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Therapeutic effects of *Erythrina variegata* on primary dysmenorrhea and endometriosis: An *in silico* analysis

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Primary dysmenorrhea (PD) is painful menstrual cramps, while endometriosis (EM) is a condition where tissue like the uterine lining grows outside the uterus, causing pain and infertility. EM and PD commonly associated in nociceptive pathways and inflammatory responses. In this study, phytochemicals such as erycrystagallin, erystagallin A, eryvarine A, 4-hydroxy-6, 3, 5 triprenyl isoflavonone, eryvarinol A, orientanol B from *E. variegata* have been evaluated for its potential to regulate TNF, COX-1, COX-2, PGF 2α involved in the inflammatory pathway resulting in hypersecretion of prostaglandins thereby causing excessive constriction of uterine muscle, nerve sensitization which leads to unbearable pain in PD. Similarly, employing network analysis in STRING and Cytoscape along with molecular docking in PyRx, the phytochemicals were investigated for their potential against PSR, ESR1, SF1, CYP19A1, GATA6, and MMP2, which creates an epigenetic abnormality converting stromal cells to endometriotic lesions and triggering inflammation through excessive production of estrogen. Using Cytoscape, the ligands with strong ADMET characteristics were found, and their interactions with the targets were verified. Molecular docking was performed to visualise the target-ligand complexes with the lowest binding affinities between -9 and -9.9. Hydrophobic and electrostatic interactions confirmed the ligands' influence on the targets. *E. variegata* ceases primary dysmenorrhea from developing into endometriosis. Additionally, it minimises estrogen and progesterone imbalances associated with EM & PD.

Keywords: Molecular docking, Cyclooxygenases, Prostaglandins, Estrogen receptors, Aromatase, 4-hydroxy-6,3,5 triprenyl isoflavonone

Primary dysmenorrhea (PD), a menstrual pain because of overproduction of prostaglandins, can cause uterine hypercontractility, leading to 8-72 hour pain and negatively affecting school and work attendance[1]. The on-trend and first-line treatment suggested by physicians is non-steroidal anti-inflammatory drugs (NSAIDs)[2]. Endometriosis (EM) is a gynaecologic disease affecting 10% of women globally; affecting 190 million women from menarche to menopause[3]. Genetics-based theories suggest that endometriosis-related pelvic discomfort is caused by pathogenic processes in the extra uterine and intracavitary endometrium. Overexpression of targets, such as steroidogenic factor-1 and GATA-binding factor-6 can lead to increased estrogen levels and inflammation. Treatments include cyclooxygenase inhibitor medications and surgical excision of pelvic lesions to prevent menses during ovulation and inhibit excess estrogen secretion[4].

Primary dysmenorrhea and endometriosis are conditions characterized by increased production of pro-inflammatory cytokines, such as Interleukin (IL)-1B, Tumour Necrosis Factor (TNF), IL-6, and IL-8, and downregulation of anti-inflammatory responses, such as ILF5 and IL11[5]. These cytokines increase myometrial contractions and nerve hypersensitivity, leading to insufficient defense against anti-inflammatory responses[6]. In endometriosis patients, endometrial cells increase macrophage contribution, synthesizing cytokines like IL-1, IL-6, IL-8, IL-10, and TNF-α, and nerve growth factor VEGF. VEGF promotes COX-2 secretion, leading to the production of prostaglandin, which are responsible for menstrual pain. Excessive production of uterine prostaglandins, specifically PGF 2α and PGE2, increases pain severity and hypercontractility of uterine muscles. This negatively impacts nerve endings, causing pain to be stronger and more unbearable[7]. Endometrial lesions in women suffering from endometriosis cause severe pain due to the release of prostaglandins, leading to hyperalgesia in both primary dysmenorrhea and endometriosis cases[8,9].

Pain in women with primary dysmenorrhea is influenced by central sensitization, peripheral pain-responsive messages, and excitability of somato-visceral convergent neurons in the spinal cord. Studies show variations in cerebral activity, metabolism, and pain processing methods[10]. Women with endometriosis show decreased volume of gray matter in pain-related brain regions, while increase in gray matter volume is observed in pain modulation and endocrine function regulating regions. Chronic

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pain perception in endometriosis patients is influenced by brain alterations, particularly declined gray matter volume[11]. Suppression in the hypothalamic-pituitary-adrenal axis promotes lower cortisol levels, decreasing pain-stress handling through the 'fight-flight response'[12]. Dysmenorrhea may initially alter brain structure and metabolism, leading to maladaptive changes and chronic pain. Prolonged nociceptive input can impair pain inhibition, contributing to its persistence.

Genus *Erythrina*, a Fabaceae family, includes many tropical and subtropical plants with significant traditional medicine applications[13]. *Variegata*, a prominent species, contains abundant alkaloids and other substances like flavonoids and polyphenols[14]. Alkaloids such as ,erysotine, erythratidine, erysotrine, erysodine, erythrodiol erythratidine abyssinone V, erycrystagallin and 4′hydroxy6,3′,5′- triprenyl isoflavonone, erythrinin B, genistein, laburnetin, erysopine, erysovine etc. and other substances such as flavonoids, poly phenols erythrinins A, B, C, alpinum isoflavone, osajin, etc were rich in *E. variegata*[15]. Its leaves, flowers, and bark have been traditionally used as analgesic, anti-asthmatic, antiseptic, and anti-inflammatory properties in its native areas[16].

The study investigates *E. variegata*'s potential to regulate hormones and inflammatory signalling in PD & EM. Targets and ligands will be selected based on literature and databases, and ligands will be examined for ADMET properties. Network interaction among targets and target-ligands will be analyzed using String and Cytoscape. The impact of ligands on targets will be assessed through virtual screening and molecular docking in PyRx.

Materials and Methods

Target identification

The potential targets were found based on the earlier works[17,18], which affected the inflammatory pathway during primary dysmenorrhea. TNF, COX-1, COX-2, PGF 2α were considered as the four leading targets[19]. The protein targets such as PSR, ESR1, SF1, CYP19A1, GATA6, MMP2[20-23] were considered as they play a key role in endometrial receptivity, estrogen expression and in formation of steroidogenic tissues (Table 1)[24]. Along with literature studies, gene card (https://www.genecards.org/) and therapeutic target database (https://db.idrblab.net/ttd/) were referred to identify targets. The 3D protein structures were retrieved from PDB (https://www.rcsb.org/) and Alfa fold. (https://alphafold.ebi.ac.uk/).

Ligand selectionADMET profiling

The phytochemicals from leaves of *E. variegata* were retrieved from GCMS analysis of different *E. variegata* solvent extracts as secondary data from various research literature[25-28]. The compound structures were retrieved from PUBCHEM (https://pubchem.ncbi.nlm.nih.gov), based on their availability and concentration in solvent extracts, 17 promising compounds from *E. variegata* were shortlisted. Out of 17 compounds, 15 phytochemicals follow Lipinski's rule of drug likeliness validated via SWISS ADME (http://www.swissadme.ch/) and

Table 1 — Selected targets for primary dysmenorrhea and endometriosis

Target Name	PDB/Alpha fold ID	Role
Tumor Necrosis Factor	1TNF	TNFα inhibits endometrial proliferation and initiate apoptosis. Endometrial cells produce Higher levels of TNFα during menses
Cyclooxygenase (COX)-2	5F19	It involves in the conversion of arachidonic acid (AA) from membrane phospholipid to unstable endoperoxide PGG2
Cyclooxygenase (COX)-1	6Y3C	One of the mandatory enzymes essential for the conversion of arachidonic acid to prostaglandins
Prostaglandin f2 alpha receptor	8IUK	PGF 2α regulates the contraction of arcuate vessels which leads to endometrial local hypoxia
PGR, progesterone receptor	1A28	Establishes progesterone resistance, which results in poor control of inflammation and abnormal tissue development
ESR1, Estrogen receptor 1	1A52	ESR1 is the primary mediator of the estrogenic action
Steroidogenic factor 1 SF1	1 YOW	Plays a key role in the development and function of steroidogenic tissues
Aromatase	3EQM	The enzyme responsible for converting testosterone and androgen to estrogen in estrogen biosynthesis
MMP2- Matrix Metallopeptidase 2	H3BV48	Matrix metalloproteinases (MMPs), enzymes that turns normal tissue into endometrial tissue
GATA binding protein 6	Q92908	GATA6 can transform endometrial stromal cells into endometriotic like cells by introducing an estrogen-producing phenotype

with further investigation in ADMET lab 2.0 (https://admetmesh.scbdd.com/), with higher GI absorption, bioavailability, solubility, and lower hERG blocking, carcinogenicity, acute oral toxicity, and with moderate TPSA were considered as ligand with appropriate ADMET properties[29-31].

Virtual screening The shortlisted ligands with good ADMET properties and the chosen targets of primary dysmenorrhea and endometriosis were virtually screened to look for models with minimal binding affinity and to select the ligands which interacts excellently well with low binding energy. This analysis was carried out using PyRx (https://pyrx.sourceforge.io/), virtual screening software for Computational Drug Discovery. Based on the ADMET profiling and virtual screening (Table 2), erycrystagallin (10362969), erystagallin A (10410005), eryvarine A (44448173), 4-hydroxy-6, 3, 5 triphenyl isoflavonone (10435114), eryvarinol A (637476), orientanal B (467497) were finalised for docking[32].

Protein-protein interactions Protein-protein interactions studies were conducted using STRING database (https://string-db.org/). The interactions among the targets of primary dysmenorrhea, as well as the interactions between the targets of endometriosis were observed. In both cases of Primary dysmenorrhea and endometriosis, hyperalgesia (increased sensitivity to pain) and inflammation are common physiological responses. Hence, the interaction between the protein targets of PM and EM was analyzed for correlation[33].

Network construction

The network was constructed with the help of Cytoscape (https://cytoscape.org/). The ligands and the target proteins were assigned as the edges and *E. variegata* was assigned as node. The network was constructed among erycrystagallin, erystagallin A, eryvarine A, 4-hydroxy-6,3,5 triphenyl isoflavonone, eryvarinol A for primary dysmenorrhea targets as they were the best interacting ligands for Primary dysmenorrhea targets and similarly the network was established among erycrystagallin, orientanal B, eryvarine A, 4-hydroxy-6, 3, 5 - triprenyl isoflavonone, eryvarinol A and targets of endometriosis[34].

Molecular docking From Table 3, the highlighted ligand–target models with lowest binding energy and were docked to visualize the exact chemical interactions. The (.pdb) files were converted into (.pdbqt) files, which included the entire protein molecule prepared and validated previously with added hydrogens and charges. The structural files of the prepared ligands were then assessed for their inhibition potential against validated protein targets involved in the inflammatory pathway and the formation of endometrial lesions. The docking was carried out in Auto Dock Vina through PyRx (https://pyrx.sourceforge.io/), an open-source virtual screening platform using the genetic algorithm parameters. The results provided various conformations accompanied by binding affinity values against their respective protein targets. These docked poses were interpreted with the help of BIOVIA Discovery Studio (https://discover.3ds.com/

Table 2 — Lipinski properties of phytochemicals from *Erythrina variegata*

Compound name	Pubchem ID	Molecular weight (Da)	H-bond acceptor	H-bond donor	Log p	LipinskiViolation
Erythraline	5317205	297.35	4	0	3.23	0
Erythramine	101289752	299.36	4	0	3.27	0
Erythrinine	3084503	313.35	5	1	2.92	0
Erysonine	442218	285.34	4	2	2.54	0
Erysodine	169017	299.36	4	1	2.93	0
Erysovine	5317203	299.36	4	1	3.05	0
Erythratidine	442220	331.41	5	1	3.14	0
Erysotrine	442219	313.39	4	0	3.32	0
Gallic Acid	370	170.12	5	4	0.21	0
3-eicosyne	549159	278.52	0	0	5.41	1
Erycricstagallin	10362969	390.47	4	2	4.26	0
Orientanol B	467497	338.4	4	1	3.52	0
Erystagallin A	10410005	422.51	5	2	3.95	0
4-hydroxy-6,3,5 triprenyl isoflavonone	10435114	476.6	5	3	4.98	0
Eryvarin A	44448173	370.4	6	2	3.31	0
Eryvarinol A	637476	470.47	9	4	3.18	0
Eryvarinol B	11114230	538.59	9	4	2.89	1

Table 3 — Depicts values and variables of several parameters for ADMET prediction

Compound name	GI absorption	BBB permeant	Bio-availability	TPSA	ESOL solubility (mg/mL)	hERG blocker	Carcinogenicity	Oral acute toxicity
Erythraline	High	Yes	0.55	30.9	2.41E-01	-	++	--
Erythramine	High	Yes	0.55	30.9	1.87E-01	---	+++	-
Erythrinine	High	Yes	0.55	51.1	1.05E+00	--	++	--
Erysonine	High	Yes	0.55	52.9	7.84E-01	--	++	--
Erysodine	High	Yes	0.55	41.9	3.66E-01	--	++	---
Erysovine	High	Yes	0.55	41.9	3.66E-01	--	++	---
Erythratidine	High	Yes	0.55	51.1	7.63E-01	-	+	---
Erysotrine	High	Yes	0.55	30.9	2.34E-01	--	++	--
Gallic acid	High	No	0.56	97.9	3.90E+00	---	---	---
3-eicosyne	Low	No	0.55	0	3.84E-05	--	---	---
Erycricstagallin	High	No	0.55	62.8	1.02E-04	---	---	+
Orientanol B	High	Yes	0.55	47.9	3.02E-03	---	--	--
Erystagallin A	High	Yes	0.55	68.1	6.61E-04	-	---	++
4-hydroxy-6,3,5 triprenyl isoflavonone	Low	No	0.55	86.9	8.80E-06	---	---	++
Eryvarin A	High	No	0.55	77.3	2.66E-02	---	---	---
Eryvarinol A	High	No	0.55	134.	9.66E-03	--	---	---
Eryvarinol B	Low	No	0.55	134.9	3.87E-04	---	---	---

+ positive, ++ moderately positive, +++ highly positive; - negative, -- moderately negative, --- highly negative

discovery-studio-visualizer-download) to better understand the molecular interactions between the docked ligands and the protein[35].

Results and Discussion

Target identification

The Identified targets have been listed out in Table 1 with their Name, PDB ID, and the crucial role they possess in primary dysmenorrhea and endometriosis.

Ligand selectionADMET profilingFrom Table 2, log p value of 3-eicosyne is 5.41 which is greater than 5 and Eryvarinol B with a molecular weight of 538.59 Da which is more than 500 Da, both the phytochemicals disobey rule of five. Hence, they were eliminated from the list of ligands. Erythrinine, erythraline, erythramine, erysonine, erythraline, erythrodine, erythrovine, erysotrine were found to be carcinogenic based on the prediction of ADMET lab 2.0. From the results of ADMET profiling and virtual screening six potential ligands, erycrystagallin (10362969), erystagallin A, eryvarine A,eryvarinol A, orientanal B, 4-hydroxy-6,3,5 triprenyl isoflavonone, gallic acid was finalized for virtual screening as they contain higher GI absorption, bioavailability, with minimal toxicity and moderate TPSA values which denotes proper solubility. They neither cross the BBB nor block hERG (Table 3).

Virtual screening

The selected ligands were subjected to virtual screening, to examine their interactions with the targets and to find their binding affinity. The identified binding affinity scores from PyRx for ligand–target models were compared. From Table 4, we can conclude that ligand–target models eryvarinol A–TNF (-9.4) b) erycrystagallin –COX-2 (-9.9) c) erystagallin A–COX-2 (-9.6) d) 4-hydroxy-6, 3, 5 triprenyl isoflavonone–COX-2 (-9.7) e) eryvarin A–PTGFR (-9.4) f) 4-hydroxy-6, 3, 5 triprenyl isoflavonone–PTGFR (-9.6). 4-hydroxy-6,3,5 triprenyl isoflavonone–SF1 (-9.1) b) eryvarine A–SF1(-9.4) c) erycristagallin –aromatase (-9.8) d) eryvarine A–aromatase (-9) e) orientanal B–aromatase (-9.2) f) erystagallin A–SF1(-9.1)} interacts with lowest binding energy and they were docked to visualize the exact chemical interactions.

Protein-protein interactions

The STRING database finds a network of proteins (Fig. 1A) that regulate inflammation and lipid metabolism. PLA2G2F releases arachidonic acid, which COX-2 converts into prostaglandins, driving inflammation. The interaction with TNF reinforces COX-2's role in immune and inflammatory signaling. PTGFR modulates prostaglandin-driven effects in tissues. In endometriosis targets (Fig 1B), ESR1 plays a crucial role in estrogen signalling,

Table 4 — Binding affinity of shortlisted ligands and targets of primary dysmenorrhea and endometriosis

Phytochemicals from *E.variegata*	Significant targets of primary dysmenorrhea				Significant targets of endometriosis					
	TNF	COX-2	COX-1	PGF 2α	PGR	ESR1	Aromatase	SF1	MMP2	GATA
Erycricstagallin	-7.8	-9.9	-8.5	-8.9	-8.2	-7.5	-9.8	-8.9	-8.6	-6.8
Erystagallin A,	-6.1	-9.6	-8.4	-8.8	-8.2	-6.9	-7.7	- 9.1	-7.6	-7.1
4-hydroxy-6,3,5 triprenyl isoflavonone	-7.8	-9.7	-8.8	-9.6	-8.6	-7.8	-8.5	-9.1	-8.7	-7.5
Gallic acid	-6.6	-6.5	-6.2	-6.1	-6.2	-5.8	-6.1	-5.6	-5	-5.9
Eryvarin A	-7.7	-8	-8.1	-9.4	-8.7	-7.2	-9	-9.4	-7.3	-7.2
Orientanol B	-7.5	-7.5	-8	-8	-7.9	-7.2	-9.2	-8.5	-7.5	-6.8
Eryvarinol A	-9.4	-8.8	-6.9	-7	-7.6	-6.7	-7.3	-8	-6.5	-6.1

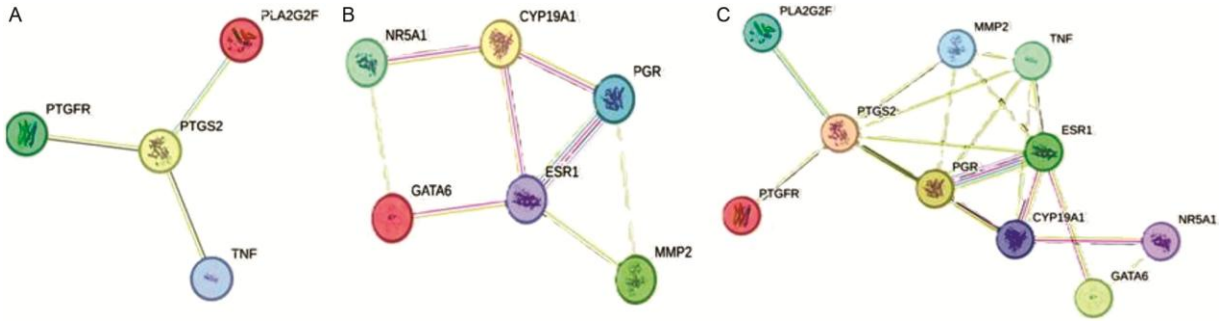

Fig. 1 — (A) Interaction between targets of primary dysmenorrhea; (B) Interaction between targets of endometriosis; (C) Interaction between targets of primary dysmenorrhea and endometriosis.

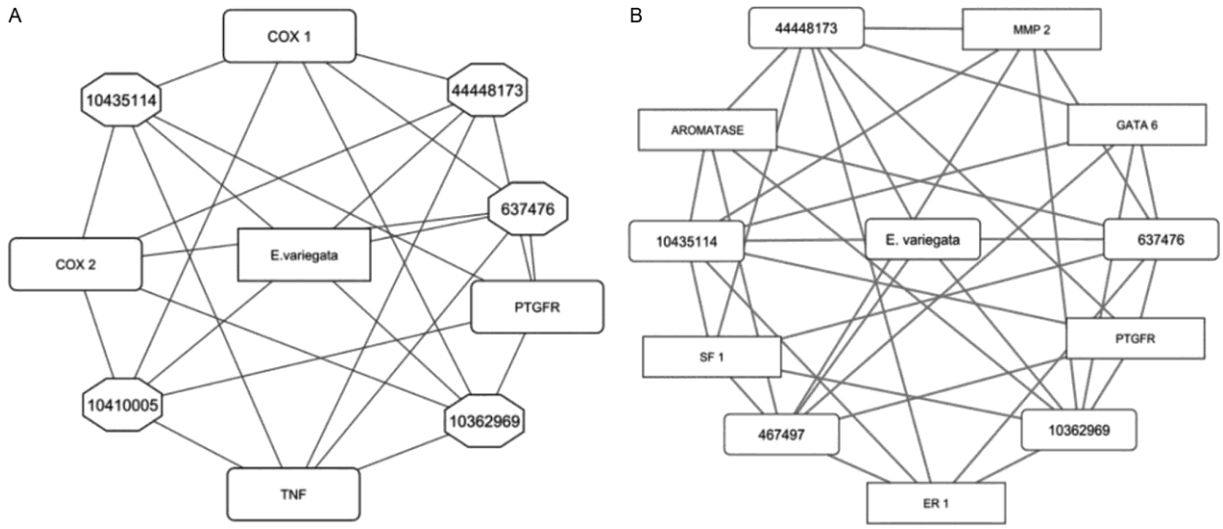

Fig. 2 — (A) Interaction between targets of primary dysmenorrhea and phytochemicals of *Erythrina variegata;* (B) Interaction between targets of endometriosis and phytochemicals of *E.variegata.*

affecting endometrial tissue expansion, NR5A1 and GATA6 for hormone production and reproductive tissue development, and MMP2 for the breakdown of the extracellular matrix. ESR1 interacts with CYP19A1 for local estrogen production, PGR for progesterone resistance these interactions reveal how estrogen dominance, progesterone resistance, and tissue remodelling contribute to endometriosis progression, resulting in chronic pain and infertility.

Network construction

The analysis of ligand interactions with primary dysmenorrhea targets, illustrated in the Cytoscape network (Fig. 2A), shows that phytochemicals interact with key proteins such as COX-1, COX-2, TNF, and PGI2. These interactions suggest that the plant compounds may show anti-inflammatory and cardiovascular effects by modulating pathways involved in inflammation and vascular regulation.

Similarly, network diagrams (Fig 2B) highlight the potential therapeutic role of *E. variegata*. It interacts with aromatase and ER1 to regulate estrogen levels, a critical factor in endometriosis management. Its influence on COX enzymes and TNF indicates anti-inflammatory properties, while its links with MMP2 indicate a role in endometriosis progression by inhibiting tissue remodelling and adhesion formation.

Molecular docking

In the molecular docking analysis, the interaction between Eryvarinol A and TNF SER C:99, GLN B:102, GLU C:116, GLN C:102, and π-π stacking are involved, indicating strong hydrogen bonds and ARG A:103, CYS C:101, CYS C:69 non-covalent interactions suggests that eryvarinol A can down-regulate TNF (Fig. 3A). Erycrystagallin and COX-2 (Fig. 3B) interactions involve hydrogen bonds TRP A:387 and π-stacking with amino acids such as HIS A:207, HIS A:386, indicating strong inhibition of COX-2 Similarly, (Fig 3C) erystagallin A forms non-polar bonds with amino acids, ALA A:199, A:202, LEU A:391, HIS A:386 strongly binding to COX-2. The interaction of 4-hydroxy-6, 3, 5 triprenyl isoflavonone with COX-2 (Fig 3D) also relies on hydrogen bonds and hydrophobic interactions with residues GLN A: 203, TYR A: 385, ALA A: 202, suggesting potent inhibition of COX-2. Eryvarin A interacts with PTGFR (Fig 3E) through hydrogen bonds and non-covalent interactions with amino acids VAL B: 325, ARG B: 155, CYS B:238, which could down-regulate the PGF 2α activity. In interaction between 4-hydroxy-6,3,5 triprenyl isoflavonone and PTGFR (Fig 3F), hydrogen bonds with LEU B:323, along with hydrophobic interactions CYS B:238, PRO B:241, LEU B:197, down-regulates PTGFR activity. To be precise, these bonds especially the hydrogen bonds, play a crucial role in stabilizing the binding of these phytoconstituents to their respective targets, highlighting their potential efficacy in down-regulating key inflammatory and pain pathways associated with dysmenorrhea.

The interaction between 4-hydroxy-6, 3, 5 triprenyl isoflavonone and SF1 *(*Fig. 4A) involves hydrogen bonds with CYS A: 266 and hydrophobic interactions, primarily with amino acids, ALA A: 433 A: 269, VAL 326, 307, 348 indicating a strong binding affinity. Eryvarine A also forms Pi stacking with LEU A: 306, hydrophobic interactions with (Fig 4B), ALA A: 269, HIS A: 310 residues. Both molecules aim to down-regulate SF1, potentially reducing aromatase expression and lowering estrogen production. Erycristagallin's interaction with aromatase (Fig 4C)

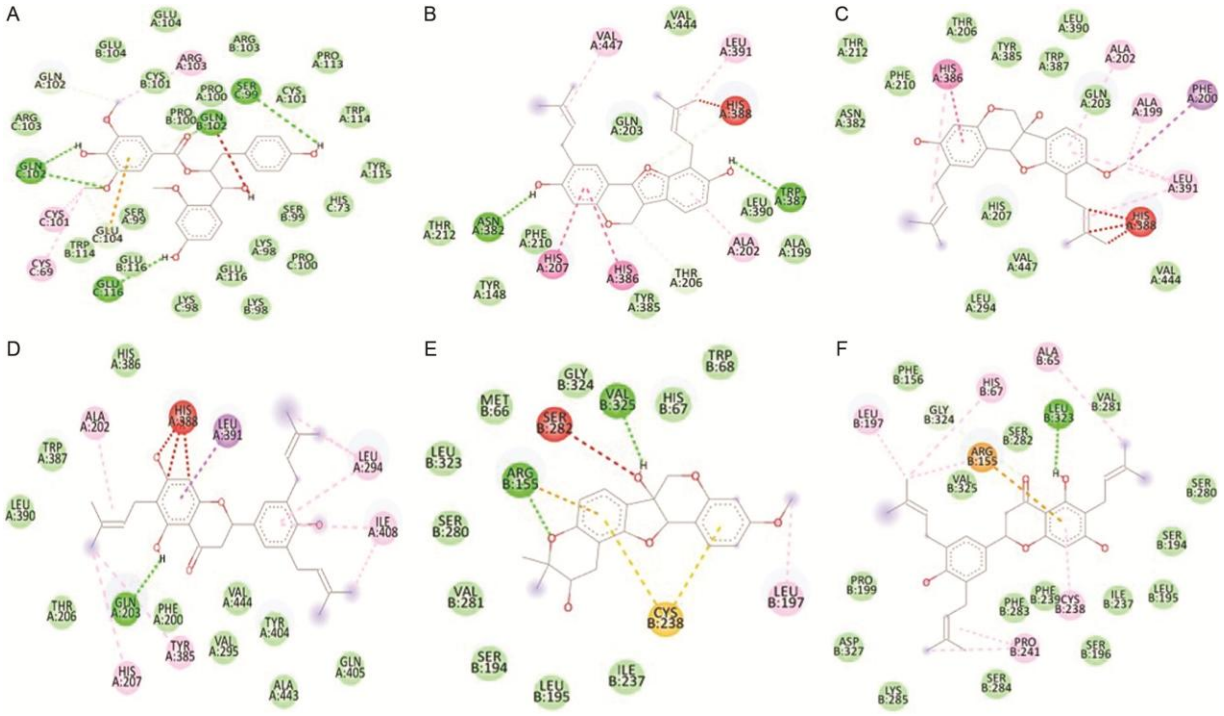

Fig. 3 — Interaction between phytoconstituent leads of *Erythrina variegeta* and targets of primary dysmenorrhea (A) Eryvarinol A- TNF; (B) Erycrystagallin–COX2; (C) Erystagallin A–COX-2; (D) 4-hydroxy-6,3,5 triprenyl isoflavonone–COX-2; (E)Eryvarin A–PTGFR; (F) 4-hydroxy-6,3,5 triprenyl isoflavonone–PTGFR.

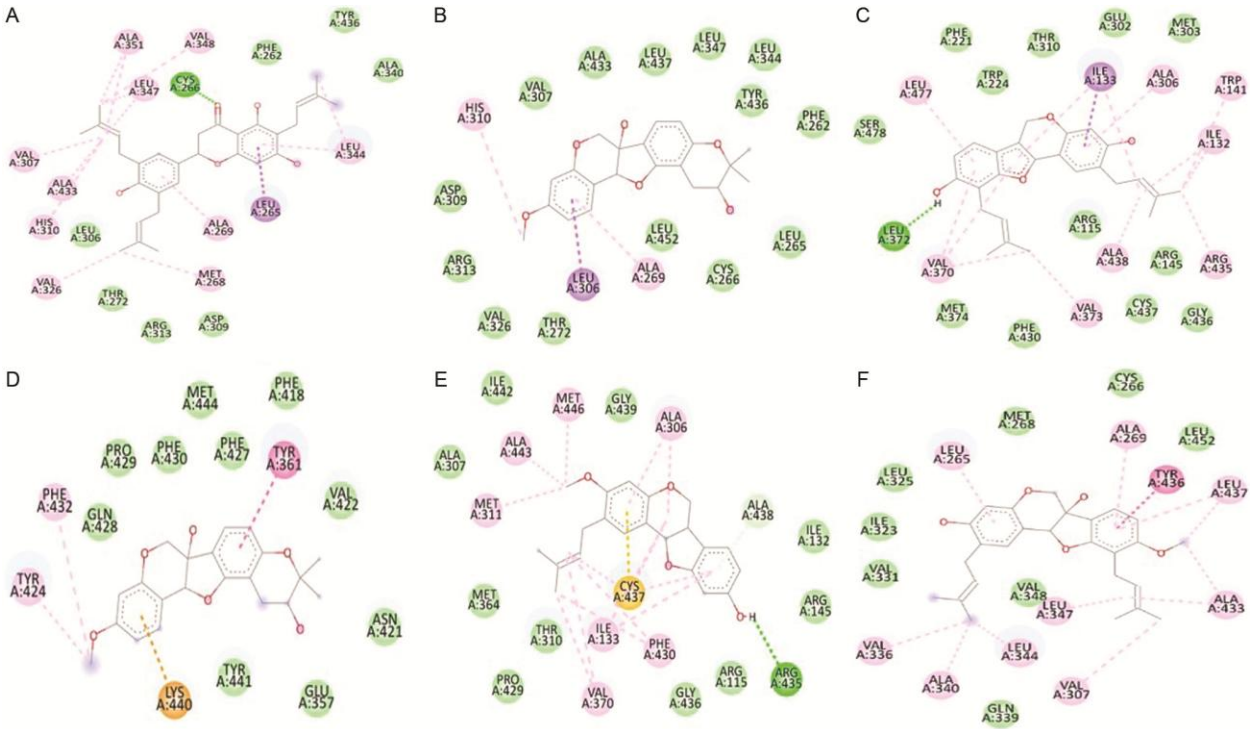

Fig. 4 — Interaction between phytoconstituent leads of *Erythrina variegeta* and targets of endometriosis. (A) 4-hydroxy-6,3,5 triprenyl isoflavonone–SF1; (B) Eryvarine A–SF1; (C) Erycristagallin–Aromatase; (D) Eryvarine A–Aromatase; (E) Orientanol B– romatase; (F) Erystagallin A–SF1.

is reinforced by hydrogen bonds, LEU A: 372, π-π stacking with ILE A: 133, and hydrophobic interactions with VAL 370, 373, ALA 306,438, making it a potent aromatase inhibitor. Eryvarine A similarly inhibits aromatase (Fig 4D), with significant hydrophobic interactions involving PHE A: 432, TYR A: 424, as well as a key interaction with LYS A: 440, which form a salt bridge, further stabilizing the binding. Orientanol B (Fig 4E) exhibits strong hydrogen bonds and possible covalent-like interactions with CYS A: 437, alongside non-covalent interactions with, MET 446, 311, PHE A: 430, ILE A: 133, ALA A: 306, 443, making it an effective aromatase inhibitor. In the interaction between erystagallin A and SF1 (Fig 4F), hydrophobic interactions involve LEU A: 265, ALA A: 269, 433, and 340. These bonds and interactions stabilize erystagallin A binding to SF1, potentially down-regulating its activity. Overall, the hydrogen bonds, π-π stacking, hydrophobic interactions contribute to their ability to inhibit SF1 and aromatase, thus reducing estrogen production, a critical factor in managing endometriosis progression.

Dysmenorrhea, a prevalent issue among adolescents, affects 70-93% of the population and is the leading cause of absenteeism from school. Around 20-40% struggle to manage dysmenorrhea, with 40% reporting negative impacts on academic performance. Those suffering from severe dysmenorrhea face diminished quality of life and a heightened risk of anxiety and depression[36]. A balanced lifestyle, a nutritious diet, stabilizing hormone levels, and supporting menstrual health deserve special attention[37,38] and it is imperative to re-examine and acknowledge qualities of medicinal plants considering the realities of modern living. This study focuses on the medicinal significance of *E. variegata* in treating primary dysmenorrhea and endometriosis. Shrinitha & Aruna[39] studies confirm the use of *E. variegata* for dysmenorrhea among the people of Natham village, Dindigul, Tamil Nadu. The findings from this *in silico* study of *E. variegata* suggest exactly that phytoconsituents of the Erythrina genus have significant potential to modulate therapeutic targets, largely due to their anti-inflammatory properties.

The initial analysis of primary dysmenorrhea and its targets in this study implies that the unbearable pain is because of hypersecretion of prostaglandins and higher perception of pain in nerves by receiving prostaglandin signals. Barcikowska Z *et al.*, discusses

that women suffering from pain had a higher concentration of prostaglandins in the endometrium. The study compared prostaglandin levels in women with menstrual pain versus healthy controls and found a significant difference in plasma concentration of PGF 2α prostaglandin between pain and pain-free women. In molecular docking analysis, selected phytochemicals (Fig. 3) formed hydrophobic interactions to inhibits the targets such as COX-2, TNF and PGF 2α which controls the prostaglandins production there by reducing severe pain. In the same way, Zhou, *et al*. [40], confirm the reduction of pain on down-regulation of COX-2, PGF 2α, TNF. In the study, Taohong Siwu decoction (TSD) alleviates primary dysmenorrhea by targeting key inflammatory pathways. It inhibits COX-2, which reduces the production of PGF 2α, a prostaglandin responsible for uterine contractions and pain. Additionally, TSD lowers the levels of TNF-α, a pro-inflammatory cytokine that contributes to the inflammatory response and worsens menstrual pain.

In the case of endometriosis, the phytochemicals interacted with GATA 6 (Fig 2), inhibiting its activity. Similarly, Cheng *et al*. [41], confirms that down-regulating GATA 6 reduces endometriotic lesion formation. Huayu Jiedu formula has shown a positive therapeutic impact on endometriosis by reducing GATA 6 expression in ectopic tissues, which promoted cell apoptosis and had anti-inflammatory properties[42].In the molecular docking studies of phytochemicals against endometriosis targets (Fig 4), they interacted strongly with aromatase and SF1 suggesting that inhibiting them can prevent endometrial lesion progression. Monnin. *et al*. [43], summarizes the causes of endometriosis, where regulation of these targets plays an inevitable role. SF1 promotes steroidogenesis by increasing CYP19A1 expression, CYP19A1 (aromatase) leads to excessive estrogen production, driving inflammation. ESR (estrogen receptors), especially ESR β, is upregulated, enhancing estrogen´s effects on proliferation and apoptosis resistance. Down-regulating inflammatory pathway targets decrease the overproduction of interleukins and other macrophage secretions in response to inflammatory signals. VEGF pathway is also disrupted as excessive interleukins are one of the multiple triggers that start the VEGF pathway of endometriosis[44]. GPER receptors are also down-regulated as a counteraction to decreased interleukin production prohibiting endometriosis.

In alliance with these findings, an earlier study stands as proof of E.variegata's potential for primary dysmenorrhea. The study on the effectiveness of *E. variegata* juice for dysmenorrhea among final-year nursing students showed promising results. Participants who consumed the juice reported a significant reduction in pain intensity and duration during menstruation compared to those who did not. The study concluded that *E. variegata* juice may be an effective natural remedy for alleviating dysmenorrhea symptoms, potentially due to its anti-inflammatory and analgesic properties[45].

Conclusion

Erythrina variegeta phytoconsituents have demonstrated significant binding affinity and show proper ADMET properties. By analyzing how phyto-compounds interact with the targets of primary dysmenorrhea and endometriosis, it has been proven that these bioactive substances lower the risk of developing both conditions by controlling targets such as COX-2, TNF, PGF 2α in the inflammatory prostaglandin pathways and potentially inhibit the targets SF1 and aromatase which are responsible for forming endometrial lesion. Moreover, they hold the ability to prevent the progression of primary dysmenorrhea to endometriosis by reducing nerve sensitization and regulating pro-inflammatory cytokines. Nevertheless, further pre-clinical research is needed to confirm these phytochemicals as drugs.

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