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Phaleria macrocarpa Flavonoid as a Potent MMP-1 Inhibitor for Endometriosis Therapy: In silico Study

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ABSTRACT

Introduction: Endometriosis is a gynaecological disorder in women and causes infertility. Several therapeutic were developed to reduce endometriosis cases, one of them was matrix metalloproteinase (MMP) inhibitor. This study investigated the potential activity of the flavonoids in *Phaleria macrocarpa* fruit as MMP1 inhibitors for endometriosis.

Methods: In silico modelling was used in this study. Six flavonoid structures were retrieved from PubChem NCBI database. A targeted protein, MMP1 was taken out from Protein Data Bank with ID 1CGE and predicted the active sites. Six flavonoids and MMP1 was interacted by molecular docking using Molegro virtual Docker version 5 and analyzed using PyMol 2.2 and Discovery Studio version 21.1.1. Three-dimensional complex structure of flavonoids – MMP1 showed interaction in the same active sites and performed an amino acid residue Glu219 as catalytic site.

Results: Six flavonoids of *Phaleria macrocarpa* were divided into two patterns and generated varied binding energy. Glycitin and Catechin 7-O-beta-D-xyloside showed low score of binding energy and depicted similar structure with four aromatic rings. 8-Prenylnaringenin performed lower binding energy than naringenin, eriodictyol, and 5-O-methylgenistein.

Conclusion: *In silico* analysis suggested that six flavonoids compounds is potent as MMP1 inhibitor and might be interfered endometriosis pathophysiology. *In vivo* and *in vivo* investigations are required for further analysis.

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INTRODUCTION

Endometriosis defines as endometrium at the outside of the uterine cavity. Endometriosis cases occur in around 5-20% of reproductive women [1–6]. Some therapeutic drugs, herbs, and treatments for endometriosis were reported in recent studies. Those treatments include surgery, laparoscopy, ablation, draining, and drug therapy [3,7,8]. Drug therapy was modeled due to the endometriosis mechanism and targeted protein. A matrix metalloproteinase is an essentials enzyme that regulates the pathophysiology of endometriosis. Matrix metalloproteinase has several types, involving collagenases, gelatinases, stromelysins, and matrilysins. Matrix metalloproteinase also played roles in tissue remodeling, angiogenesis, inflammation, cell proliferation and movement, embryogenesis, and ovulation. MMP activities were regulated by several factors: cytokines, hormones, and growth factors [9–11]. MMP levels increased in cell cancer and were used as a therapeutic target biomarker. Some MMPs was reported that overexpressed in ectopic tissue, peritoneal fluid, and sera of endometrium patients. In ectopic endometrium, MMP1, MMP13, MMP2, MMP9, MMP3, MMP10, MMP7, MMP26, MMP12, and MMP23 were elevated. While, several MMPs in the peritoneal fluid was decreased, involving MT1-MMP, MMP2, and MMP13. MMPs inhibitor is an alternative therapeutic drug for endometriosis [9,10].

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The [N-(2-((2-methoxyphenyl)amino)-4'-methyl-[4,5'-bithiazol]-2'-yl)acetamide] compound was reported inhibiting proMMP-9 and interfering HT1080 cells migration. A synthetic compound, [3,4-dichloro-N-(1-methylbutyl)benzamide] inhibited MT1-MMP activity by binding the HPX domain in MCF7-β3/MT tumor xenograft. Hydroxylamine, hydroxamic acid, and carboxylic acid formed a complex with Zn2+ to reduce non-specific inhibition of MMPs [11–13]. *Phaleria macrocarpa* fruit is a traditional herb found in some Asia regions, especially Indonesia. *Phaleria macrocarpa* was traditionally used for reducing cholesterol, atherosclerosis, and diabetes mellitus [14–17]. Our previous study revealed that *Phaleria macrocarpa* contained six flavonoids, including eriodictyol, glycitin, 5-O-methylgenistein, catechin 7-O-beta-D-xyloside, 8-prenylnaringenin, and naringenin and inhibited peritoneal damage in the endometriosis mice model [18]. A flavonoids compound has been reported high activity to reduce cancer proliferation.

However, flavonoids from *Phaleria macrocarpa* compounds as MMP1 inhibitors was limited information. Therefore, this study identified six flavonoid compounds identified in the *Phaleria macrocarpa* fruit as endometriosis therapy.

MATERIAL AND METHODS

Structure Retrieval

Six flavonoid compounds of *Phaleria macrocarpa* fruit were retrieved from their three-dimensional structures in PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). Those compounds were eriodictyol (CID 440735), glycitin (CID 187808), 5-O-methylgenistein (CID 5748551), catechin 7-O-beta-D-xyloside (CID 73533), 8-prenylnaringenin (CID 480764), and naringenin (CID 932) [18]. Matrix metalloproteinase–1 (MMP1) as the targeted protein was taken out of the structure from Protein Data Bank with ID 1CGE [19].

Docking Simulation

Matrix metalloproteinase–1 (MMP1) was identified the active sites using Molegro Virtual Docker version 5.0 with van der Waals as a parameter [20–23]. Six flavonoids were interacted with MMP1 in the specific grid, which was X -2.92; Y 51.58; Z 52.09; radius 10. Score based on MolDock Score [Grid], Grid resolution 0.30A; Algorithm MolDock SE; Number of runs 10, Max iteration 1500, max population size 50; max step 300; neighbor distance factor 1.00; max number model 5, energy threshold 0.00; RMSD threshold 1.00.

Data Analysis

Docking models were superimposed between interacted ligand with prepared MMP1 using PyMol 2.2. data was analyzed using Discovery Studio version 21.1.1. binding energy was generated from the summary of MolDock Score, MolDock Grid score, and Rerank score.

Ethics

This study used bioinformatic approach, which were not require any ethic clearance or informed consent.

RESULTS

According to the three – dimensional complex structure, six flavonoids bound to MMP1 in the same active sites (**Fig. 1**). Same active sites of six flavonoids in MMP1 protein were LEU181. GLU219 and VAL215 were identified on five complex, involved Naringenin - MMP1, Catechin 7-O-beta-D-xyloside - MMP1, Eriodictyol - MMP1, 8-Prenylnaringenin - MMP1, 5-O-methylgenistein - MMP1. Ligand–protein complexes performed varied binding energy and interactions, with the lowest binding energy being Catechin 7-O-beta-D-xyloside - MMP1 complex (**Table 1**). Catechin 7-O-beta-D-xyloside - MMP1 produced -326,4 kJ/mol with nine

Table 1. Binding Interaction Between Flavonoids Compound in *Phaleria macrocarpa* with MMP1 Protein

Complexes	Binding Energy (kJ/mol)	Hydrogen Bond	Hydrophobic	Electrostatic
Naringenin - MMP1	-264,4	ASN180, LEU181, ALA182, GLU219, HIS222, HIS228	VAL215	GLU219
Catechin 7-O-beta-D-xyloside - MMP1	-326,4	ASN180, ALA184, ARG214, ARG214, GLU219, HIS222, HIS228, TYR237, PRO238	LEU181, VAL215	GLU219
Glycitin - MMP1	-319,4	TYR210, ALA184, SER239, PRO238	HIS222, LEU181	
Eriodictyol - MMP1	-285	LEU181, ARG214, ALA182, HIS222, HIS228, TYR237, ASN180, GLU219	VAL215	GLU219
8-Prenylnaringenin - MMP1	-290,8	ASN180, ALA182, TYR240, GLY179, LEU181, TYR240	VAL215, HIS218, LEU181	
5-O-methylgenistein - MMP1	-240	LEU181, GLU219	HIS218, HIS222, HIS228, LEU181, VAL215	GLU219

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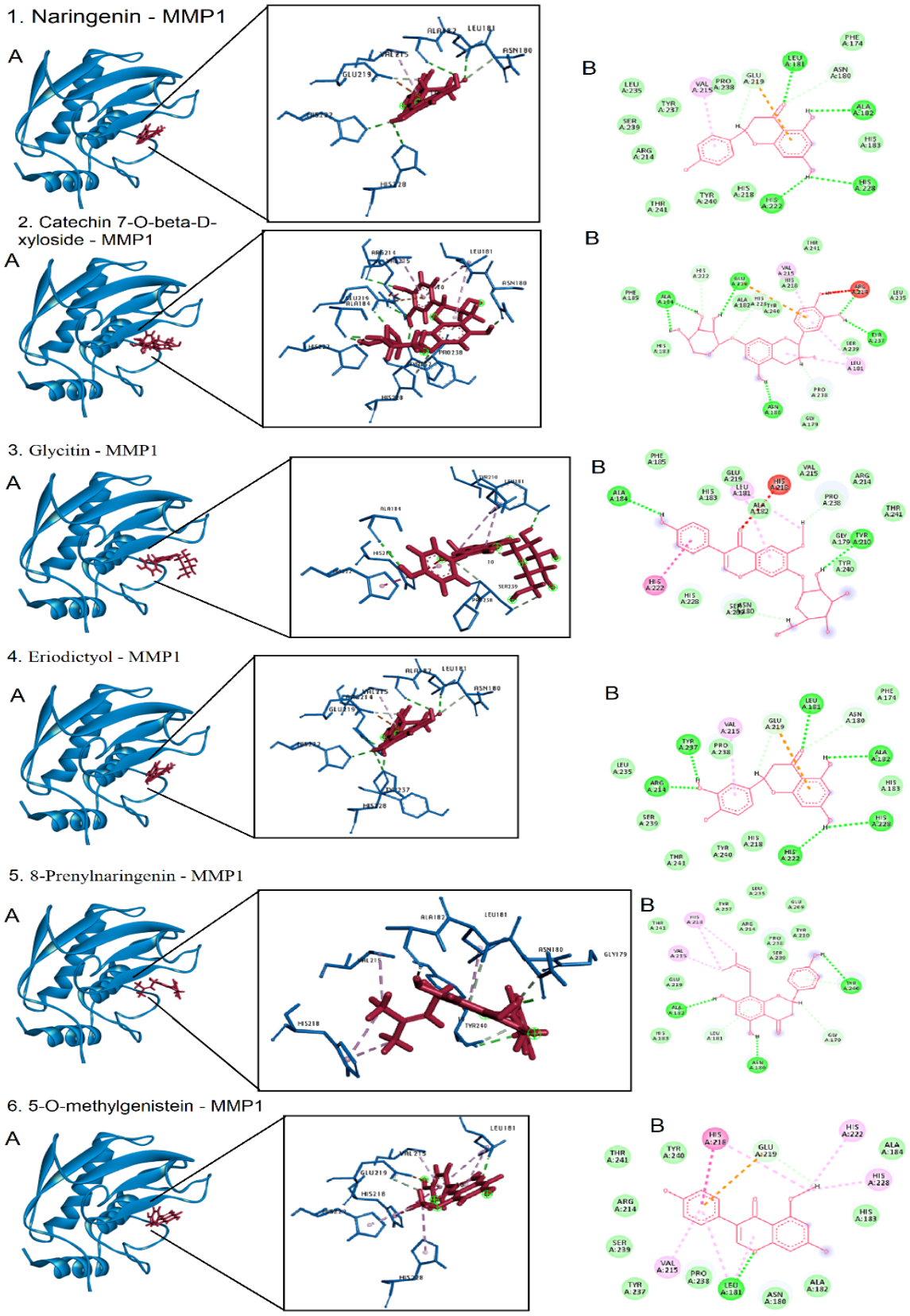

Fig. 1. Binding poses of flavonoids compound in *Phaleria macrocarpa* with MMP1 protein: A. Three-dimensional view; B. Two-dimensional view

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electrostatic. The binding energy ranged from -326.4 kJ/mol – 240 kJ/mol. 5-O-methylgenistein - MMP1 demostrated the higher binding energy and performed two hydrogen bonds, five hydrophobic interactions and an electrostatic interaction. Hydrogen bonds and hydrophobic interactions were mostly identified in all complex, while electrostatic interaction did not show in glycitin – MMP1 and 8-Prenylnaringenin - MMP1 complexes. Besides that, van der Waals force also performed in all complexes based on the two dimensional structure of complex (**Fig. 1**).

The binding energy might be correlated with the number of active sites of complex, type of interactions, and ligand structure. Glycitin and Catechin 7-O-beta-D-xyloside have similar structure with four aromatic rings, produced lower score of binding energy. 8-Prenylnaringenin showed lower binding energy than naringenin, Eriodictyol, and 5-O-methylgenistein. Eventhough, all of four flavonoids have similar pattern. Low energy indicated tight interactions of ligand – protein complex. The binding energy score was affected by type of interactions, number of hydrogen and hydrophobic interactions [21,22,24–26].

DISCUSSION

Our docking study revealed several active sites of MMP1 protein. A previous study reported that Zink catalytic ion bound to MMP1 in His201, His205, and His211, HIS151, HIS166 and HIS179. Astaxantin interacted with MMP1 in some binding residues, including VAL312, GLY72, GLN257, ARG262, GLY261, GLU311, LYS276, GLU113, ASP254, and ALA25.[27,28] Similar with our study, quercetin and wogonin showed interaction in several amino acid residues, which were SER239, VAL215, TYR240, ASN180, PRO238, ALA182, and GLU219 [27]. The amino acid residue Glu219 was reported as catalytic sites of MMP1 protein [29]. Inhibition of flavonoid compounds in catalytic sites indicated a proper inhibitor for MMP1 and prevented collagen degradation and cell proliferation. Matrix metalloproteinase was a protease that degrading collagen or fibrinogen. Matrix metalloproteinase also key protein for cell proliferation [27–29]. Inhibiting MMP1 activity by flavonoids of *Phaleria macrocarpa* interfered cell lead to metastasis, and might inhibit endometriosis.

CONCLUSION

Six flavonoids inhibited matrix metalloproteinase in several binding sites by two alternative inhibition mechanism. Naringenin, Catechin 7-O-beta-D-xyloside, Eriodictyol, 8-Prenylnaringenin, and 5-O-methylgenistein inhibited MMP1 in catalytic site, while glycitin interfered MMP1 activity in allosteric site.

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest regarding the publication of this article.

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