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Original Article

Network pharmacology and molecular docking of *Phaleria macrocarpa* (Scheff.) Boerl. bioactive compounds involved in endometriosis pathway

[Farmacología en red y acoplamiento molecular de compuestos bioactivos de *Phaleria macrocarpa* (Scheff.) Boerl. implicados en la vía de la endometriosis]

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Abstract

Context: Endometriosis is the presence of endometrial glands and stromal lesions outside the uterus.

Aims: To investigate the network pharmacology and molecular docking of *Phaleria macrocarpa* bioactive compounds on selected endometriosis pathways.

Methods: *P. macrocarpa* compounds were obtained from PubChem. The compounds were analyzed for their potential using the WAY2DRUG PASS prediction. Protein targets were predicted using the Comparative Toxicogenomic Database (CTD), STITCH, and Open Target databases. The pharmacology network analysis was conducted using STRING. Molecular docking analysis was performed between tolfenamic acid, naringenin, eriodictyol, 5-O-methylgenistein, and 8-prenylnaringenin against postaglandin-endoperoxide synthase 2/cyclooxygenase-2 (PTGS2/COX-2) and progesterone receptor (PGR) protein using AutoDock Vina integrated into PyRx v.0.9.8. The visualization was performed using BioVia Discovery Studio 2019 software.

Results: For the PTGS/COX-2 pathway, the compounds that have the highest affinity for interaction to occur are tolfenamic acid and naringenin. Against PGR, the phytochemicals with the highest interaction affinity are naringenin and 8-prenylnaringenin.

Conclusions: According to the molecular docking results, *P. macrocarpa* compounds are predicted to have activity as PTGS2/COX-2 inhibitors, especially (±)-naringenin and eriodictyol. Narigenin and 8-prenylnaringenin showed activity to PGR. Compared to 8-prenylnarigenin, naringenin binds more strongly to PGRs.

Keywords: angiogenesis; endometriosis; inflammation; pathomechanisms; proliferation.

Resumen

Contexto: La endometriosis se define como la presencia de glándulas endometriales y lesiones estromales fuera del útero.

Objetivos: Investigar la farmacología en red y la unión molecular de los compuestos bioactivos de *Phaleria macrocarpa* en vías específicas de la endometriosis.

Métodos: Los compuestos de *P. macrocarpa* se obtuvieron de PubChem. Se analizó el potencial de los compuestos mediante la predicción WAY2DRUG PASS. Las dianas proteicas se predijeron utilizando las bases de datos Comparative Toxicogenomic Database (CTD), STITCH y Open Target. El análisis de redes farmacológicas se realizó con STRING. Se realizó un análisis de acoplamiento molecular entre ácido tolfenámico, naringenina, eriodictiol, 5-O-metilgenisteína y 8-prenilnaringenina frente a la proteína postraglandina-endoperóxido sintasa 2/ciclooxigenasa-2 (PTGS2/COX-2) y el receptor de progesterona (PGR) utilizando AutoDock Vina integrado en PyRx v.0.9.8. La visualización se realizó con el software BioVia Discovery Studio 2019.

Resultados: En la vía PTGS/COX-2, los compuestos con mayor afinidad para la interacción son el ácido tolfenámico y la naringenina. Frente al PGR, los fitoquímicos con mayor afinidad de interacción son la naringenina y la 8-prenilnaringenina.

Conclusiones: Según los resultados del acoplamiento molecular, se predice que los compuestos de *P. macrocarpa* tienen actividad como inhibidores de PTGS2/COX-2, especialmente la (±)-naringenina y el eriodictiol. La narigenina y la 8-prenilnaringenina mostraron actividad sobre los PGR. En comparación con la 8-prenilnarigenina, la naringenina se une con mayor fuerza a los PGR.

Palabras Clave: angiogénesis; endometriosis; inflamación; patomecanismos; proliferación.

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Abbreviations: COX-2: Cyclooxygenase-2; CTD: Comparative Toxicogenomic Database; ERE: Estrogen responsive element; ESR: Estrogen receptor; MAPK: Mitogen-activated protein kinase; MMP: Matrix metalloproteinases; NF-κB: Nuclear factor *kappa*-B; NLRP3: NLR family pyrin domain containing 3; PGR: Progesterone receptor; PPI: Protein-protein interaction; PTGS2: Prostaglandin-endoperoxide synthase 2; RMSD: Root mean square deviation; TNF: Tumor necrosis factor; TP53: Tumor protein p53; VEGF: Vascular endothelial growth factor.

INTRODUCTION

Endometriosis is the presence of endometrial glands and stromal lesions outside the uterus. The lesions may be peritoneal, superficial implants, or cysts on the ovary, or deep-infiltrating disease. While there is no proven cause of endometriosis, a few theories exist about how endometriotic lesions form. Retrograde menstruation, which occurs throughout the menstrual cycle in women and non-human primates, is an outflow of the endometrial lining into the pelvic area via the patent fallopian tubes (Parasar et al., 2017). Endometriosis is a neuro-inflammatory illness that causes chronic pelvic pain and affects around 6%-10% of women of reproductive age. The diagnosis of endometriosis is often delayed because of infertility (Saunders and Horne, 2021). In fact, male infertility accounts for about 20-30% of all infertile couples (Agarwal et al., 2015). Endometriosis affects women's immunity, making it difficult to discharge endometrial cells and fragments during retrograde menstruation. This might help explain why some women with retrograde menstruation develop endometriosis (Macer and Taylor, 2012)

Endometriosis' molecular features include a hormone-dependent (estrogen reliance, progesterone resistance) and inflammatory syndrome with an epigenetic propensity. Newly discovered molecular ideas with broad implications are also pertinent to endometriosis pathogenesis (Klemmt and Starzinski-Powitz, 2018). Women with endometriosis have an immunological microenvironment characterized by inflammation, angiogenesis, and endocrine signals. The molecular profile demonstrates the variations in immune response and inflammatory gene expression between normal endometrium and endometriosis patients, as evidenced by the presence of leukocyte signal dysregulation and cytokine receptor interaction on endometriosis (Vania and Hutagalung, 2023).

Endometriosis treatment options include surgery and medication therapy. Surgical treatment can be conducted in conjunction with diagnostic surgery. It may consist of the destruction of endometriotic tissue (ablation), the division of adhesions (scar tissue), or the removal of endometriotic cysts. The preferred therapy for advanced endometriosis (AFS stage III or IV) is laparoscopic surgery to remove (excise) visible endometrial implants, split adhesions (scar tissue), or physically block neural connections (Yap et al., 2000). The therapy of endometriosis has numerous disadvantages, including easing discomfort and causing side effects, and treatment is carried out continually since endometriosis might reoccur (Saunders and Horne, 2021).

Phaleria macrocarpa (Scheff.) Boerl. (family *Thymelaeaceae*) is an edible and medicinal plant that is endemic to Indonesia. This plant includes tannins, terpenes, alkaloids, flavonoids, acids, benzophenones, lignans, phenolics, sugars, and xanthones (Ahmad et al., 2023; Hendra et al., 2011). Research on endometrial cells RL95-2 has shown that the bioactive portion of *P. macrocarpa* is both anti-angiogenic and pro-apoptotic (Tjandrawinata et al., 2015). Mahkota Dewa (*Phaleria macrocarpa*) contains phytochemicals in its fruit, seeds, leaves, and fruit peels, including alkaloids, tannins, terpenoids, and flavonoids (Altaf et al., 2013). *P. macrocarpa* contains flavonoids, which weaken proinflammatory cytokines (Mendes et al., 2019). Flavonoids from the *P. macrocarpa* fruit extract can also reduce the expression of Matrix metalloproteinases-7 (MMP7), MMP1, and MMP3 in a mouse model of endometriosis (Irwanto et al., 2023). The content of bioactive compounds in *P. macrocarpa* encourages the development of therapies using this herbal plant. This study aimed to investigate the docking molecular of *Phaleria macrocarpa* phytocompounds on selected endometriosis pathways.

MATERIAL AND METHODS

Selection of phytochemical compounds

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used for chemical searches of canonical or isomeric simple molecular-input line-entry system (SMILES). Previous research identified the phytochemical compounds (Maharani et al., 2021).

Activity prediction of bioactive compounds with structure analysis relationship (SAR) approach

For each compound was needed to find the SMILE structure obtained from the PubChem database. Then, the compound was analyzed for its potential using the WAY2DRUG PASS prediction (http://www.pharmaexpert.ru/passonline/predict.php) to determine its possible action for metabolic syndrome treatment. The probability to be active (Pa) value was a value that described the possibility of a compound being tested. Determination of this value was done by comparing the structure of the input compound with compounds that have been tested as

specific treatments. Suppose the Pa value was more than 0.7, it indicated that the compound was predicted to have high potential, for example, anti-inflammatory, due to the high similarity with compounds in the database that have been tested as a treatment. The Pa value gives a sense of the accuracy of the prediction function obtained; the higher the Pa value of a function, the better the accuracy (Filimonov et al., 2014).

Protein target prediction

Analysis of target proteins from the Comparative Toxicogenomics Database (http://ctdbase.org/voc.go?type=chem). Screening was done by appearing in at least three interactors from the database. Proteins/Genes whose value was less than three interactors will be eliminated. Target protein analysis was also performed using the STITCH database (http://stitch.embl.de/). Analysis on STITCH was carried out by entering compound names in the multiple names menu in STITCH. Additional settings were made by selecting the type of organism to *Homo sapiens*, and the minimum required interaction score value was changed to 0.700. The Open Target database (https://www.opentargets.org/) screened known drugs and endometriosis targets. Endometriosis targets used were those with an overall score of ≥ 0.5. Furthermore, all endometriosis targets and known drugs were processed and visualized with a Venn diagram.

Pharmacology network analysis

The compiled list of target proteins from both databases was then input into the multiple protein menu of the STRING database (https://string-db.org/). In addition, additional settings in the form of organism type selection were set to Homo sapiens, the meaning of network edges was set to confidence, and the minimum required interaction score was set to 0.700. After updating the network based on these additional settings, the visualization results were downloaded as TSV (tab-separated values) in the table/export menu. The downloaded (.tsv) file was then imported into the Cytoscape v.3.9.1 software to continue analyzing the network procedure on the tools menu. An analysis network is used to determine the degree of protein nodes, illustrating that the compound interacts more and more with other proteins (Table 1).

Molecular docking analysis

The 3D structures of the selected target proteins were obtained from the RSCB PDB database (https://www.rcsb.org/), namely PTGS2 (PDB ID: 5IKT) and PGR (PDB ID: 1A28). In contrast, the 3D structures of each active compound (±)-naringenin, eriodictyol, 5-O-methylgenistein, 8-prenylnaringenin, and control (tolfenamic acid) were obtained from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov). Furthermore, the protein was pretreated by removing water molecules in Discovery Studio 2019 software, while the ligand was energy minimized using PyRx v.0.9.8 software. Docking was performed using Autodock Vina integrated in PyRx v.0.9.8. Docking was performed with the targeted docking method with an exhaustive parameter of 50. Based on the literature, the grid box size was adjusted to the position of amino acid residues.

Molecular docking visualization

The results of the docking analysis were visualized with the BioVia Discovery Studio 2019 software. Protein-ligand interactions were investigated to determine the number and types of bonds formed, including hydrogen bonds, hydrophobic bonds, and van der Waals bonds.

RESULTS

Bioactive compounds selection

Data mining was conducted to obtain the list of *P. macrocarpa* compounds, including each compound's SMILES information (Table 2).

Prediction of bioactive compound activity as metabolic syndrome therapy

The probability to be active (Pa) value is a value that describes the potential of a compound being tested. Determination of this value is done by comparing the input compound structure with compounds that have been proven as specific treatments. Analysis of herbal bioactive potential is carried out using the Way2Drug Pass Server. Suppose the Pa value is more than 0.7 (green). In that case, the compound is predicted to have high potential as an immunomodulator due to its high similarity to compounds in the database that have been proven to be effective treatments. Meanwhile, the compound computationally has activity if the Pa value is more than 0.3 but less than 0.7. Still, the similarity is low with compounds proven as treatments. The prediction results of the analyzed *P. macrocarpa* compounds show that the bioactives contained have potential as an apoptosis agonist, TP53 expression enhancer, anti-inflammatory, proliferative disease treatment, free radical scavenger, antioxidant, and vasoprotective because they show Pa values of more than 0.5 cut off (Fig. 1).

Table 1. The grid coordinates for specific docking.

Protein target	Grid coordinate	
	Center	**Dimension**
PTGS2	X: 165.331	X: 10.031
	Y: 186.126	Y: 4.411
	Z: 192.885	Z: 6.509
PGR	X: 23.2463408977	X: 16.1657870164
	Y: 10,8405216281	Y: 16.5498423419
	Z: 59.3761620592	Z: 23.1454308387

Table 2. Data mining result of *P. macrocarpa.*

Chemical name	Classification	CID	Canonical	Isomeric
Glycitin	Flavonoid (isoflavone)	187808	COC1=C(C=C2C(=C1)C(=O)C(=CO2)C3=CC=C(C=C3)O)OC4C(C(C(C(O4)CO)O)O)O	COC1=C(C=C2C(=C1)C(=O)C(=CO2)C3=CC=C(C=C3)O)O[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O
Eriodictyol	Flavonoid (flavonone)	440735	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC(=C(C=C3)O)O	C1[C@H](OC2=CC(=CC(=C2C1=O)O)O)C3=CC(=C(C=C3)O)O
(±)-Naringenin	Flavonoid (flavonone)	932	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O	
5-O-Methylgenistein	Flavonoid (isoflavone)	5748551	COC1=CC(=CC2=C1C(=O)C(=CO2)C3=CC=C(C=C3)O)O	
(þ)-Catechin 7-O-beta-D-xyloside	Glycoside (beta-D-xyloside)	73533	C1C(C(OC2=CC(=CC(=C21)O)OC3C(C(C(CO3)O)O)O)C4=CC(=C(C=C4)O)O)O	C1[C@@H]([C@H](OC2=CC(=CC(=C21)O)O[C@H]3[C@@H]([C@H]([C@@H](CO3)O)O)O)C4=CC(=C(C=C4)O)O)O
8-Prenylnaringenin	Flavonoid (flavonone)	480764	CC(=CCC1=C2C(=C(C=C1O)O)C(=O)CC(O2)C3=CC=C(C=C3)O)C	CC(=CCC1=C2C(=C(C=C1O)O)C(=O)C[C@H](O2)C3=CC=C(C=C3)O)C

Protein prediction

The target protein prediction list was obtained from three databases, namely CTD, SEA Server, and STITCH. CTD and SEA Search Server analysis requires each selected compound to be predicted separately with a search key as a SMILES structure. Furthermore, TC or Tanimoto similarity (SEA) of >40% (>0.4) is determined as the basis for selecting the list of target proteins for each bioactive compound. Meanwhile, through the multiple names menu on STITCH, the list of names of bioactive compounds can

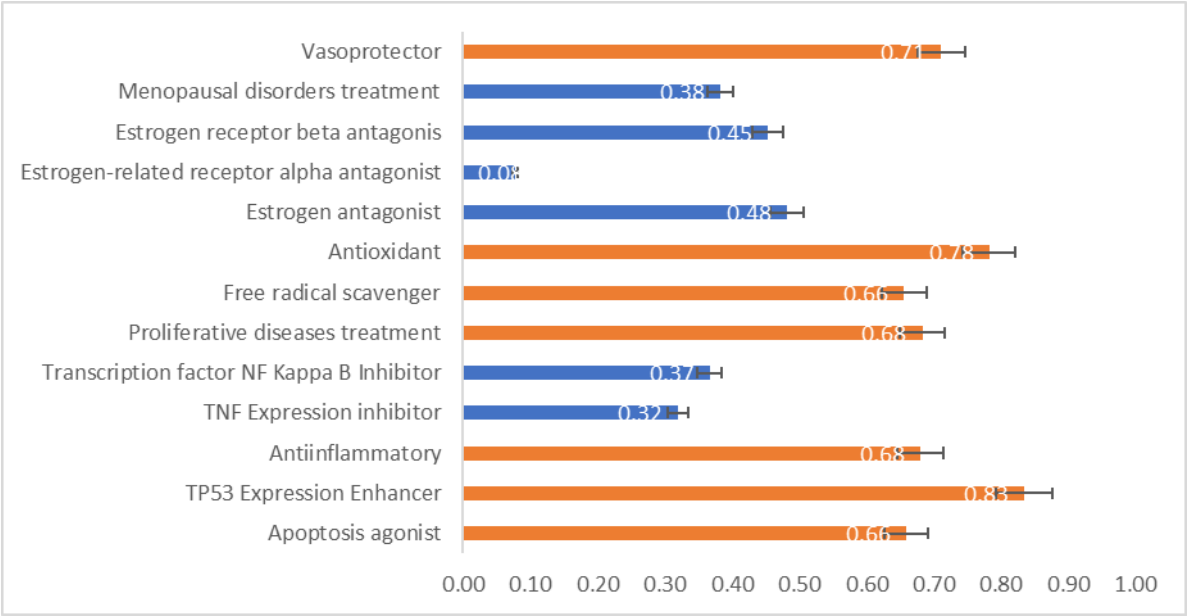


Figure 1. Activity prediction analysis results of *P. macrocarpa* compounds using PASS Online

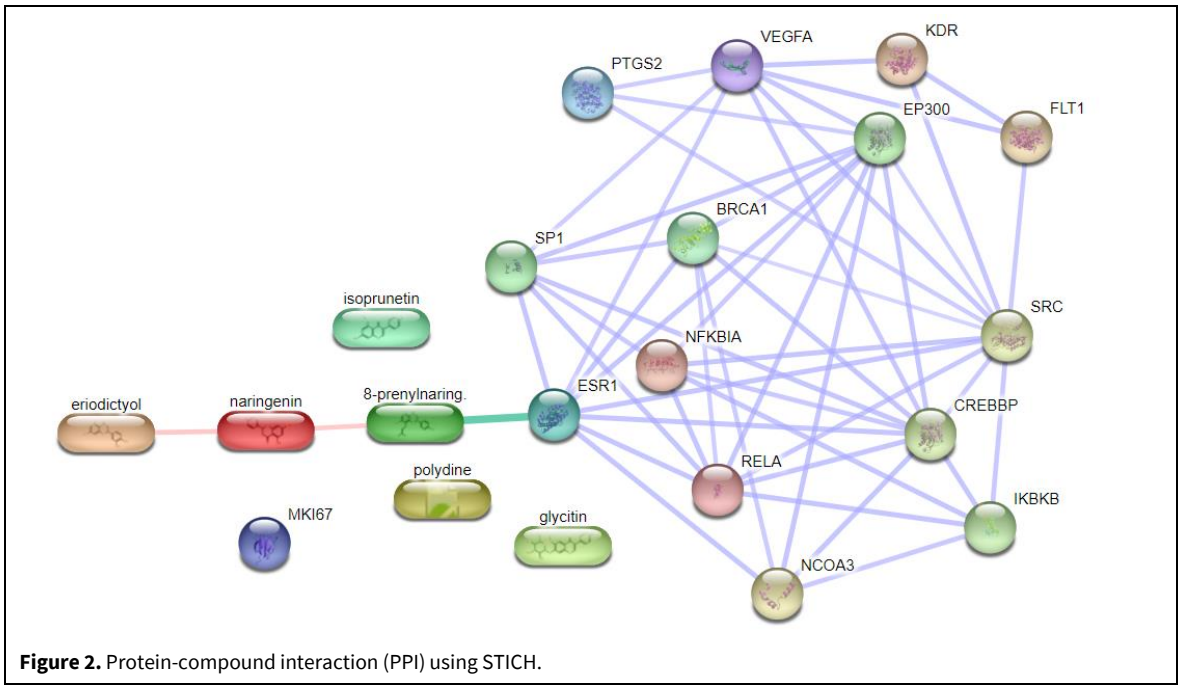


Figure 2. Protein-compound interaction (PPI) using STICH.

be inputted, as well as keywords with additional settings in the form of selecting the type of organism made into *Homo sapiens* and the minimum required interaction score value made 0.700.

The analysis results show that almost all compounds from the extraction results have the potential to interact with endometriosis treatment target proteins, except glycitin compounds. Furthermore, STITCH results align with target predictions using CTD and SEA Target. STITCH results show that of the six predicted compounds, 8-prenylnaringenin can bind/interact directly with target proteins, especially ESR1. Naringenin and eriodictyol are the potential compounds predicted to have good activity with target proteins involved in endometriosis pathways (Fig. 2).

There were 69 drug targets from endometriosis and six targets with an overall score > 0.5 (maximum score is 1) detected in the Open Target database (https://www.opentargets.org/) (Fig. 3). As for the protein/gene that changes due to the administration of eriodictyol, naringenin, and 8-prenylnaringenin, at

least three interactors in the CTD database (http://ctdbase.org/) are 38.

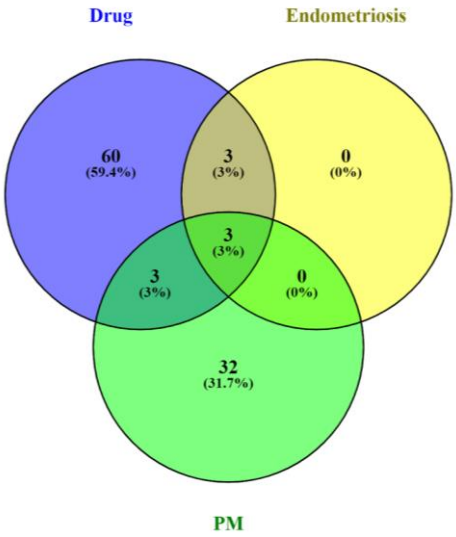


Figure 3. Venn diagram of proteins/genes that are relevant to endometriosis, known drug endometriosis, and *P. macrocarpa*

Fig. 4 shows the network analysis graph processed from CytoScape V.3.9.1 CytoScape input data obtained from STRING V.11.5 (https://string-db.org/), and PM relevance protein with a minimum interaction score of 0.7. ESR1 and PTGS2 are in the blue range (high degree), while ESR2 and PGR are in the green range (medium degree). ESR1 and PTGS2 interact with 14 other proteins. The darker and more significant the size and color of the node, the higher the degree (Fig. 4).

Molecular docking analysis

The compounds selected as ligands are the four most potent bioactive compounds, namely, (±)-naringenin, eriodictyol, 5-O-methylgenistein, and 8-prenylnaringenin, while glycitin and (þ)-catechin 7-O-beta-D-xyloside cannot interact directly with PTGS2 according to the results of STRING and Network Analysis. Meanwhile, the control ligand, tolfenamic acid, was obtained based on previous research as a PTGS2/COX-2 inhibitor (Orlando and Malkowski, 2016). The control RMSD results follow the standard of less than 2 Å (Nafisah et al., 2022). The results of molecular docking of the PTGS2 protein with bioactive compounds from *P. macrocarpa* showed that the three compounds had lower binding affinity values than the control. However, the binding affinity value of (±)-naringenin-PTGS2 complex and eriodictyol-PTGS2 complex was more significant than the threshold of -7 kcal/mol (Table 3).

Based on the type of bonds produced, van der Waals and hydrophobic bonds are the most dominant bonds (Table 4). Residues with bold font are amino acid residues from the control retained sample. Meanwhile, residues with red italic font indicate changes in the type of bond formed. The analysis showed that the 8-prenylnaringenin sample retained the most active site residues from the control, namely 14 residues. However, 8-prenylnaringenin does not form hydrogen bonds and instead forms unfavorable bonds, so that its binding affinity is lower than the control and actually the lowest of all samples. While (±)-naringenin, eriodictyol, and 5-O-methylgenistein formed 9, 8, and 9 amino acids, respectively, which were the same as the control.

The 3D visualization of the PTGS2 protein complex with the control ligand and the four most potent compounds is shown in Fig. 5. The surface hydrophobicity is shown based on the color of the protein surface, with brown color indicating hydrophobic, towards white color (intermediates) to hydrophilic blue color. The more hydrophobic the interaction, the more stable the conformation of the complex. The visualization results show that although the three sample ligands have more binding affinity than the control, the binding pocket formed for all three is more hydrophobic than that of the control. Whereas 8-prenylnaringenin can form bonds with more amino acid residues than the other three samples due to its larger molecular size, 8-prenylnaringenin can form bonds with more amino acid residues than the control.

The compounds selected as ligands are the two most potent bioactive compounds, namely, (±)-naringenin and 8-prenylnaringenin. Recorded in the CTD database, treatment with these bioactives is able to increase PGR. Meanwhile, the control agonist ligand is Progesterone, obtained from PDB ID 1A28. The control RMSD results follow the standard of less than 2 Å (Nafisah et al., 2022). The results of molecular docking of the PGR protein with bioactive compounds from *P. macrocarpa* show that it has a binding affinity value close to the control. Still, the interaction is not as strong as the control (Table 5).

Just like the PGR results, van der Waals and hydrophobic bonds are the most dominant based on the type of bond produced (Table 6). Residues with bold fonts are the identical amino acid residues between the control and bioactive *P. macrocarpa*. Meanwhile, residues with red italic font indicate changes in the type of bond formed. The analysis shows that the naringenin sample gives results close to the control's. Ten residues are the same as the control, and of the 10 residues, 6 have different interactions. While 8-prenylnaringenin has one unfavorable interaction and 17 residues that are the same as PGR, where 7 out of 10 residues have interaction changes.

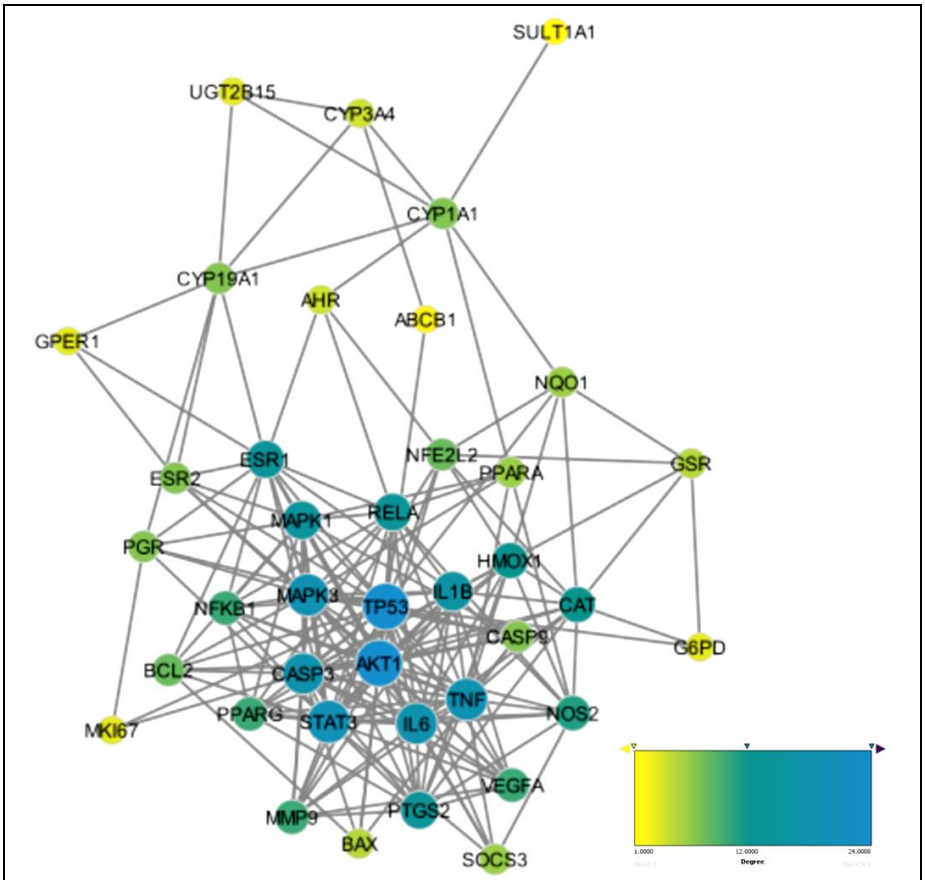


Figure 4. Network analysis targets *P. macrocarpa* (8-prenylnaringenin, naringenin, and eriodictyol).

Table 3. Binding affinity between PTGS2/COX-2 protein and ligands from bioactive compounds *P. macrocarpa.*

Compound	Binding affinity (kcal/mol)	RMSD (Å)
Tolfenamic acid	-8.6	0.2399
(±)-Naringenin	-7.2	
Eriodictyol	-7.2	
5-O-methylgenistein	-4.8	
8-Prenylnaringenin	-3.4	

The results of 3D visualization of PGR protein complexes with control ligands and two *P. macrocarpa* compounds show surface hydrophobicity based on the color of the protein surface, with brown color indicating hydrophobicity towards white color (intermediate) to hydrophilic blue color (Fig. 6). The more hydrophobic the discovered interaction, the more stable the complex conformation. The hydrophobic map shows that the docking results between PGR-bioactive *P. macrocarpa* and the control have a fairly stable interaction. However, this needs to be further proven by molecular simulation.

DISCUSSION

Endometriosis is a benign gynecological disorder characterized by endometrial tissue outside the uterus. However, it can be aggressive. Ectopic endometrium exhibits proliferation, implantation, angiogenesis, and the ability to survive in various conditions. Endometriosis pathogenesis is complex and multifactorial in etiology (Jouhari et al., 2018). Flavonoids from *Phaleria macrocarpa* fruit extract reduced proinflammatory factors MMP-1, MMP-3, and MMP-7 in mice with endometriosis (Irwanto et al., 2023). *Phaleria macrocarpa* inhibits the expression of NF-κB, MMP-2, and MMP-9 in endometriosis mouse models. *P. macrocarpa* can effectively reduce inflammation in endometriosis (Febriani et al., 2022). *In silico* studies revealed that

Table 4. Interaction of amino acid residues generated in PTGS2/COX-2 protein with bioactive compounds *P. macrocarpa.*

Ligand	Interaction					
	Van der Waals	**Conventional hydrogen**	**Carbon hydrogen**	**Hydrophobic**	**Other**	**Unfavorable**
Tolfenamic acid	**A:SER353, A:LEU384, *A:MET522, A:GLY526* A:TRP387, A:PHE381, A:TYR348, A:SER530**	*A:TYR385*		**A:VAL349, A:LEU531,** *A:VAL116, A:LEU359,* ***A:TYR355,*** **A:LEU352, A:ALA527,** ***A:VAL523***		
(±)-Naringenin	A:LEU534, A:MET113, A:LEU117, A:LEU359, *A:VAL116,* A:LEU93, *A:TYR355,* A:ARG120, *A:TYR385,* **A:PHE381, A:TRP387, A:LEU384, A:MET522,** *A:VAL523,* **A:SER530**			**A:LEU352, A:ALA527,** *A:GLY526,* **A:VAL349, A:LEU531**		
Eriodictyol	A:TRP387, **A:PHE381,** A:TYR385, A:LEU534, **A:SER530,** A:MET113, A:LEU359, *A:VAL116,* A:ARG120, *A:TYR355, A:VAL523,* A:PHE518, **A:MET522, A:LEU384**			**A:LEU352, A:ALA527,** *A:GLY526,* **A:VAL349, A:LEU531**		
5-O-methylgenistein	**A:TRP387,** A:PHE381, A:SER530, A:ILE345, A:MET113, *A:LEU359, A:VAL116,* A:ARG120, **A:SER353,** A:PHE518, **A:LEU384**		*A:TYR355*	**A:VAL349,** *A:GLY526,* **A:ALA527, A:LEU352, A:LEU531, A:VAL523, A:TYR355**	*A:MET522*	
8-Prenylnaringenin	**A:TRP387, A:MET522, A:GLY526,**A:PHE518, **A:SER353,** A:HIS90, A:ARG120, A:LEU93, *A:VAL116, A:LEU359,* A:LEU117, A:MET113, A:ILE345, A:LEU534, **A:TYR348, A:SER530,** A:TYR385, **A:PHE381, A:LEU384**			**A:VAL523, A:LEU352, A:VAL349, A:ALA527, A:LEU531,A:TYR355**		*A:LEU352*

genistein inhibits VEGF-A pathway in endometriosis at its active locations. Furthermore, genistein inhibited ER-α, competing with oestradiol as the native ligand (Sutrisno and Maharani, 2024).

P. macrocarpa exhibits a variety of biological functions, and substantial toxicity and anti-proliferative investigations have been conducted to investigate its potential as an alternative medicinal agent. Toxicity tests have shown that the leaves and fruits of *P. macrocarpa* are safe to consume based on harmful dose levels, but more research is needed to determine the toxicity of other plant parts (Ahmad et al., 2023). *P. macrocarpa* has the potential to treat endometriosis by addressing numerous molecular targets. This plant's extracts may have anti-inflammatory, anti-angiogenic, and anti-proliferative properties. The extract activities may have influenced the composition of its phytocompounds. The bioactive fractions, dubbed DLBS1442, have entered a clinical trial, with the latest results indicating hope (Maharani, 2023).

The flavonoid eriodictyol (ER) [(S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one] belongs to the flavanones subclass and is commonly found in citrus fruits, vegetables, and most medicinal plants. Flavonoids are polyphenols commonly found in the human diet, particularly in natural herbs. Phytomedicine is an important component of the traditional Chinese medicine system that works by scavenging free radicals and has anti-inflammatory properties. Eriodictyol's activity is thought to be mediated by various cellular signaling cascades. Eriodictyol is an effective natural drug source to maintain higher health standards because of its excellent therapeutic roles in neuroprotection, car-

dioprotective activity, hepatoprotective activity, anti-diabetes and obesity, and skin protection, as well as having highly analgesic, antioxidant, and anti-inflammatory effects, antipyretic and antinociceptive actions, antitumor activity (Deng et al., 2020).

Based on the CTD database, eriodictyol has a role in several inflammatory targets such as IL-6, NF-κBIA, PTGS2, RELA, NLRP3, and TNF. Eriodictyol treatment can inhibit the upregulation of IL-6 and NF-κBIA protein and mRNA expression due to the presence of LPS (Lee, 2011). In addition, eriodictyol is also able to suppress inflammation by inhibiting NLRP3 (NLR family pyrin domain containing 3)/COX-2/NF-κB inflammasome signaling in lung tissue in ALI (LPS-induced acute lung injury) mice (Wang et al., 2020). Eriodictyol is also able to inhibit and affect the reaction of tetradecanoylphorbol acetate, which acts to increase the phosphorylation of RELA protein and VEGFA mRNA (Suttana et al., 2010).

Naringenin, a plant-derived flavonoid, exhibits antioxidant, anti-proliferative, anti-inflammatory, and anti-angiogenic activities in chronic and metabolic disorders. It frequently works as a pro-oxidant in malignancies and inhibits cell proliferation by causing ROS-mediated cell death. Naringenin has been shown to inhibit the migration and invasion of cancer cells. A recent study demonstrates the antiproliferative effects of naringenin on endometriotic cells, mediated mainly by the MAPK and AKT cell signaling pathways (Kapoor et al., 2019).

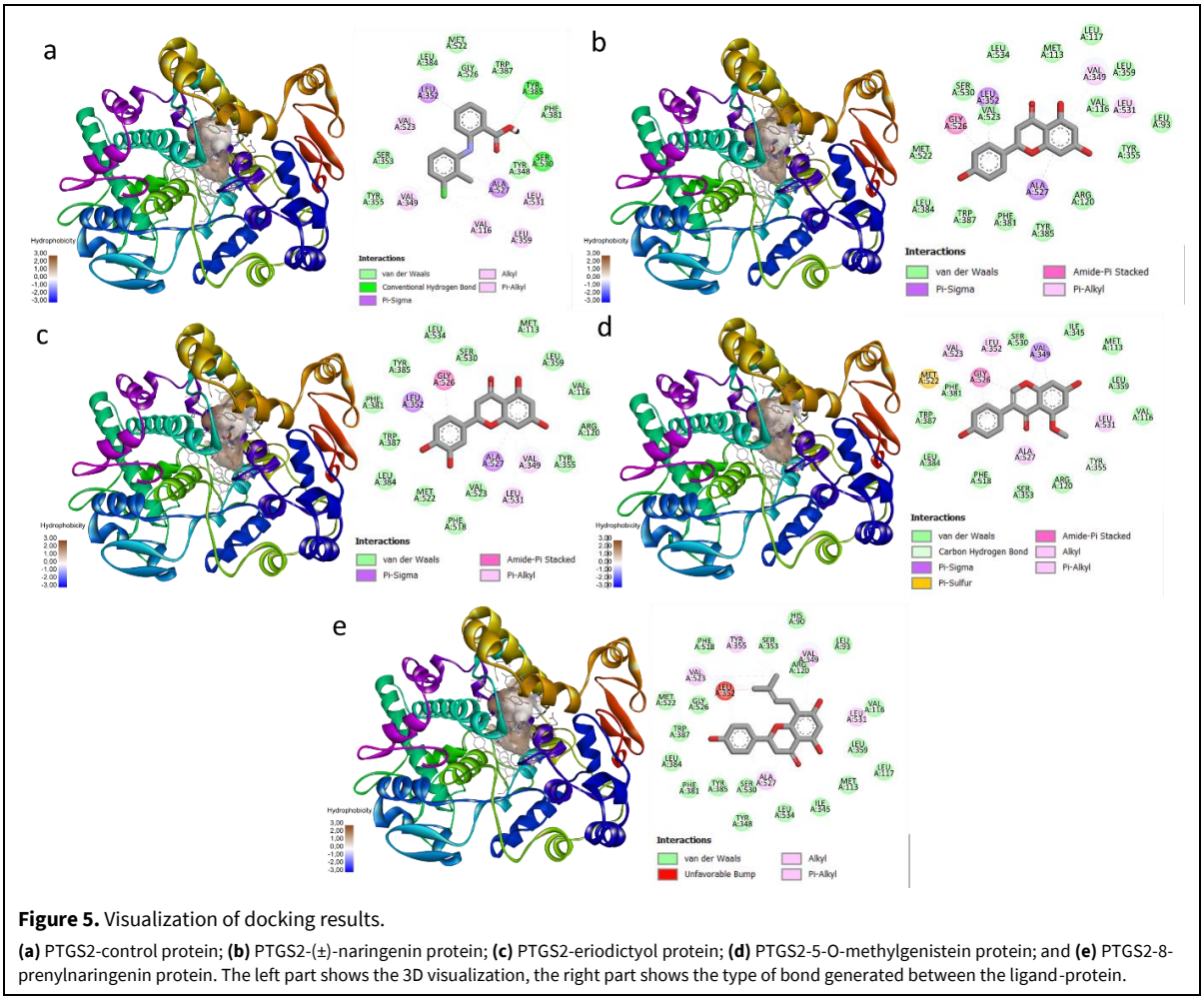


Figure 5. Visualization of docking results.

(a) PTGS2-control protein; **(b)** PTGS2-(±)-naringenin protein; **(c)** PTGS2-eriodictyol protein; **(d)** PTGS2-5-O-methylgenistein protein; and **(e)** PTGS2-8-prenylnaringenin protein. The left part shows the 3D visualization, the right part shows the type of bond generated between the ligand-protein.

Table 5. Binding affinity between PGR proteins and ligands from *P. macrocarpa* bioactive compounds.

Compound	Binding affinity (kcal/mol)	RMSD (Å)
Progesterone (control)	- 11.2	0.60
(±)-Naringenin	-9.2	
8-Prenylnaringenin	-8.3	

Table 6. Interaction of amino acid residues generated in PGR proteins with *P. macrocarpa* bioactive compounds.

Ligand	Interaction					
	Van der Waals	**Conventional hydrogen**	**Carbon hydrogen**	**Hydrophobic**	**Other**	**Unfavorable**
Progesterone (control)	A:THR:894 A:PHE:905 A:LEU:715 **A:VAL:903** **A:ASN:719** A:MET:909 A:TRP:755 A:GLY:722 A:LEU:721	***A:GLN725*** A:ARG766	***CYS891***	**A:LEU718** ***A:LEU797*** **A:MET756** ***A:MET759*** ***A:PHE794*** A:TYR890		
Naringenin	**A:VAL:903** **A:ASN:719** **A:LEU:721** ***A:GLN:725*** ***A:ARG:766*** AlVAL:760 ***A:MET:756*** A:LEU:887 ***A:LEU:797*** ***A:CYS:891***	***A:MET759*** A:LEU715		A:PHE778 **A:LEU718** A:LEU763		
8-Naringenin	A:VAL;760 ***A:ARG:766*** **A:LEU:721** **A:GLY:722** ***A:LEU:718*** A:MET:801 ***A:PHE:794*** ***A:LEU:797*** **A:LEU:715** ***A:TYR:890*** **A:PHE:905** **A:THR:894**	***A:ASN719***		A:PHE778 **A:MET756** **A:MET759** ***A:MET909*** ***A:CYS891*** A:LEU763 ***A:TRP755***		***GLN725***

Naringenin in the CTD database is recorded as having 31 interactors with ESR1, some of these studies state that naringenin has a role in increasing ESR1 expression. However, in other studies such as stating that naringenin can reduce ESR1 mRNA expression (La Rosa et al., 2014). ESR1 mRNA and alternative form protein (Xu et al., 2018). Naringenin also plays a role in reducing TNF protein activity (Chen et al., 2012). Naringenin can inhibit the reaction of LPS in increasing the expression of PTGS2 mRNA (Hämäläinen et al., 2011). Naringenin can inhibit the reaction of LPS in localizing and increasing the activity of RELA protein (Hämäläinen et al., 2007).

8-Prenylnaringenin, a flavonoid, is the most potent phytoestrogen (plant-derived estrogen mimic) for therapeutic purposes (Wilhelm and Wessjohann, 2006). 8-PN is one of the most powerful phytoestrogens currently known. *In vitro* assays for oestrogenic activity have confirmed this, including yeast-based screens expressing the human estrogen receptor (ER) and a reporter gene (usually luciferase, β-galactosidase, or chloramphenicol acetyltransferase) under the control of estrogen-responsive sequences (ERE), human cell lines responsive to oestrogenic stimulation, and ER binding (Pohjanvirta and Nasri, 2022). 8-Prenylnaringenin is recorded in the CTD database as having 14 interactors with ESR1, and most of these studies provide data that show that 8-prenylnaringenin can increase ESR1. However, some studies prove that 8-prenylnaringenin can reduce ESR1 protein expression (Sim et al., 2022). *P. macrocarpa* showed some flavonoids, including naringenin, catechin 7-O-beta-D-xyloside, eriodictyol, 8-prenylnaringenin, and 5-omethylgenistein, which inhibited MMP1 in the catalytic site, while glycitin interfered with MMP1 activity in the allosteric site (Maharani and Sutrisno, 2022).

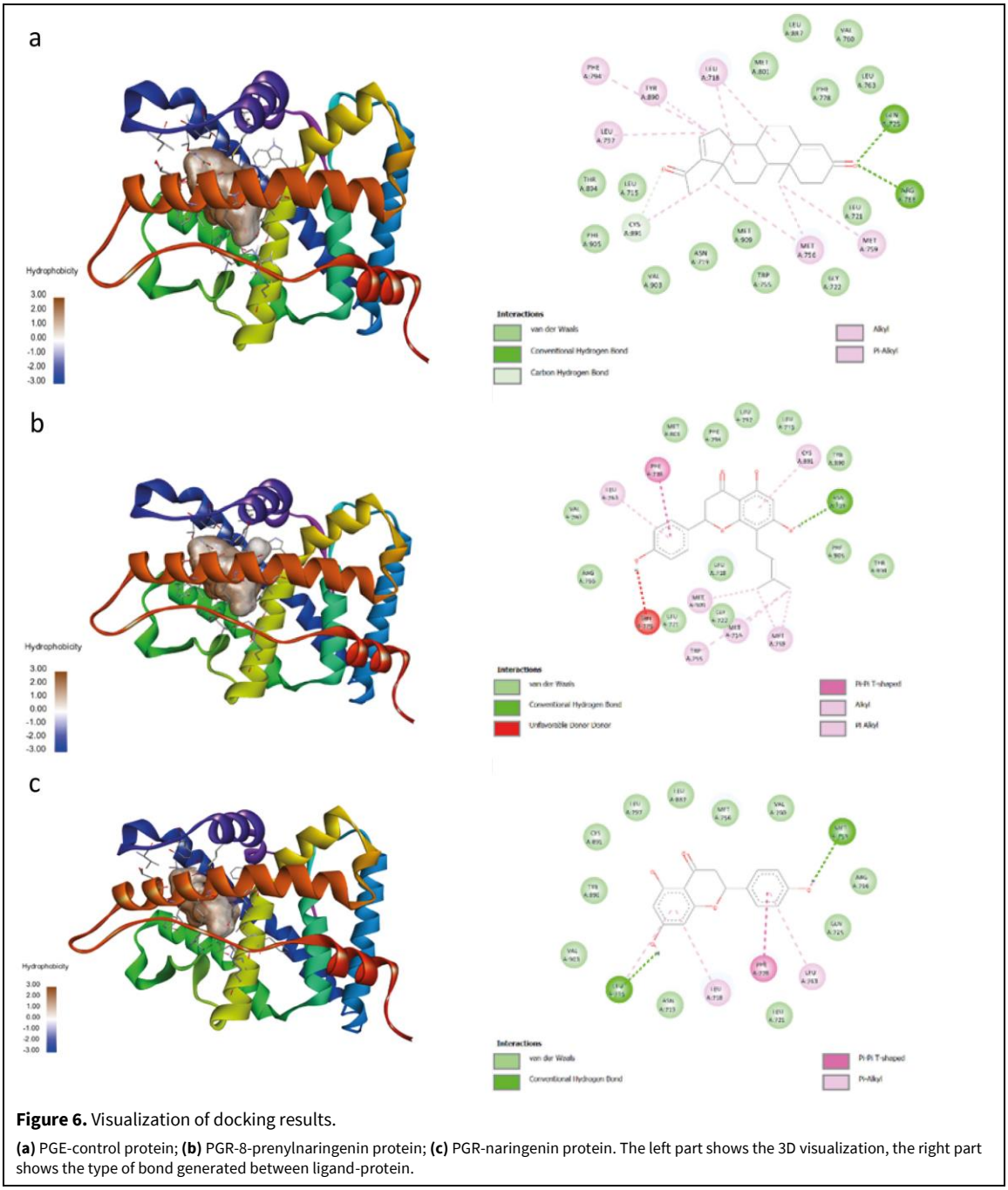


Figure 6. Visualization of docking results.

(a) PGE-control protein; **(b)** PGR-8-prenylnaringenin protein; **(c)** PGR-naringenin protein. The left part shows the 3D visualization, the right part shows the type of bond generated between ligand-protein.

CONCLUSION

P. macrocarpa compounds are predicted to have activity as PTGS2/COX-2 inhibitors, especially (±)-naringenin and eriodictyol. Naringenin and 8-prenylnaringenin showed activity to PGR. Compared to 8-prenylnaringenin, naringenin binds more strongly to PGRs.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sutrisno S	Maharani M
Concepts or ideas	x	x
Design	x	x
Definition of intellectual content	x	x
Literature search	x	
Experimental studies		
Data acquisition	x	x
Data analysis	x	x
Statistical analysis	x	x
Manuscript preparation	x	x
Manuscript editing	x	x
Manuscript review	x	x

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