6	Ethinyl estradiol (Kontrol-1)	296.40	3.90	2	2	88.64
7	17β Estradiol (Kontrol-2)	272.38	3.53	2	2	81.03

* = not the requirements RO5.

the amino acid residues Glu353, Leu387, and Arg394 in the receptor protein. The distance of the hydrogen bonds formed is 2.55, 2.33, and 2.98 Å. In addition to hydrogen bonds, zearalenone also forms alkyl and pi-alkyl bonds with the amino acid residues Met388, Leu346, Leu391, Ala350, Leu384, Trp383, and Leu525. The van der Waals interaction between zearalenone and the receptor protein occurs at the amino acid residues Leu428, Ile424, Gly521, Met421, Met343, Val533, Thr347, and Leu349 (Fig. 6).

The interaction of ethylestrenol with the estrogen receptor protein (Fig. 7) occurs through the formation of alkyl and Pi-Alkyl bonds with the amino acid residues Leu346 (4.47 Å), Leu 384 (4.38 Å), Leu428 (5.42 Å), Met388 (4.72 Å), Leu391 (4.53 Å), Ala350 (5.15 Å), Leu349 (4.60 Å), Phe404 (5.41 Å), and Leu387 (4.98 Å). In addition to alkyl and pi-alkyl bonds, ethylestrenol also produces van der Waals interactions

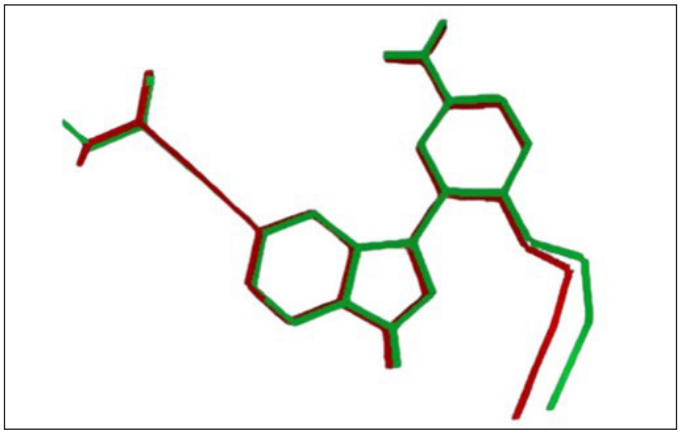

Figure 4. Comparison of the conformations of the standard 13V ligand before (red) and after (green) redocking.

Table 2. Molecular formula, molecular weight, PubChem CID, and canonical smiles of test and control compounds.

Compounds	Molecular formula	Molekular Weight (g/mol)	PubChem CID	Canonical smiles
Zearalenone	$C_{18}H_{22}O_5$	318.4	5281576	CC1CCCC(=O)CCCC=CC2=C(C(=CC(=C2)O)O)C(=O)O1
Ethylestrenol	$C_{20}H_{32}O$	288.5	13765	CCC1(CCC2C1(CCC3C2CCC4=CCCCC34)C)O
Aromadendrin	$C_{15}H_{12}O_6$	288.3	122850	C1=CC(=CC=C1C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Cianidanol	$C_{15}H_{14}O_6$	290.3	9064	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C=C3)O)O)O
Pinoresinol	$C_{20}H_{22}O_6$	358.4	73399	COC1=C(C=CC(=C1)C2C3COC(C3CO2)C4=CC(=C(C=C4)O)OC)O
Ethynil estradiol (Control 1)	$C_{20}H_{24}O_2$	296.4	5991	CC12CCC3C(C1CCC2(C#C)O)CCC4=C3C=CC(=C4)O
Estradiol (Control-2)	$C_{18}H_{24}O_2$	272.4	5757	CC12CCC3C(C1CCC2O)CCC4=C3C=CC(=C4)O

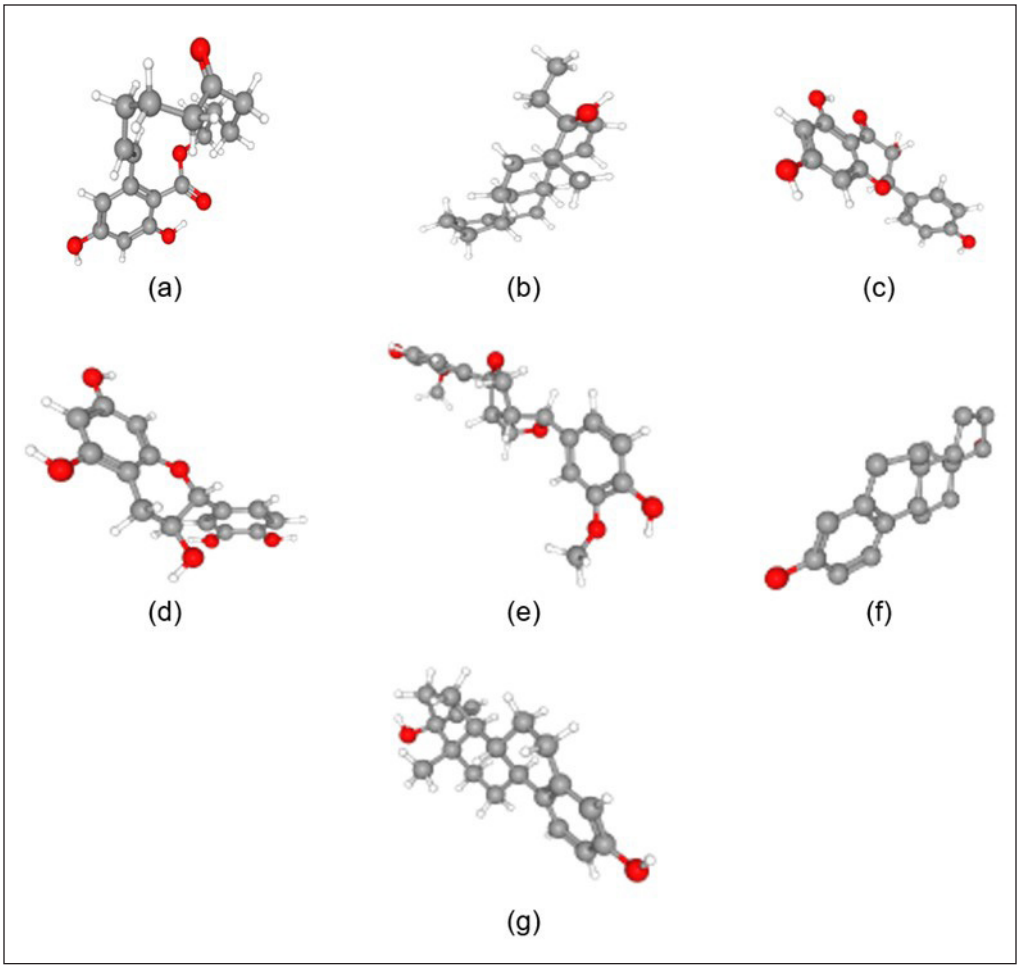

Figure 5. 3D molecular structures of (a) zearalenone, (b) ethylestrenol, (c) aromadendrin, (d) cianidanol, (e) pinoresinol, (f) 17β estradiol/control-1, and (g) ethynil estradiol/control-2.

with the amino acid residues Gly521, His524, Glu353, Met343, Met421, Arg394, and Ile424.

Figure 8 shows the interaction between aromadendrin and the receptor protein through the formation of hydrogen bonds with the amino acid residues Glu353 (2.36 Å) and Gly521 (2.49 Å). In addition, pi-alkyl bonds are also formed with amino acid residues Leu387 (5.34 Å), Leu346 (5.42 Å), Leu391 (5.13 Å), and Ile424 (5.41 A). Meanwhile, van der Waals interactions occur with the amino acid residues Trp383, Ala360, Leu384, Leu525, Leu349, His524, Arg394, and Met421.

The interaction of cianidanol with the estrogen receptor protein (Fig. 9) occurs through the formation of hydrogen bonds to the amino acid residues Gly521 and Glu353. The hydrogen bond distances formed are 2.43 and 3.00 Å. The pi-alkyl bond between cianidanol and the estrogen receptor protein occurs at amino acid residues Leu346 (5.37 Å), Leu391 (5.21 Å), and Leu387 (5.04 Å).

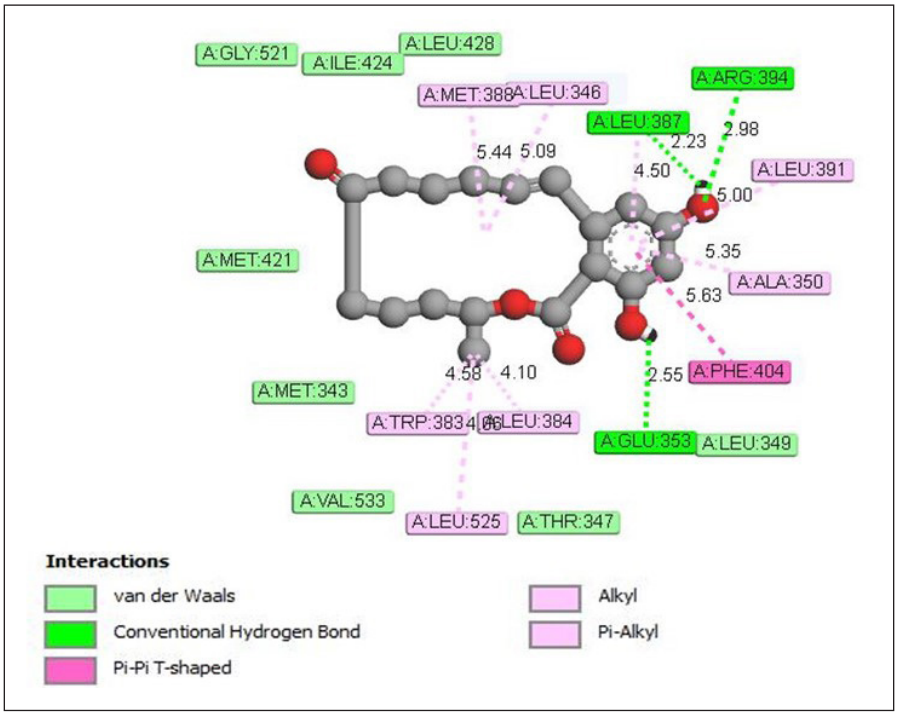

Figure 6. Interaction of zearalenone with estrogen receptor protein.

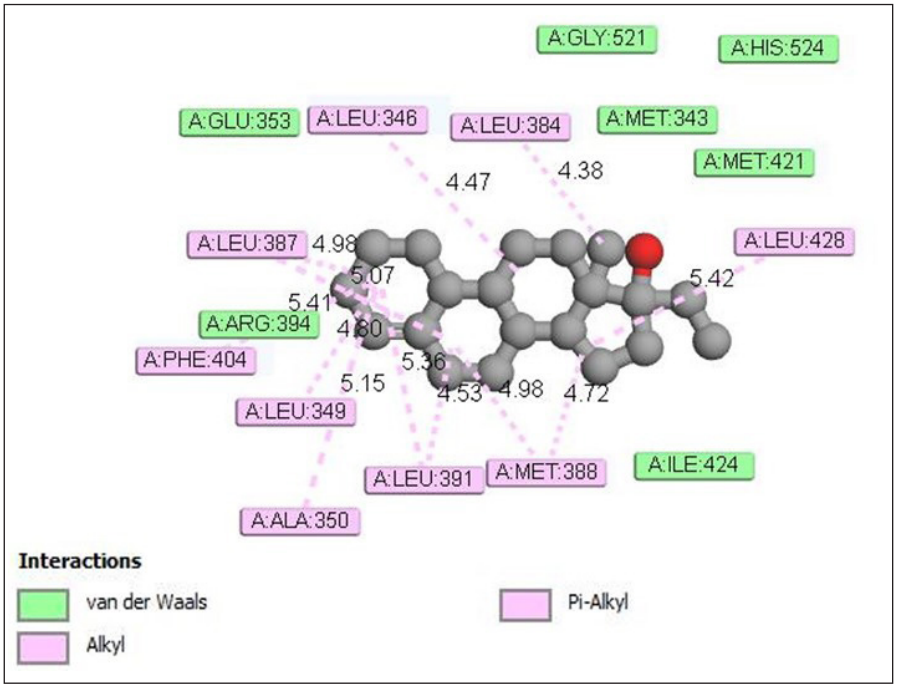

Figure 7. Interaction of ethylestrenol with estrogen receptor protein.

The interaction of pinoresinol with the estrogen receptor protein (Fig. 10) occurs through the formation of hydrogen bonds with the Arg394 amino acid residue with a bond distance of 2.80 Å. Apart from that, carbon–hydrogen bonds are also formed with amino acid residues Trp393 (3.39 Å), Pro324 (5.40 Å), and Pro325 (3.51 Å), as well as alkyl and pi-alkyl bonds to Phe445 (5.30 Å) and Pro324 (4.24 Å). Meanwhile, van der Waals interactions occur on the amino acid residues His356, Glu353, Glu323, Ile326, Met357, Leu387, Ile386, Lys449, and Gly390.

Based on the results of molecular docking studies, it is known that the compounds, zearalenone, ethylestrenol, aromadendrin, cianidanol, and pinoresinol, form alkyl bonds with several amino acid residues found in the

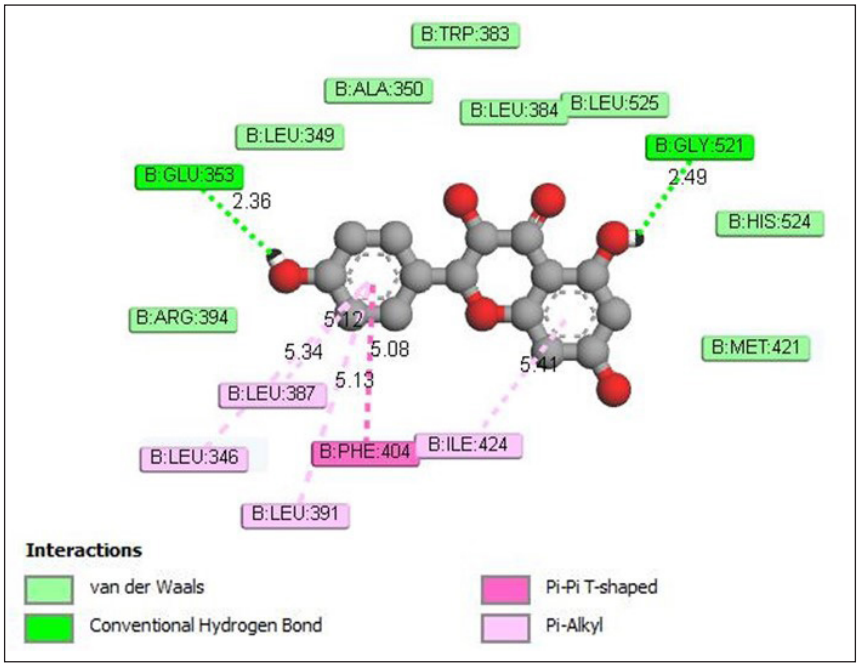

Figure 8. Interaction aromadendrin with estrogen receptor protein.

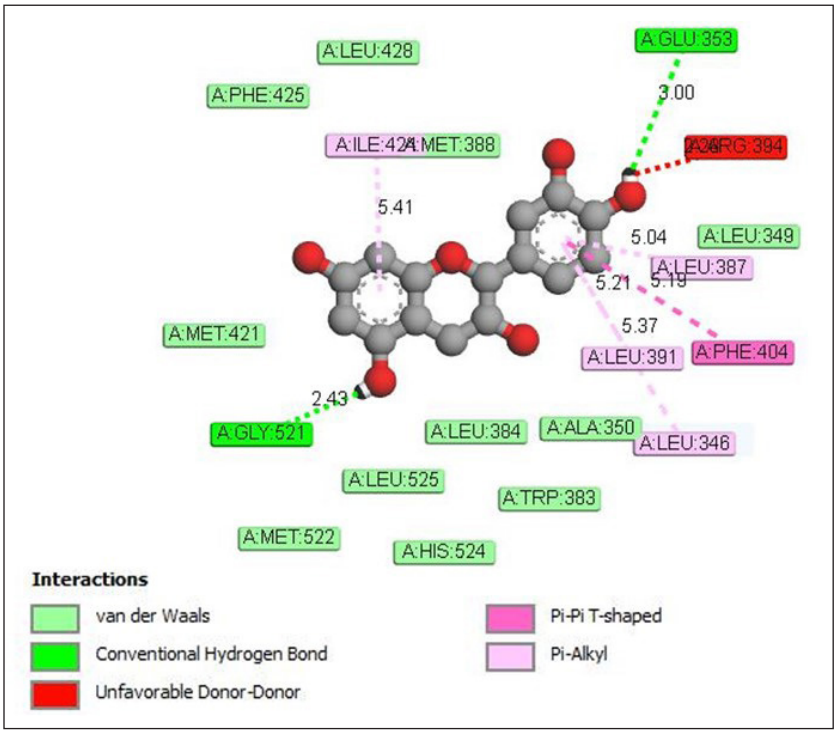

Figure 9. Interaction of estrogen receptor protein with the cianidanol compound.

estrogen receptor protein. The formation of hydrophobic or fat-soluble alkyl bonds plays a role in the drug–receptor interaction mechanism. Of the five test compounds, zearalenone produces a greater number of hydrogen bonds than the other four compounds, namely, three hydrogen bonds. The more bonds produced, the better the interactions that occur between a molecule and the target protein. Apart from that, hydrogen bonds are also known to be the dominant interaction in studying the activity of a medicinal compound [30,31]. The strength of hydrogen bonds varies greatly and generally occurs in groups of electron-rich heteroatoms and electron-deficient hydrogen atoms [32,33]. Most hydrogen bonds have a binding energy of 16 to 60 kJ/mol or about 10 times less than covalent bonds.

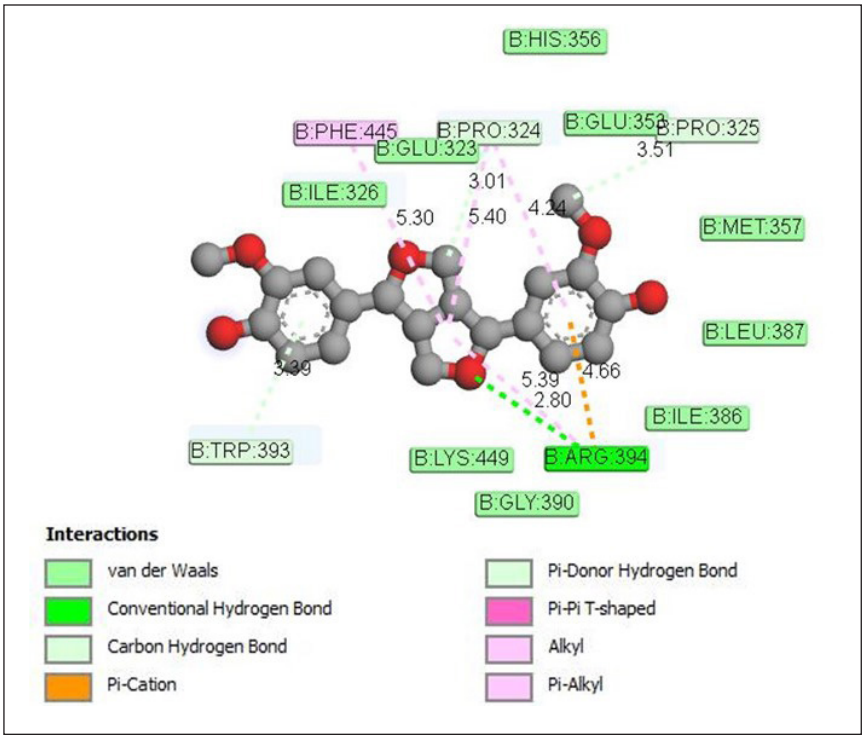

Figure 10. Interaction pinoresinol with estrogen receptor protein.

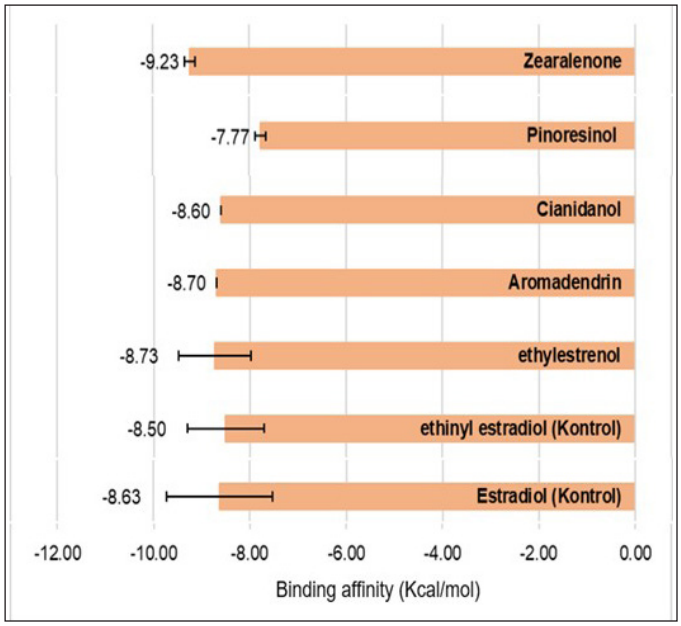

Figure 11. Binding affinity of estrogen receptor protein with test and control compounds.

The strong interaction between zearalenone and the estrogen receptor protein can also be seen from the large binding affinity produced (Fig. 11). The binding affinity value between zearalenone and the estrogen receptor protein is –9.23 kcal/mol. This value is higher than the control compounds (17β estradiol and ethynyl estradiol), which have binding affinity values of 8.63 and 8.83 kcal/mol, respectively. Thus, the interaction

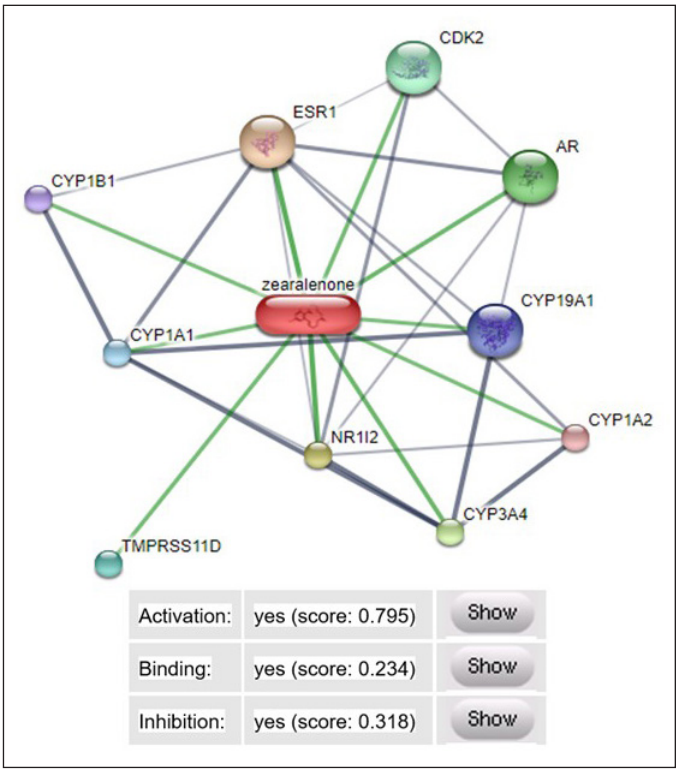

Figure 12. Mechanism of interaction of zearalenone with estrogen receptor protein. ESR1 = estrogen reseptor, CYP1A1 = cytochrome P450 family 1 subfamily A1, CYP1B1 = cytochrome P450 family 1 subfamily B1, TMPRSS11D = transmembrane protease serine 11D, NR112 = nuclear receptor subfamily 1, AR = androgen receptor, CDK2 = cyclin dependent kinase 2.

produced by zearalenone with the estrogen receptor protein is better than the control compound.

Pathway analysis

The results of the molecular docking analysis showed that the interaction produced by zearalenone with the estrogen receptor protein was better when compared to control compounds (ethinyl estradiol and 17β estradiol), as well as four other test compounds (pinoresinol, cianidanol, aromadendrin, and ethylestrenol). Therefore, the study continued with stitch analysis to predict the interaction mechanism between zearalenone and receptor proteins. The results of the stitch analysis are presented in Figure 12.

The prediction results for zearalenone interactions through stitch analysis obtained an activation score of 0.795, a binding strength of 0.234, and an inhibition of 0.318. The strongest interaction is shown by the thick and short green line, which indicates that zearalenone has estrogenic potential through the activation of the estrogen receptor (ESR1), nuclear receptor subfamily 1 (NR112), and androgen receptor (AR). Another interaction that occurs is through activation of the zearalenone compound with CDK2, CYP1A1, CYP1B1, TMPRSS11D, CYP3A4, CYP1A2, and CYP19A1.

CONCLUSION

Zearalenone, pinoresinol, aromadendrin, and ethylestrenol from the ethyl acetate and ethanol fractions of *R. mucronata* fruit flour are known to have potential estrogenic activity. The strongest interaction with the estrogen receptor protein was shown by zearalenone with a binding affinity of –9.23 Kcal/mol and the formation of hydrogen bonds to the amino acid residues Glu353, Leu387, and Arg394. The estrogenic potential of zearalenone is through activation of the estrogen receptor (ESR1), nuclear receptor subfamily 1 (NR112), and AR.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

AVAILABILITY OF DATA AND MATERIALS

The corresponding author can provide access to the data used to support the findings of this research upon request.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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