PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 259-267
https://doi.org/10.14719/pst.3224

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RESEARCH ARTICLE

A molecular docking insight: *Phyllanthus niruri* L. constituents targeting MMP-9 for angiogenesis inhibition and IL-1β for anti-inflammatory action in endometriosis therapy

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OPEN ACCESS

ARTICLE HISTORY

Received: 25 December 2023
Accepted: 09 June 2024

Available online
Version 1.0 : 30 September 2024
Version 2.0 : 01 October 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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CITE THIS ARTICLE

Wulandari E T, Soetrisno, Purwanto B, Reviono, Ertanto Y, Sunowo J. A molecular docking insight: *Phyllanthus niruri* constituents targeting MMP-9 for angiogenesis inhibition and IL-1β for anti-inflammatory action in endometriosis therapy. Plant Science Today. 2024; 11(4): 259-267. https://doi.org/10.14719/pst.3224

Abstract

Endometriosis, characterized by inflammatory lesions resembling endometrium outside the uterine cavity, induces chronic inflammation, anatomical changes and persistent pain. The expression of Interleukin 1 β (IL-1β) is significantly associated with endometriosis, contributing to inflammatory process and fertility issues. Matrix metalloproteinase 9 (MMP-9) is crucial in the adhesion and angiogenesis of endometrial tissue. This study investigates the potential of *Phyllanthus niruri* L. in inhibiting MMP-9 and IL-1β through molecular docking analysis.

Molecular docking was performed using Discovery Studio Visualizer, Open Babel, PyRx and AutoDock Vina. The inhibitory activities of bioactive compounds on MMP-9 and IL-1β were predicted. Biological activity and cytotoxicity were assessed using PASS and CLC-Pred respectively. Kaempferol and quercetin from *P. niruri* exhibited significant MMP-9 expression inhibitory activity. Search Tool for Interacting Chemicals (STITCH) analysis revealed interactions of quercetin and galangin with specific proteins involved in pathways related to endometriosis. Biological activity analysis indicated that quercetin, kaempferol, herbacetin and galangin show potential as MMP-9 expression inhibitors. CLC-Pred analysis suggested high cytotoxicity of kaempferol against glioma.

Molecular docking results showed quercetin's potential as an MMP-9 inhibitor and galangin's potential as an IL-1β inhibitor. These findings support the therapeutic potential of *P. niruri* for endometriosis, providing insights for further research in developing innovative therapies targeting endometriosis-related inflammation and angiogenesis.

Keywords

Endometriosis; *Phyllanthus niruri* ; molecular docking; MMP-9; IL-1β

Introduction

Endometriosis is a gynaecological disorder characterized by an inflammatory condition marked by the presence of tissue lesions resembling the endometrium located outside the uterine cavity (1-3). Endometriosis induces chronic inflammation, leading to prolonged anatomical changes and persistent pain as well as infertility in women affected by the condition. Endome-

triosis is found in 30 % of women with infertility issues and 10-15 % in women of reproductive age (2). The precise etiology of endometriosis remains elusive, despite various hypotheses proposed and no definitive explanation established. Sampson's theory of retrograde menstruation is among the most widely accepted. According to this theory, during the menstrual cycle, endometrial-like tissue migrates outside its normal uterine location, implanting on pelvic structures and forming lesions. This process subsequently triggers an immune response, which in the case of endometriosis, leads to chronic inflammation and associated symptoms (3).

Some evidence suggest a multifactorial impact of endometriosis that lasts throughout a woman's life, from the point of onset of symptoms onwards (4). Analyses of the economic impact associated with endometriosis reveal a pronounced increase in the number of workdays lost among affected women compared to their healthy counterparts, consequently leading to a substantial decline in work productivity. Moreover, the research outlines the average annual economic burden attributed to endometriosis cases, amounting to $16573 USD in the United States and Int$9864 within the Eastern Mediterranean population (5, 6).

The increased expression of IL-1β is commonly linked to endometriosis. This pro-inflammatory cytokine, also known as katabolin, can promote the development of endometriosis by inducing endometriotic cells to release growth factors and other cytokines. These substances play a pivotal role in processes such as adhesion, growth, invasion, inflammation and angiogenesis in endometriotic tissue (7, 8). Previous studies have observed that the analysis of peritoneal fluid in women with endometriosis reveals elevated levels of IL-1β compared to those without endometriosis (8). Additionally, the observed increase in IL-1β levels has the potential to induce inflammation in the uterine environment. This inflammation may negatively impact embryo implantation and ultimately serve as a factor hindering fertility in affected women with endometriosis (9).

Matrix Metalloproteinase-9 (MMP-9) plays a crucial role in the breakdown and remodelling of the extracellular matrix around endometriosis lesions, facilitating the invasion and spread of endometriosis cells into the surrounding tissue (10). The formation of new blood vessels (angiogenesis) is necessary for endometriosis tissue to support lesion formation. Increased MMP-9 in endometrial tissue of women with endometriosis, suggests its role in driving cell movement and invasion in endometriosis lesions. In other words, MMP-9 may facilitate cell movement and tissue invasion in the context of endometriosis (11).

Meniran or *Phyllanthus niruri* L., is a widely used herbal plant in traditional Indonesian medicine, cultivated extensively in Indonesia's natural environment. Research has confirmed its bioactive compounds, including phenolics, flavonoids, tannins, steroids, saponins and alkaloids, with notably elevated levels of flavonoids (12). Several active phytochemical compounds have been identified in *P. niruri*, enhancing our understanding of its health benefits (13, 14). In this study, we use docking analysis to predict compounds that show potential as modulators for MMP-9 and IL-1β, targeting the treatment of endometriosis.

Materials and Methods

Plant materials and Q-TOF (Quadrupole Time-of-Flight Mass Spectrometry) analysis of P. niruri

Phyllanthus niruri used in this study was obtained from the Research and Development of Medicinal Plants and Traditional Medicine Centre (B2P2TOOT) in Tawangmangu, Indonesia. The *P. niruri* extraction process began with grinding the raw material into a fine powder, followed by its placement in an Erlenmeyer flask and covering it with 60 % ethanol solvent. Continuous shaking for 2-3 h ensured thorough extraction. Subsequently, the mixture underwent a 24 h maceration process, protected by aluminium foil to prevent contamination. After maceration, filtration yielded a concentrated extract devoid of solvent residues, suitable for further analysis and experimentation. Furthermore, Q-TOF analysis was conducted at the Saraswanti Indo Genetech (SIG) laboratory in Bogor, Indonesia, to identify the compound content in *P. niruri*.

Modelling software such as Discovery Studio Visualizer 3.0 (https://discover.3ds.com/discovery-studio-visualizer-download) Open Babel (https://openbabel.org/wiki/Main_Page), PyRx (https://pyrx.sourceforge.io/) AutoDock Vina (https://vina.scripps.edu/) were used in this study. The 3D structures of MMP-9 (PDB ID: 4XCT) and IL-1β (PDB ID: 1I1B) were retrieved from the Protein Data Bank, as illustrated in Fig. 1.

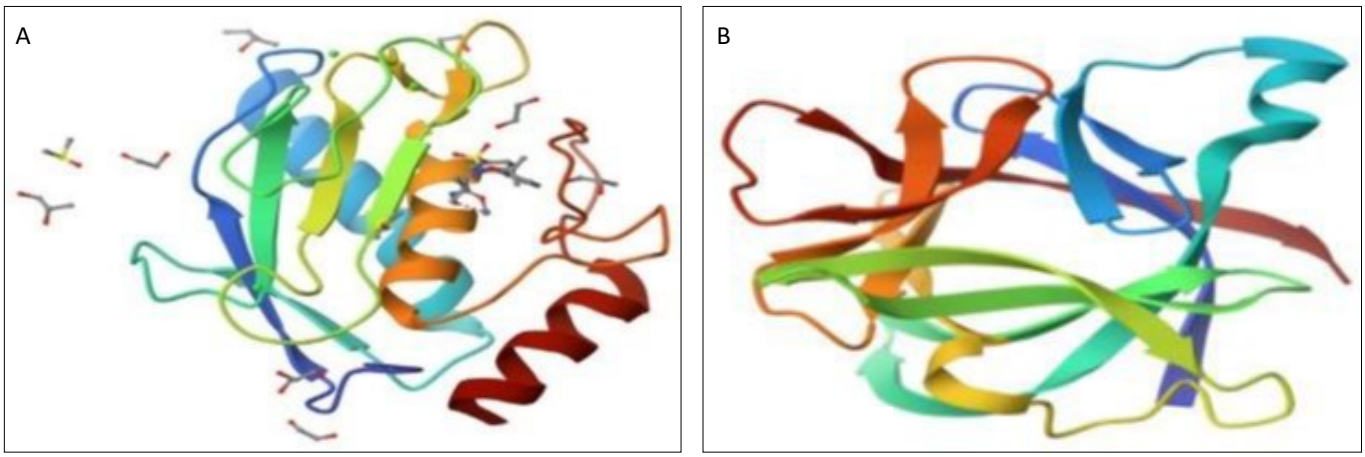

Fig. 1. (**A**) MMP-9 and (**B**) IL-1β 3D structures.

The study was a preliminary study and screening method for the prediction of MMP-9 and IL-1β inhibiting agents from bioactive compounds of herbal *P. niruri*. We used anticancer activity to predict cytotoxic activity. The similarity between endometriosis and cancer lies in the uncontrolled growth of cells or tissue, where both are characterized by excessive proliferation beyond normal limits. Anti-cancer activity of *P. niruri* compounds such as quercetin, kaempferol, herbacetin and galangin was conducted through an *in silico* study. The examination in this research encompassed biological activity, cytotoxicity activity on cell lines, SwissADME, STITCH and molecular docking.

Data collection

The data collection process encompassed the retrieval of essential information, including the canonical SMILES (Simplified Molecular Input Line Entry System) of the compounds utilized for the analysis of biological activity, evaluation of cell line cytotoxicity activity and assessment through SwissADME as shown in Table 1. Furthermore, comprehensive details regarding the 3D structures of the compounds under investigation were sourced from PubChem, accessible through the link (https://pubchem.ncbi.nlm.nih.gov). In addition to this, the 3D molecular structures of MMP-9 (PDB ID: 4XCT) and IL-1β (PDB ID: 1I1B) were downloaded from Protein Data Bank, contributing to the comprehensive dataset employed in our research endeavours.

Table 1. Data collection of *Phyllanthus niruri* extract compounds and native ligands.

No.	Compound	Pubchem ID	Molecular Weight (g/mol)	Canonical SMILES
1	Quercetin	5280343	302	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
2	Kaempferol	5280863	286	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
3	Herbacetin	5280544	302	C1=CC(=CC=C1C2=C(C(=O)C3=C(O2)C(=C(C=C3O)O)O)O)O
4	Galangin	5281616	270	C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O
5	N73 (MMP-9 native ligand)	138753324	404	CC(C)C(C(=O)N=O)N(OC(C)C)S(=O)(=O)C1=CC=C(C=C1)C2=CC=CC=C2
6	Dexamethasone (IL-1β ligand)	5743	392	CC1CC2C3CCC4=CC(=O)C=CC4(C3(C(CC2(C1(C(=O)CO)O)C)O)F)C

Data analysis

The examination of the biological activity of the bioactive compounds under investigation was conducted using the PASS web server available at http://way2drug.com/PassOnline/index.php. The graphical representations of the molecular structures of each bioactive compound were included by incorporating their canonical SMILES. This process allowed the acquisition of parameters, with Pa indicating "probable activity" and Pi indicating "probable inactivity." In this study, the obtained Pa values played a crucial role, as a higher Pa value indicated a greater potential for the biological activity of the compound. The study implemented a cut-off value for the Pa parameter set at 0.7. The evaluation of cytotoxic activity was extended using the PASS web server through diverse web links accessible at http://way2drug.com/cell-line/. This approach aimed to predict the cytotoxic potential of the compounds across both cancer and normal cell lines. The identification of the highest Pa value, indicating the greatest potential for anticancer activity against a specific protein according to PASS analysis, served as a basis for subsequent analysis. Consistent with these findings, the reported flavonoid activity within *P. niruri* was recognized for its capability to inhibit cancer cell migration by suppressing MMP-2 and MMP-9 through the inhibition of Ras *in vitro*.

Results and Discussion

Identification of flavonoid compounds in Phyllanthus niruri

Based on Table 2, there are 15 identified compounds in *P. niruri* that are classified as flavonoids. The presence of several identified compounds in the flavonoid class indicate the potential significant benefits of *P. niruri*. Flavonoids are known as phytochemical compounds with antioxidant and anti-inflammatory properties (14). The diversity of flavonoids revealed can have a positive impact on health. In this study, we specifically selected quercetin, kaempferol, herbacetin and galangin. Our selection of these active compounds was based on an extensive review of literature supporting their reported bioactivities.

For instance, quercetin has been widely recognized for its potent anti-inflammatory effects (15, 16). Kaempferol has shown promising anticancer activity. It works through mechanisms targeting specific pathways by regulating tumor cell cycle progression, proliferation, apoptosis, migration and invasion as well as by inhibiting angiogenesis (17). Herbacetin has shown promising antioxidant properties (18) and galangin has also demonstrated significant antioxidant properties (19).

Biological activity analysis using PASS Online

According to the findings from PASS analysis, kaempferol demonstrated elevated likelihoods for its anti-mutagenic properties (0.948/0.001). Additionally, quercetin also exhibited biological activity as an antineoplastic agent (0.797/0.012). The prediction of the bioactive compounds' biological activity through PASS analysis is presented in Table 3. In line with these results, the flavonoid activity contained in the bioactive compounds, particularly kaempferol and quercetin, aligns with their potential as anti-mutagenic agents, MMP-9 expression inhibitors and antineoplastic agents as indicated by the PASS analysis.

Cell line cytotoxicity prediction through CLC-Pred analy-

Table 2. Identification of flavonoid compounds in *Phyllanthus niruri* using Q-TOF analysis.

No	ESI	Compound name	Chemical formula	Mol. Weight (g/mol)	RT	m/z Value (Isotope Match Mz RMS)	Adduct	Result
Flavonoid								
1	(-)	(-)-Epiafzelechin-3-O-β-D-allopyranoside	C21H24O10	436.45	11.44	162.96	-H	Positive
2	(-)	(-)-Epigallocatechin	C15H14O7	306.29	4.01	89.01	-H	Positive
3	(-)	Galangin (Norizalpinin)	C15H10O5	270.25	13.08	16.86	-H	Positive
4	(-)	Hibiscetin-3-O-glucoside	C21H20O14	496.41	4.17	198.47	-H	Positive
5	(-)	Isoastilbin	C21H22O11	450.43	6.72	37.1	-H	Positive
6	(-)	Kaempferol-3,7-di-O-β-D-glucopyranoside	C27H30O16*	610.57	9.26	2.80	-H	Positive
7	(-)	Nobiletin-3-O-β-D-glucoside	C27H32O14	580.59	8.91	5.35	-H	Positive
8	(-)	Quercetin 3-O-neohesperidoside	C27H30O16*	610.57	8.72	3.71	-H	Positive
9	(-)	Quercetin-3,7-O-β-D-diglucopyranoside	C27H30O17	626.57	8.36	2.67	-H	Positive
10	(+)	Herbacetin	C15H10O7	302.25	13.05	422.32	+H	Positive
11	(+)	Isohyperoside	C21H20O13	480.41	8.25	720.74	+H	Positive
12	(+)	Isoquercitrin	C21H20O12	464.41	9.35	291.338	+H	Positive
13	(+)	Kaempferol-3,7-di-O-β-D-glucopyranoside	C27H30O16*	610.57	9.05	693.105	+H	Positive
14	(+)	Quercetin-3,7-O-β-D-diglucopyranoside	C27H30O17	626.57	8.11	711.06	+H	Positive
15	(+)	Quercetin-3-O-α-L-rhamnose-7-O-β-D- glucoside	C27H30O16*	610.57	8.49	315.34	+H	Positive

*Additional Notes: different sugar moiety.

Table 3. Biological activity of Quercetin, Kaempferol, Herbacetin and Galangin.

No	Compound	Anticancer Activity	Pa	Pi
1	Quercetin	Antimutagenic	0.940	0.001
		Antineoplastic	0.797	0.012
		MMP-9 expression inhibitor	0.734	0.005
2	Kaempferol	Antimutagenic	0.948	0.001
		Antineoplastic	0.791	0.013
		MMP-9 expression inhibitor	0.738	0.005
3	Herbacetin	Antimutagenic	0.939	0.001
		Antineoplastic	0.792	0.013
		MMP-9 expression inhibitor	0.728	0.005
4	Galangin	Antimutagenic	0.940	0.001
		Antineoplastic	0.787	0.013
		MMP-9 expression inhibitor	0.722	0.005

sis

The prediction of cell-line cytotoxicity against normal cells helps to evaluate the possible toxic effects of the compounds. In this study, the CLC-Pred tool, renowned in cheminformatics and medicinal chemistry, was employed to forecast the toxicity of compounds against specific cell line types and tissues associated with corresponding tumor types. A comprehensive dataset, including the maximum number of diverse cell line predictions, was compiled and organized. The presented data can be found in Table 4. The CLC-Pred analysis showed that kaempferol exhibited the highest cytotoxicity against the glioma cell line Hs 683, with a Pa value of 0.555. This indicates significant potential for kaempferol as a cytotoxic agent against glioma cells, warranting further investigation into its mechanisms of action and therapeutic applications. These findings suggest kaempferol's bioactivity could substantially contribute to inhibiting tumor growth, positioning it as a promising anticancer candidate.

Investigated bioactive compound-protein interaction analysis using STITCH

Table 4. CLC-Pred analysis of cell line cytotoxicity prediction.

No	Compound	Cell Line	Tumor Type	Pa	Pi
1	Quercetin	Hs 683	Glioma	0.523	0.049
2	Kaempferol	Hs 683	Glioma	0.555	0.038
3	Herbacetin	Hs 683	Glioma	0.505	0.057
4	Galangin	Hs 683	Glioma	0.505	0.057

The STITCH Assessment result exclusively revealed compound-protein interactions involving quercetin and galangin. In contrast, kaempferol and herbacetin exhibited no interactions with any proteins. According to the data, numerous proteins interacted with quercetin and galangin as shown in Fig. 2. The network illustrated multi-

ple biological processes within the body that can be succinctly summarized and validated using the KEGG pathway as shown in Table 5.

The interactions identified through STITCH analysis emphasize the potential roles of quercetin and galangin in various biological processes, suggesting their relevance in the treatment of conditions such as endometriosis. The KEGG pathway analysis provides further insights into the specific pathways and biological functions these compounds may influence.

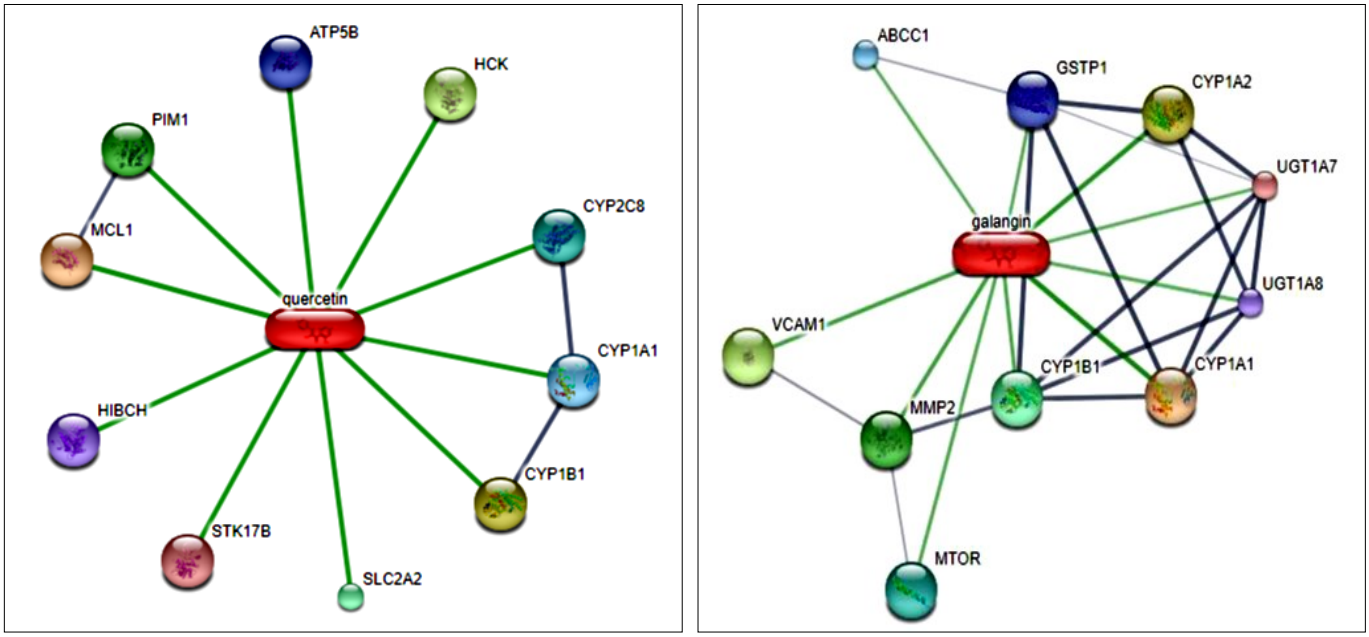

Fig. 2. STITCH analysis result of Quercetin and Galangin- protein interaction.

Table 5. Protein involvement based on the KEGG pathway.

No	Pathway ID	Pathway Description	Gene Set	False Discovery
A	**Quercetin**			
1	05204	Chemical carcinogenesis	CYP1A1, CYP1B1, CYP2C8	0.00186
2	05206	MicroRNAs in cancer	CYP1B1, MCL1, PIM1	0.0076
3	00380	Tryptophan metabolism	CYP1A1, CYP1B1	0.0184
4	00140	Steroid hormone biosynthesis	CYP1A1, CYP1B1	0.0204
5	00830	Retinol metabolism	CYP1A1, CYP2C8	0.0204
6	04913	Ovarian steroidogenesis	CYP1A1, CYP1B1	0.0204
7	00980	Metabolism of xenobiotics by cytochrome P450	CYP1A1, CYP1B1	0.0234
B	**Galangin**			
1	00980	Metabolism of xenobiotics by cytochrome P450	CYP1A1, CYP1A2, CYP1B1, GSTP1, UGT1A7, UGT1A8	9.21e-11
2	05204	Chemical carcinogenesis	CYP1A1, CYP1A2, CYP1B1, GSTP1, UGT1A7, UGT1A8	9.21e-11
3	00140	Steroid hormone biosynthesis	CYP1A1, CYP1A2, CYP1B1, UGT1A7, UGT1A8	4.2e-09
4	00830	Retinol metabolism	CYP1A1, CYP1A2, UGT1A7, UGT1A8	1.36E-06
5	00982	Drug metabolism – cytochrome P450	CYP1A2, GSTP1, UGT1A7, UGT1A8	1.52E-06
6	00380	Tryptophan metabolism	CYP1A1, CYP1A2, CYP1B1	5E-05
7	05206	MicroRNAs in cancer	ABCC1, CYP1B1, MTOR	0.00217
8	00053	Ascorbate and aldarate metabolism	UGT1A7, UGT1A8	0.0028
9	00040	Pentose and glucuronate interconversions	UGT1A7, UGT1A8	0.00439
10	00860	Porphyrin and chlorophyll metabolism	UGT1A7, UGT1A8	0.00612
11	00983	Drug metabolism – other enzymes	GT1A7, UGT1A8	0.00612
12	00500	Starch and sucrose metabolism	UGT1A7, UGT1A8	0.00701
13	04913	Ovarian steroidogenesis	CYP1A1, CYP1B1	0.00701
14	05200	Pathways in cancer	GSTP1, MMP2, MTOR	0.0117
15	05215	Prostate cancer	GSTP1, MTOR	0.0175
16	04670	Leukocyte transendothelial migration	MMP2, VCAM1	0.0289
17	01100	Metabolic pathways	CYP1A1, CYP1A2, UGT1A7, UGT1A8	0.0417

Ligand selection, retrieval and preparation

Comprehensive literature review was conducted to identify and detail the active compounds in *P. niruri*. Through this research, ligands were selected for use in the docking process against MMP-9 and IL-1β. Four specific compounds were chosen and information regarding their chemical structures and IDs was obtained from PubChem. Illustrations of the structures of the extracted compounds and the selected ligands can be seen in Fig. 3. The ligands were then imported into the Open Babel suite of PyRx (https://pyrx.sourceforge.io/). Energy minimization, under default parameters, was done for all the ligands. They were further converted to the appropriate docking format using the make ligand option available in PyRx. For specific details related to this process, the information is presented in Table 6.

Docking results of Phyllanthus niruri extract compounds and native ligands

Table 7 illustrates the docking results of selected compounds present in the extract (*P. niruri*) on the protein target. The rmsd values approaching zero indicate that the conformation of the compounds in the complex with the protein target is in a favorable position. Negative docking scores indicate high affinity, with lower values indicating stronger affinity.

Based on these results, the best conformation was shown by quecetin against MMP-9 (rmsd value 0.0 and docking score -9.9) and galangin against IL-1β (rmsd value 0.0 and docking score -8.1). The full description is explained below:

Binding to MMP-9

The interaction involving MMP-9 is depicted in Fig. 4. Quercetin exhibited binding to MMP-9 with a binding energy of -9.9 kcal/mol and formed hydrogen bonds with amino acids, including Met247 at 5.35 Å, Leu188 at 3.15 Å and Ala189

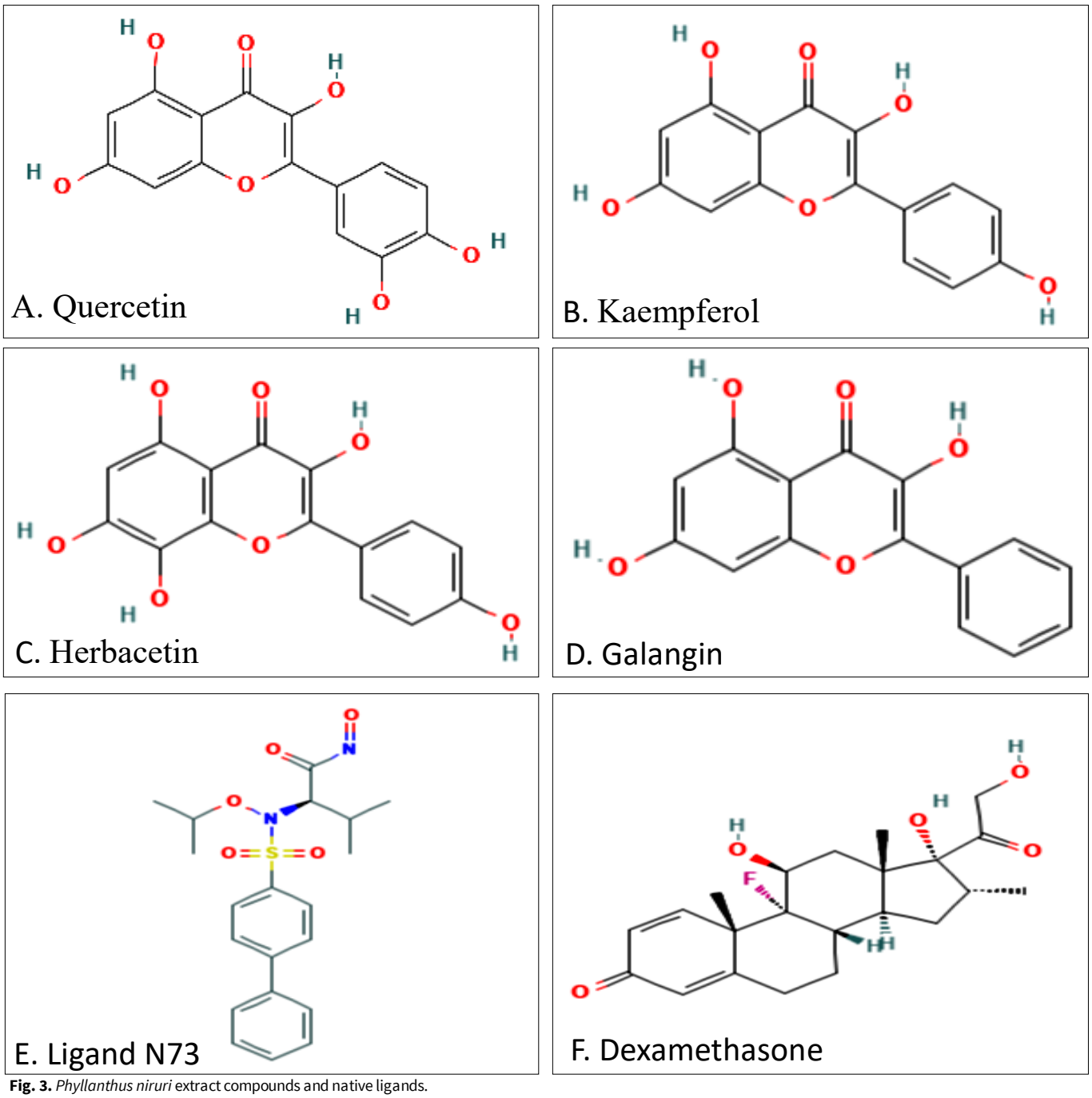

Fig. 3. *Phyllanthus niruri* extract compounds and native ligands.

Table 6. Coordinates of the X, Y, and Z centers of grid boxes for MMP-9 and

Protein Molecule	X-center (Dimension)	Y-center (Dimension)	Z-center (Dimension)
MMP-9	19.653	-10.832	14.832
IL-1β	45.325	6.820	14.918

Table 7. Docking results of selected compounds with target proteins

Compound	Target proteins	rmsd value	Score docking (kcal/mol)
Quercetin	MMP-9	0.0	-9.9
	IL-1β	0.0	-7.7
Kaempferol	MMP-9	0.0	-9.3
	IL-1β	0.0	-7.8
Herbacetin	MMP-9	0.0	-9.4
	IL-1β	0.0	-7.2
Galangin	MMP9	0.0	-9.4
	IL-1β	0.0	-8.1
N73 (MMP-9 native ligand)	MMP-9	0.0	-9.1
Dexamethasone (IL-1β ligand)	IL-1β	0.0	-7.5

at 3.65 Å. The formation of hydrogen bonds between ligands and proteins plays a pivotal role in these interactions, as hydrogen bonds significantly contribute to the structural integrity of the bonds, thereby enhancing the overall stability of the interaction.

Binding to IL-1β

The interaction between IL-1β and galangin is illustrated in Fig. 5. Galangin exhibited binding to IL-1β with a binding energy of -8.1 kcal/mol, forming hydrogen bonds with amino acid residues Gln236 at distances of 2.24 Å and 2.68 Å as well as Asn299 at a distance of 3.06 Å. To increase the stability of the interaction, it is imperative that hydrogen bonds form between ligands and proteins. These bonds play a crucial role in shaping the overall structure of the interaction, contributing significantly to its robustness.

Based on these results, all compounds in the *P. niruri* extract tested exhibited significantly higher scores compared to the native ligands N73 and Dexamethasone. Particularly, the compound quercetin in *P. niruri* stood out with a significantly higher degree score than the reference compound N73, indicating its strong potential as an MMP-

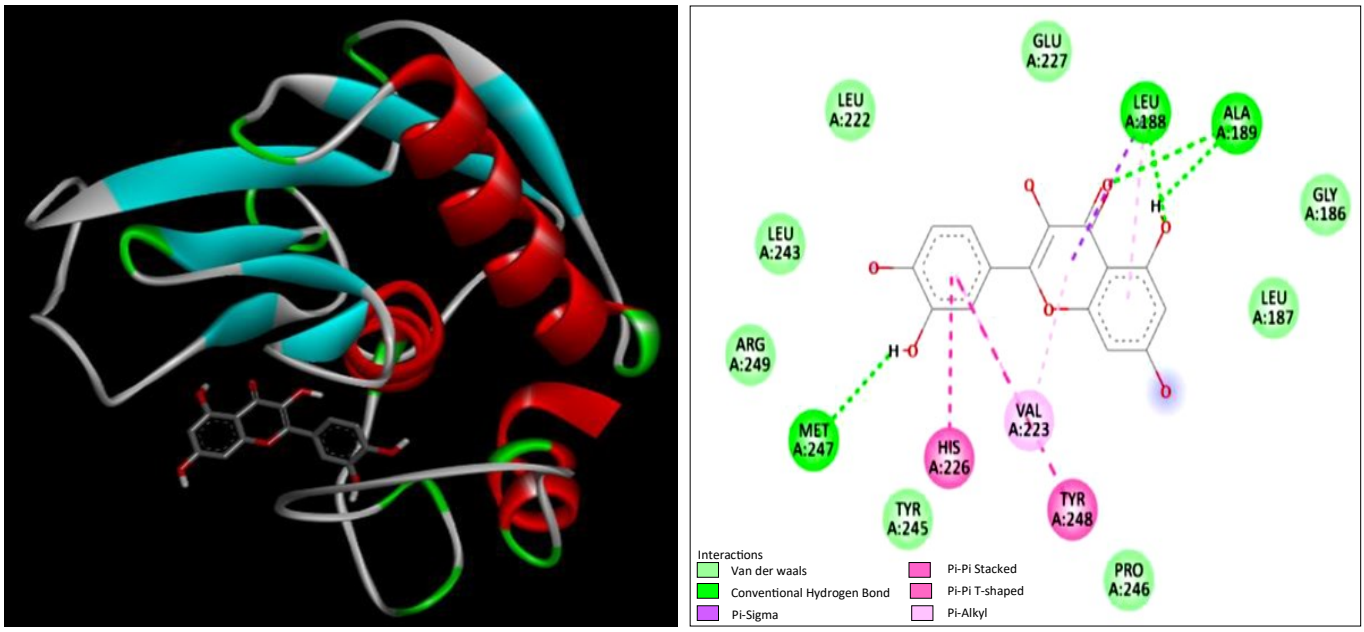

Fig. 4. Docking pose of hydrogen bonding interaction of MMP-9 with quercetin.

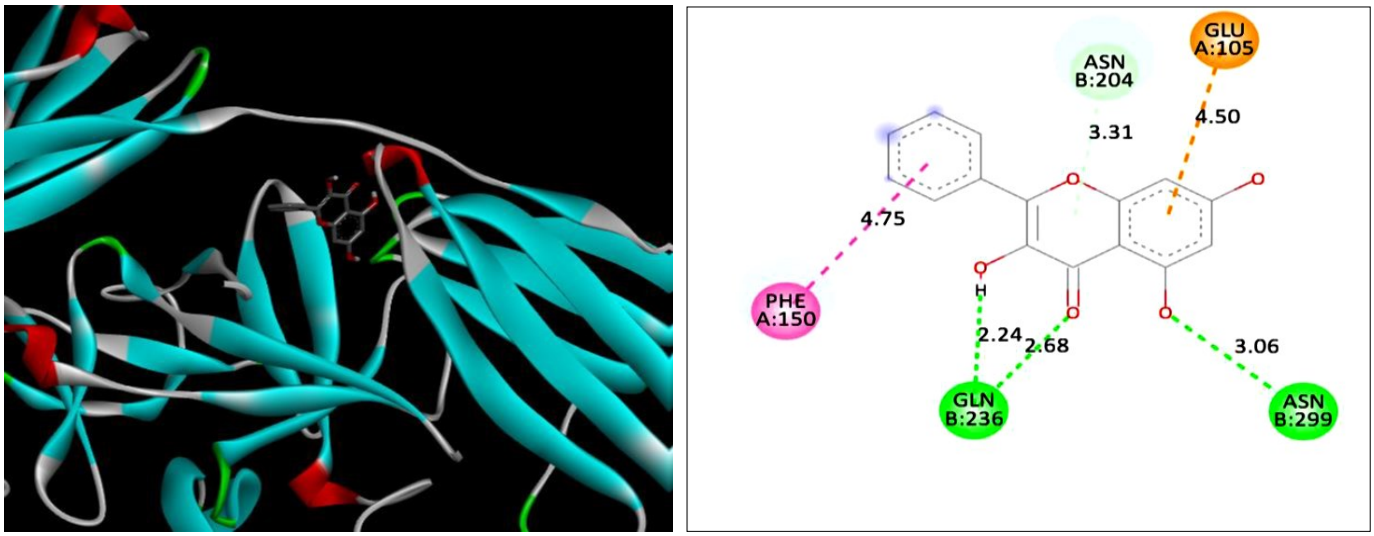

Fig. 5. Docking pose of hydrogen bonding interaction of IL-1β with galangin.

9 inhibitor. These findings align with the results of a recent study, which demonstrated that kaempferol and quercetin have high binding affinities to interleukin-1β (IL-1β), interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9) and tumor necrosis factor-alpha (TNF-α) (20). This similarity in findings reinforces the potential of these compounds in targeting inflammatory and angiogenic pathways in endometriosis therapy. Furthermore, MMP-9 can be regarded as one of the most typical downstream biomarkers in the progression of endometriosis (11). MMP-9 is necessary for endometrial cells to separate from the endometrium and invade the peritoneum surface, for vascular endothelial cells to relocate to new vessels, for macrophages to identify and engulf escaped cells and for NK cells to destroy targeted cells. This suggests that MMP-9 may be a crucial target for treating endometriosis (11). The potential of quercetin to alleviate MMP-9 activity has positive implications for the development of therapies targeting the inflammatory aspects of endometriosis. Furthermore, the compound galangin in *P. niruri* demonstrated a high degree score compared to Dexamethasone, suggesting its potential as an inhibitor of IL-1β. IL-1β primarily stimulates neuroangiogenesis factors like IL-6, CXC chemokines and fractalkine. It triggers the secretion of inflammatory mediators in response to macrophages and biological molecules, including estrogen. This highlights IL-1β's role in the inflammatory environment of endometriosis, contributing to pain and disease progression. Therefore, targeting IL-1β with galangin could be a promising therapeutic strategy for endometriosis (21).

Conclusion

The docking results of this study affirm the potential of *P. niruri* constituents, particularly quercetin and galangin, in interacting with molecular markers that are widely associated with endometriosis such as IL-1β and MMP-9. These findings enrich our understanding of the therapeutic potential of *P. niruri* in reproductive health, specifically in the context of endometriosis, it is important to acknowledge the limitations of our research. One limitation is the reliance on *in silico* molecular docking analysis, which may not fully represent the complexities of *in vivo* interactions. Additionally, our study focused on specific bioactive compounds from *P. niruri* and further research is needed to explore *in vivo* studies and clinical trials to further validate these findings and explore the safety and efficacy of *P. niruri* based therapies for endometriosis management.

Acknowledgements

The authors are thankful to the administration and management of the Faculty of Medicine, Universitas Sebelas Maret, for their support during the investigation.

Authors' contributions

This study was designed, directed and coordinated by S, BP and R as the Promotors Team, providing conceptual and technical guidance for all aspects of the project and supervision. ET carried out the molecular genetic studies as part of her dissertation. YE and JS performed bioinformatics analysis. The manuscript was written by ET and reviewed by all authors

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. Annu Rev Pathol [Internet]. 2020; Available from: https://www.annualreviews.org/doi/abs/10.1146/annurev-pathmechdis-012419-032654
2. Asghari S, Valizadeh A, Aghebati-Maleki L, Nouri M, Yousefi M. Endometriosis: Perspective, lights and shadows of etiology. Biomed Pharmacother. 2018 June;106:163-74. https://doi.org/10.1016/j.biopha.2018.06.109
3. Lamceva J, Uljanovs R, Strumfa I. The main theories on the pathogenesis of endometriosis. Int J Mol Sci. 2023;24(5). https://doi.org/10.3390/ijms24054254
4. Missmer SA, Tu FF, Agarwal SK. Impact of endometriosis on life-course potential: A narrative review [Internet]. International Journal of General Medicine. ncbi.nlm.nih.gov; 2021. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7800443/
5. Soliman AM, Surrey E, Bonafede M, Nelson JK, Castelli-Haley J. Real-World evaluation of direct and indirect economic burden among endometriosis patients in the united states. Adv Ther [Internet]. 2018;35(3):408-23. Available from: https://doi.org/10.1007/s12325-018-0667-3
6. Swift B, Taneri B, Becker CM, Basarir H, Naci H, Missmer SA et al. Prevalence, diagnostic delay and economic burden of endometriosis and its impact on quality of life: Results from an Eastern Mediterranean population. Eur J Public Health. 2024;34(2):244-52. https://doi.org/10.1093/eurpub/ckad216
7. Peng B, Alotaibi FT, Sediqi S, Bedaiwy MA, Yong PJ. Role of interleukin-1β in nerve growth factor expression, neurogenesis and deep dyspareunia in endometriosis. Hum Reprod. 2021;35(4):901-12. https://doi.org/10.1093/humrep/deaa017
8. Sikora J, Smycz-Kubańska M, Mielczarek-Palacz A, Kondera-Anasz Z. Abnormal peritoneal regulation of chemokine activation—The role of IL-8 in pathogenesis of endometriosis. Am J Reprod Immunol. 2017;77(4):1-8. https://doi.org/10.1111/aji.12622
9. Matsuzaki S, Gremeau AS, Pouly JL. Impaired pathogen-induced autophagy and increased IL-1β and TNFα release in response to pathogenic triggers in secretory phase endometrial stromal cells of endometriosis patients. Reprod Biomed Online. 2020;41(5):767-81. https://doi.org/10.1016/j.rbmo.2020.06.011
10. Zheng W, Cao L, Xu Z, Ma Y, Liang X. Anti-angiogenic alternative and complementary medicines for the treatment of endometriosis: A review. 2018;2018. https://doi.org/10.1155/2018/4128984
11. Ke J, Ye J, Li M, Zhu Z. The role of matrix metalloproteinases in endometriosis: A potential target. 2021;1-16.
12. Hasan M, Safarianti S, Ramadhani AF, Khilfi S, Suryawati S, Husna F. Bioactive compounds and *in vitro* evaluation of *Phyllanthus niruri* extract as antioxidant and antimicrobial activities. Trends Sci. 2024;21(2). https://doi.org/10.48048/tis.2024.7130

13. Jeevani Maheshika Dahanayake PKP, Galappaththy P, Arawwawala AM. A mini-review on therapeutic potentials of *Phyllanthus niruri* Linn. 2020;4(3):101-08.

14. Kaur N, Kaur B, Sirhindi G. Phytochemistry and pharmacology of *Phyllanthus niruri* L.: A review. Phyther Res. 2017;31(7):980-1004. https://doi.org/10.1002/ptr.5825

15. Cholet J, Decombat C, Delort L, Gainche M, Berry A, Ogeron C et al. Potential anti-inflammatory and chondroprotective effect of *Luzula sylvatica*. Int J Mol Sci. 2023;24(1). https://doi.org/10.3390/ijms24010127

16. Rashidi Z, Khosravizadeh Z, Talebi A. Overview of biological effects of Quercetin on ovary. Phyther [Internet]. 2021; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/ptr.6750

17. de Morais EF, de Oliveira LQR, Farias Morais HG de, Souto Medeiros MR de, Freitas R de A, Rodini CO et al. The anticancer potential of Kaempferol: A systematic review based on *in vitro* studies. Cancers (Basel). 2024;16(3):1-30. https://doi.org/10.3390/cancers16030585

18. Puthanveedu V, Shameera Ahamed TK, Muraleedharan K. Theoretical insights into Zn^{2+} chelated complexes of herbacetin for the application in Alzheimer's disease. Chem Phys Impact [Internet]. 2023;6:100163. Available from: https://doi.org/10.1016/j.chphi.2023.100163

19. Jomova K, Cvik M, Orolinova T, Y Alomar S, H Alwasel S, Aldahmash W et al. Antioxidant versus prooxidant properties of the flavonoid, galangin: ROS scavenging activity, flavonoid-DNA interaction, copper-catalyzed Fenton reaction and DNA damage study. J Agric Food Res [Internet]. 2024;16:101112. Available from: https://doi.org/10.1016/j.jafr.2024.101112

20. Zheng Q han, Du L yun, Zhao Y, Zhang Z, Piao S lan, Wang Y hang et al. Mechanism of *Rhodiola rosea–Euonymus alatus* drug pair against rheumatoid arthritis: Network pharmacology and experimental validation. Immunity, Inflamm Dis. 2023;11(12):1-20. https://doi.org/10.1002/iid3.1127

21. Machairiotis N, Vasilakaki S, Thomakos N. Inflammatory mediators and pain in endometriosis: A systematic review. Biomedicines. 2021;9(1):1-18. https://doi.org/10.3390/biomedicines9010054