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Evaluating the Relative Efficacy of Synthetic and Natural Drugs in Endometriosis Adopting Molecular Modelling Approach

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Abstract

Background: Endometriosis is a chronic inflammatory condition of high incidence and with serious consequences. Several synthetic compounds proved to be useful in treating its symptoms by inhibiting aromatase, which is responsible for the pathogenesis of this painful illness. Nevertheless, synthetic drugs inflict several side effects, including headaches, osteoporosis, and so on. This scenario advocates the search for therapeutic formulations based on natural compounds. Thus, the present study was hypothesized to evaluate the comparative efficacy of the synthetic and natural drugs used in endometriosis, using the bioinformatics approach. **Methods:** CB-Dock was employed to perform molecular docking of the aromatase enzyme with two synthetic and three natural drugs for predicting their molecular interactions, and binding affinities. The curcumin-aromatase complex was further subjected to MD simulations to determine its stability, and to apply it to natural compound-based computer-aided drug discovery. **Results:** Curcumin was observed to dock with a greater binding interaction with aromatase. The RMSD profile, hydrogen bonds, and the RMSF and Rg values of the complex were stabilised after 50 ns, which was an indicator of the stable binding pose of the curcumin-aromatase complex. **Conclusion:** These in-silico findings are the basis for proposing that curcumin can be considered as a potential binding agent to inhibit the aromatase enzyme in the treatment of endometriosis. Molecular modelling and dynamics results suggest that curcumin and aromatase form a stable complex and that curcumin can be targeted as a drug in the treatment of endometriosis.

Keywords: Aromatase, Curcumin, Endometriosis, *In-silico*, Molecular Docking, Molecular Dynamics Simulation

1. Introduction

Endometriosis is a painful condition defined by chronic inflammation[1]. It is defined as the existence of functional endometrial glands and stroma outside the cavity of the uterus[2]. Certain sites, such as the uterosacral ligaments, the posterior cul-de-sac, the ovaries, and the fossa ovarica, are the locations where these endometriotic tissues are most often found[3]. Depending on the location, expanse, severity, depth, and size of the endometriotic tissues, this disease has been categorised into 4 stages, namely: Stages I, II, III, and IV, representing minimal, mild, moderate, and severe disease, respectively[3]. It is considered one of the most prevalent benign premenopausal women's gynaecological proliferative disorders, with 10-15% of women of reproductive age suffering from the condition[4]. It is a condition that is found in 176 million women of reproductive age worldwide, out of which 26 million are from India, i.e., about 14% of the total women[5].

While the cause of endometriosis is unknown, retrograde menstruation is considered the most broadly accepted mechanism for the formation of peritoneal endometriotic lesions[6]. According to Sampson, the constituents of menstrual debris, such as viable endometrial cells at eutopic sites, cytokines, and growth factors, could migrate in a retrogressive fashion via

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the fallopian tubes entering the pelvic cavity during regular menstruation, where these cells could infiltrate and multiply in surrounding tissues[7]. However, this circumstance of retrogressive menstruation affects almost all women, but not every woman is affected. As a result, it has been suggested that endometriotic patients are prone to have underlying anomalies at a molecular level, facilitating the persistent development of these endometrial tissues outside the uterus[8].

Despite its widespread occurrence, this serious disease condition is poorly understood, with recent research demonstrating no link between the disease's symptomatology and severity[9]. Although a significant number of patients with endometriosis are asymptomatic, severe pain during menstruation is termed dysmenorrhea, dysfunctional uterine bleeding, infertility, non-cyclical chronic pelvic pain, pain during sexual intercourse due to medical or psychological stress termed dyspareunia, painful or difficult urination termed as dysuria, constipation associated with a defective reflex for defecation termed as dyschezia, urinary tract symptoms, and gastrointestinal symptoms are all common symptoms[10-12].

There hasn't been an effective treatment for endometriosis up until now[13]. Pharmacological therapies aim at inhibiting the expansion of endometriotic implants, and surgical treatments aim at removing or destroying endometriotic implants, which are the two types of treatments available[14]. In addition, medical care is the greatest approach to controlling the symptoms of pain. Most patients with endometriosis-related pelvic pain appear to benefit from a combination of medication and conservative surgical treatment[15]. Since this is an estrogen-dependent disease, current medical treatments are aimed at lowering estrogen levels in the blood, which is usually accomplished by lowering the synthesis of steroid hormones (estradiol) in the ovary. Out of several types of medications available to treat endometriosis, aromatase inhibitors are one of the most effective and novel therapeutics considered, which have shown promising preliminary results[16]. Endometriosis drugs that block the aromatase enzyme systems of endometrial and endometriotic tissue should potentially render benefits since they lower estrogen production locally[17,18]. Aromatase P450 converts C19 steroids to estrogens in a catalytic manner, in a variety of organs, including placenta and ovary in premenopausal women[19]. This enzyme complex is composed of two polypeptides, namely the aromatase cytochrome P450 (CYP450arom), produced from a single gene, CYP19, and NADPH-cytochrome P450 reductase, which is a flavoprotein and is widely present in the majority of cells[17,20]. In normal endometrial and myometrial tissues, the expression of this enzyme appears to be undetectable. On the other hand, this enzyme complex was shown to be expressed in endometriosis patients' eutopic and ectopic endometrium[21]. Its overexpression in the cells of endometriotic tissues has been found, which underlines how durable and self-sustaining endometriotic cells appear to be. Endometriotic implants produce local estrogen as a result of aberrant aromatase expression. The autonomously produced estrogen, in combination with other processes such as alterations in the immune responses, angiogenetic mechanisms, and apoptosis, causes inflammation, and proliferation, and promotes survival of these implants[22-24]. Employing aromatase inhibitors to decrease the local production of estrogen by endometriotic deposits has picked up traction as a potential means of treatment for people with endometriosis who have impaired endocrine and reproductive function[24,25]. Additionally, these inhibitors are a relatively new treatment option for postmenopausal endometriosis[20].

Three generations of aromatase inhibitors have been developed. The first generation of inhibitors, such as Glutethimide, causes a clinical adrenalectomy, which has several adverse effects, such as nausea, lethargy, and skin rashes, in addition to the desired result. Fadrozole and Formestancel are second-generation inhibitors that are much more selective and have fewer negative consequences in comparison to the first. Letrozole, Anastrazole, and Examestane are third-generation inhibitors of aromatase that are derived from triazole and are potent, reversible, and selective, making them appropriate for clinical application[26]. Most side effects caused by these third-generation inhibitors are minor, with moderate headaches, stiffness or discomfort in the joints, nausea, and diarrhoea being the most prevalent. Long-term or continuous use may raise the incidence of osteopenia, bone fractures, and osteoporosis in women[20,27].

Several synthetic drugs, such as Danazol, Depo-Provera, etc., are used to treat endometriosis to lessen estrogen production and alleviate the severe symptoms of this disease. Depo-Provera, chemically known as medroxyprogesterone acetate, is a hormonal medication of

the progestin type and acts by inhibiting the production of estradiol, lowering the estradiol-dependent endometrial proliferation[28,29]. Danazol is a derivative of the synthetic steroid ethisterone, a modified form of testosterone, and has anti-estrogenic property[30].

Nonetheless, given the side effects caused by these synthetic drugs, natural drugs such as curcumin, chamomile, and lavender may serve better. Turmeric (*Curcuma longa*), a member of the Zingiberaceae family of ginger plants, contains curcumin, the main curcuminoid, which has potent anti-inflammatory, antioxidant, and anti-angiogenic properties[31]. These properties of curcumin are responsible for its role in treating endometriosis[32]. Chamomile is often used to reduce the pain symptoms of endometriosis because of its soothing properties[33]. The main chemical constituent of chamomile is chrysin. Chrysin, also called 5,7-dihydroxyflavone, is a flavone found in chamomile extracts. Lavender is a flowering plant having calming and soothing properties and is hence used in treating the pain symptoms of endometriosis[34]. Lavender oil is used to extract some 100 individual phytochemicals, comprising higher concentrations of caryophyllene (8%), tannins (5–10%), linalool (20–35%), and linalyl acetate (30–55%), with lower concentrations of sesquiterpenoid, perillyl alcohol, oxides, ketone, esters, and caryophyllene oxide[35,36]. The relative concentrations of these compounds differ significantly between species of lavender.

Keeping this perspective in mind, we have docked these synthetic as well as natural drugs with the aromatase enzyme to compare their binding efficiencies in inhibiting estrogen synthesis. Additionally, in this work, we have performed an *in-silico* Molecular Dynamics (MD) simulation for the curcumin-aromatase complex to determine the physical movement of the atoms for deciphering the stability of the docked complex.

2. Methods

A crucial tool in logical drug design and protein function studies is protein-ligand interaction[37]. A variety of intermolecular interactions, such as electrostatic, hydrophobic, van der Waals, etc., are used to characterize protein-ligand interactions. Typically, protein-ligand interaction modelling consists of two sequential steps: prediction of the binding affinity and estimation of the corresponding binding affinity. The first one forecasts the optimal location of a ligand within the protein target's binding pocket, whilst the second one determines the binding free energy between the ligand and the protein for that docking pose. One prevalent technique for determining ligand binding affinities and modes is protein-ligand docking. In this work, a convenient blind docking web server known as CB-Dock, a cavity detection-guided blind docking method, was used for the docking of the drugs with the aromatase enzyme to predict their docking efficiencies.

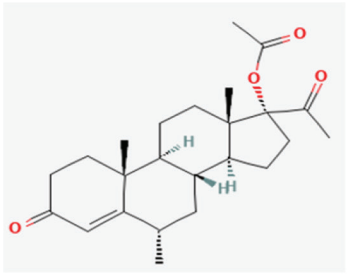
2.1 Data Retrieval

The protein sequence of the aromatase enzyme was retrieved from NCBI. The chemical structures (Figure 1) of natural drugs such as curcumin, lavender, and chamomile, as well as synthetic drugs such as depo-provera and danazol, were obtained from the PubChem database.

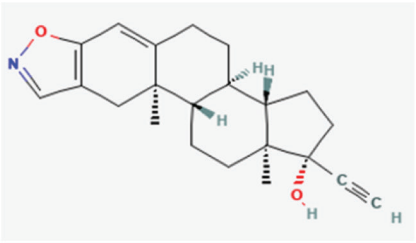
2.2 Protein Structure Prediction, Molecular Modelling, and Docking

The protein structure of aromatase was built using a modeler based on homology modelling, and an energy minimization step employing the CHARMM force field was then performed to obtain a stable structure for molecular docking investigation. The selected drugs' in-silico docking with aromatase was executed using the CB-Dock web server, which runs on the AutoDock Vina Docking algorithm of version v.1.2.0. A protein-ligand docking method called CB-Dock finds binding sites automatically (i.e., detects curvature-based cavities), computes centre and size, customises box sizes of docking based on query ligands, followed by execution of molecular docking using the prevalent AutoDock Vina docking program to determine the correct conformations and configuration of the ligands which have minimum energy structure[38]. Here, we have selected the largest cavities for flexible docking in each case. The binding modes were also ranked in this approach based on Vina scores and are displayed in an interactive 3D visual representation.

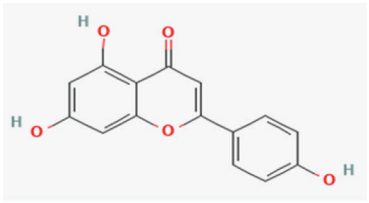
The best docking poses (conformations) were downloaded and then PyMOL and Discovery Studio 4.0 visualizer were used for post-docking analysis, visualization, and analysis of the comprehensive molecular interactions in the docked complexes. The protein-ligand interactions of the drugs with the



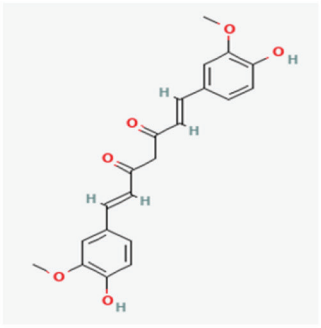
A) Depo-Provera
IUPAC name: [(6S,8R,9S,10R,13S,14S,17R)-17-acetyl-6,10,13-trimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1*H*-cyclopenta[a]phenanthren-17-yl] acetate



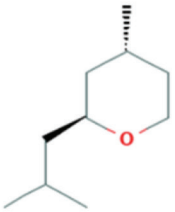
B) Danazol
IUPAC name:(1*S*,2*R*,13*R*,14*S*,17*R*,18*S*)-17-ethynyl-2,18-dimethyl-7-oxa-6-azapentacyclo[11.7.0.0^{2,10}.0^{4,8}.0^{14,18}]icosa-4(8),5,9-trien-17-ol



C) Chamomile
IUPAC name: 5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one



D) Curcumin
IUPAC name: 1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione



E) Lavender Pyran
IUPAC name: (2*S*,4*R*)-4-methyl-2-(2-methylpropyl)oxane

Figure 1. Structure and IUPAC nomenclature of the synthetic and natural drugs.

key amino acid residues on the target proteins were recorded with a special focus on non-covalent attractive interactions. All aromatase-drug complexes were analyzed for their interactions and binding affinity, and the curcumin-aromatase complex was further selected for molecular dynamics study.

2.3 Molecular Dynamics (MD) Simulation

MD simulations were done to verify that the docked complex is stable. The complexes positioned at the centre of the SPC216 water model's dodecahedron water box were subjected to an Optimized Potential for Liquid Simulations (OPLS) force field, with 9.5 Å specified as the solute-box distance. To prevent steric clashes and high energy interactions, the system was first energy-minimized using the steepest descent minimization for 50,000 steps until 10 kJ/mol tolerance was attained. By employing the GROMACS' genion program, the complex's total negative charges were balanced by an appropriate number of Na^+ ions to render the entire system neutral. Moreover, position-restrained dynamics were applied to energy-minimized structures for 80 ns, permitting water molecules to reach equilibrium while maintaining the stability of the entire protein system. The system that was optimized underwent a 100-ns MD run at 300 K and 1 atm (NPT ensemble). An estimate of the binding energy was made. To assess the simulations' stability, the root-mean-square deviation (RMSD) was determined for all the trajectories.

3. Results

3.1 Molecular Interactions Between Aromatase and Synthetic Drugs

3.1.1 Interaction between Danazol and Aromatase

The interaction between danazol and aromatase protein showed that Ile 132, Ile 133, Ala 306, Val 370, Cys 437, Ala 438, Leu 477, and Ser 478 were the key amino acids that were involved in the accommodation of danazol in the cavity of aromatase. The detailed protein-ligand interaction

types are as follows: Alanine 438 interacts via hydrogen bonds; Isoleucine 132, isoleucine 133, alanine 306, valine 370, and alanine 438 interact via alkyl bonds; Leucine 477 interacts via pi sigma bonds; Cysteine 437 interacts via carbon-hydrogen bonds; and Isoleucine 133 and Serine 478 interact via van der Waals interactions (Figure 2).

Tables 1-5, obtained from the CB-dock web server, provided information about docking centres, Vina scores, cavity sizes, and sizes of predicted cavities.

3.1.2 Interaction between Depo-Provera and Aromatase

Depo-Provera is accommodated inside the aromatase enzyme involving Gln 428, Lys 440, and Tyr 441. Amino acids which directly interact with Depo-Provera through various bonds are as follows- Glutamine 428, and Lysine 440 interact via hydrogen bonds, whereas Tyrosine 441 interacts via pi-sigma bonds (Figure 3).

3.2 Molecular Interactions between Aromatase and Natural Drugs

3.2.1 Interaction between Curcumin and Aromatase

Curcumin is a natural compound and its interaction with the aromatase involves Arg 115, Trp 224, Thr 310, Val 370, Met 374, and Cys 437. Out of all these interacting amino acids, arginine 115, threonine 310, and methionine 374 interact via five hydrogen bonds. In addition to this, cysteine 437 also interacts via pi-donor hydrogen bonds; tryptophan 224 interacts via pi-pi bonds, and valine 370 interacts via pi-sigma as well as pi-alkyl bonds (Figure 4).

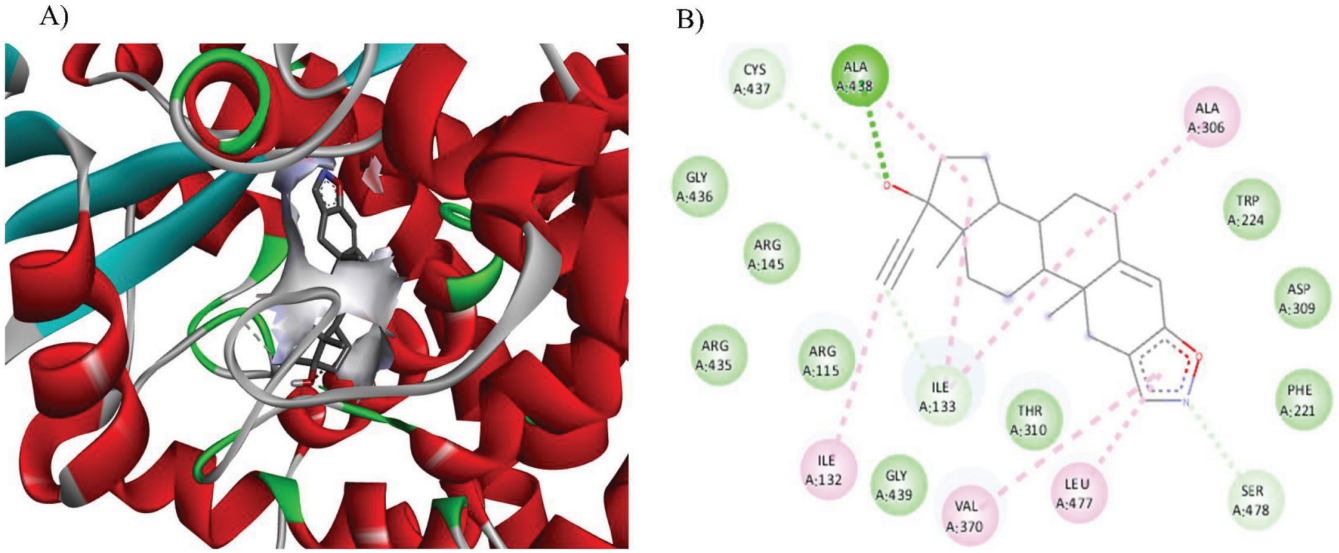


Figure 2. Docking of danazol with aromatase. **(A)** The image on the left side is the structure of the docked complex, obtained from CB-Dock. **(B)** The image on the right side shows the 2D view of the interactions between the ligand and the binding site of the protein.

Table 1. The cavity information and Vina scores of the outcomes

Cavities	Volume	Center x	Center y	Center z	Size x	Size y	Size z	Score
1	2081	84.067	50.405	49.897	21	21	31	-9
2	808	72.903	45.146	33.118	21	21	21	-8.8
3	559	98.628	37.872	37.24	21	21	21	-7
4	411	94.416	67.135	45.29	21	21	21	-8
5	289	69.464	59.317	48.617	21	21	21	-7.4

The largest cavity with a volume of 2081 was selected for flexible docking. The grid box centre values (centre X =84.067, Y = 50.405, Z = 49.897, size X = 21, Y = 21, Z = 31) were specified for a better conformational pattern in the target protein's active binding site (here, aromatase). The docking score obtained was -9 Kcal/mol.

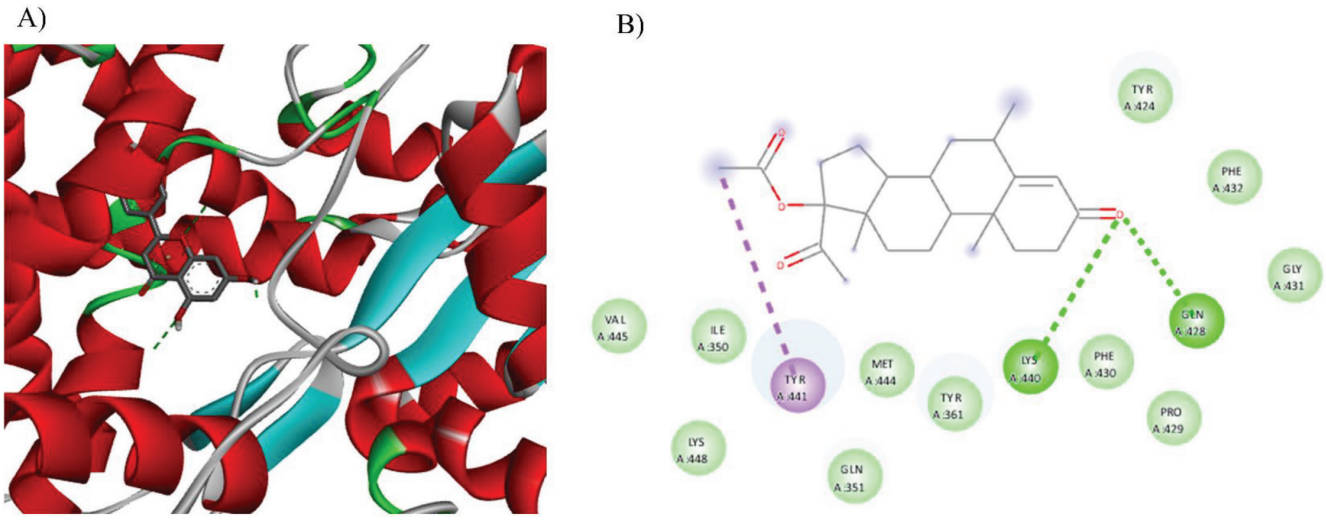


Figure 3. Docking of depo-provera with aromatase. **(A)** The image on the left side is the structure of the docked complex, obtained from CB-Dock. **(B)** The image on the right side shows the 2D view of the interactions between the ligand and the binding site of the protein.

Table 2. The cavity information and Vina scores of the outcomes

Cavities	Volume	Center x	Center y	Center z	Size x	Size y	Size z	Score
1	2081	84.067	50.405	49.897	21	21	31	-9
2	808	72.903	45.146	33.118	21	21	21	-8.8
3	559	98.628	37.872	37.24	21	21	21	-6.9
4	411	94.416	67.135	45.29	21	21	21	-8
5	289	69.464	59.317	48.617	21	21	21	-7.4

The largest cavity with a volume of 2081 was selected for flexible docking. The grid box centre values (centre X =84.067, Y = 50.405, Z = 49.897, size X = 21, Y = 21, Z = 31) were specified for a better conformational pattern in the target protein's active binding site (here, aromatase). The docking score obtained was -9 Kcal/mol.

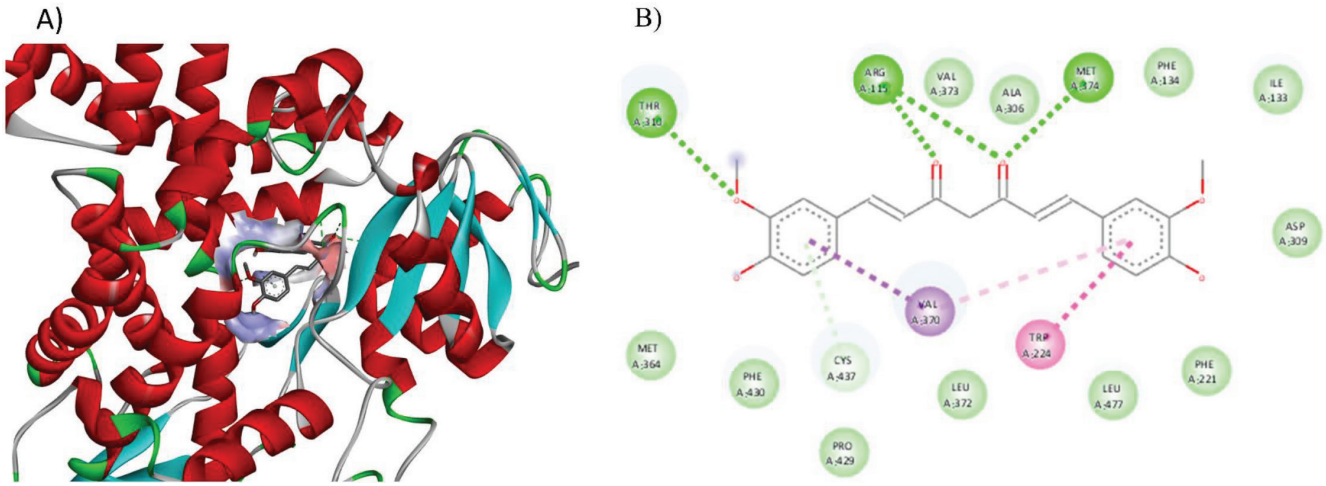


Figure 4. Docking of curcumin with aromatase. **(A)** The image on the left side is the structure of the docked complex, obtained from CB-Dock. **(B)** The image on the right side shows the 2D view of the interactions between the ligand and the binding site of the protein.

Table 3. The cavity information and Vina scores of the outcomes

Cavities	Volume	Center x	Center y	Center z	Size x	Size y	Size z	Score
1	2081	84.067	50.405	49.897	26	26	26	-8
2	808	72.903	45.146	33.118	26	26	26	-7.5
3	559	98.628	37.872	37.24	26	26	26	-6.3
4	411	94.416	67.135	45.29	26	26	26	-7
5	289	69.464	59.317	48.617	26	26	26	-7.1

The largest cavity with a volume of 2081 was selected for flexible docking.
The grid box centre values (centre X =84.067, Y = 50.405, Z = 49.897, size X = 26, Y = 26, Z = 26) were specified for a better conformational pattern in the target protein's active binding site (here, aromatase). The docking score obtained was -8 Kcal/mol.

3.2.2 Interaction between Chamomile and Aromatase

The interaction between chamomile and aromatase involves amino acids Ala 306, Ala 307, Thr 310, Met 311, Ser 314, Val 370, Pro 429, Phe 430, Cys 437, and Ala 443. Out of all these interacting amino acids, the amino acids which are directly interacting with chamomile through hydrogen bonds are Threonine 310, Serine 314, Proline 429, and Cysteine 437; Alanine 306, Alanine 307, Valine 370, Cysteine 437, and Alanine 443, interact via pi-alkyl bonds; Phenylalanine 430 interacts via pi-pi T-shaped bonds, and Methionine 311 and Cysteine 437 interact via pi-sulfur bonds (Figure 5).

3.2.3 Interaction between Lavender and Aromatase

The interaction between aromatase protein and lavender involves amino acids Ala 306, Ala 438, Gly 439, and Met 446. Here, Alanine 306, Alanine 438, and Methionine 446 interact via alkyl bonds, whereas glycine 439 interacts via hydrogen bonds (Figure 6).

Table 6 gives a comparative analysis of the drugs 'affinity and binding efficiency with the aromatase enzyme for treating endometriosis.

3.3 Molecular Dynamics (MD) Simulation Studies of Aromatase-Drug Complexes

The structural conformations and real movement of protein complexes in a biological system could be represented by MD simulations. The root mean square deviation (RMSD) is a well-known parameter of protein stability and equilibration. We determined the RMSD of the protein's C-α backbone atoms (Figure 7). The profiles of RMSD were recorded to stabilize at around 0.3 nm for the curcumin-aromatase complex and were found to be stable for the complex for the duration of the simulation, suggesting that the complex of aromatase and curcumin is stable. Hydrogen bonds are a major component that provides robust molecular interactions in biological systems. Moreover, the protein-ligand complexes are dynamic, leading to conformational changes throughout the whole molecular dynamic simulation; therefore, the number of hydrogen bonds created was calculated for the entire duration of MD simulations (Figure 7). In the curcumin-aromatase complex, the greatest number of conformations formed around 0 to 4 hydrogen bonds. Very few conformations created hydrogen bonds around 5.

The RMSD is an estimator of the protein-ligand complexes' stability during simulations. The RMSD profile of the curcumin-aromatase complex was plotted. These profiles were recorded to stabilize at around 0.3 nm for the complex. Root mean square fluctuation (RMSF) is calculated to see the fluctuation of each atom over the entire simulation time. The RMS fluctuation values for 500 amino acids were found to be between 0.08 to 0.55 nm. The average RMSF values vary from 0.08 to 0.25 nm, showing that the complex binding sites have fewer fluctuations overall. The radius of gyration(Rg) of the curcumin-aromatase complexes was found to be between 2.22 and 2.30 nm initially during the simulation period. The Rg values of the complex were stabilised after 50 ns, which served as an indicator for the stable binding pose of the curcumin-aromatase complex.

4. Discussion

Endometriosis is an estrogen-dependent inflammatory condition in which endometrial glands and stroma are found external to the uterus and cause pelvic pain and infertility[13]. It was first reported around 300 years ago. In addition to the granulosa cells of the ovary, aromatase p450,

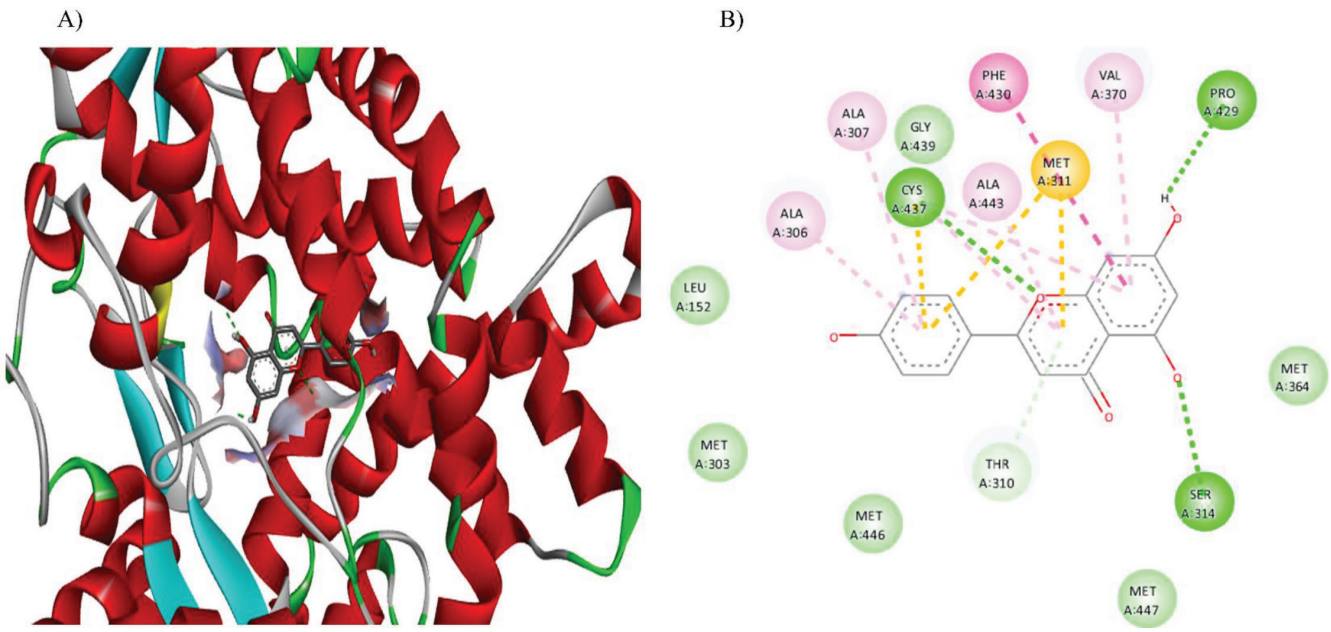


Figure 5. Docking of chamomile with aromatase. **(A)** The image on the left side is the structure of the docked complex, obtained from CB-Dock. **(B)** The image on the right side shows the 2D view of the interactions between the ligand and the binding site of the protein.

Table 4. The cavity information and Vina scores of the outcomes

Cavities	Volume	Center x	Center y	Center z	Size x	Size y	Size z	Score
1	2081	84.067	50.405	49.897	21	21	31	-8.1
2	808	72.903	45.146	33.118	21	21	21	-7.3
3	559	98.628	37.872	37.24	21	21	21	-6.8
4	411	94.416	67.135	47.29	21	21	21	-7
5	289	69.464	59.317	48.617	21	21	21	-7.8

The largest cavity with a volume of 2081 was selected for flexible docking. The grid box centre values (centre X =84.067, Y = 50.405, Z = 49.897, size X = 21, Y = 21, Z = 31) were specified for a better conformational pattern in the target protein's active binding site (here, aromatase). The docking score obtained was -8.1 Kcal/mol.

an essential enzyme in the production of E2, is aberrantly expressed in the eutopic endometrium and endometriotic deposits in endometriosis-affected women[39]. As a result, orally active third-generation Aromatase inhibitors (AIs) such as Letrozole and anastrozole, have attracted interest as adjunctive endometriosis treatment-related infertility. Chronic pelvic pain due to endometriosis may be relieved with aromatase inhibitors. Estrogens are required for endometriotic tissue growth and survival. Traditional endometriosis treatments focus on Estradiol (E2) production from the ovaries. They produce very little effect on estrogens produced by other organs. Estradiol is a form of estrogen that is biologically active. AIs are designed to target E2 production from extraovarian sources, but they also promote E2 production from the ovaries by producing an elevation in the levels of Follicle-Stimulating Hormone (FSH). As a result, coupling AI with conventional medications should be useful in treating endometriosis by blocking ovarian as well as extraovarian production of E2[18,28,40]. Nevertheless, some side effects are associated with all three generations of AIs in the management of this illness. Thus, in this work, the aromatase enzyme complex has been docked with synthetic as well as natural drugs for the comparison of their binding efficiencies to propose drug formulations based on natural compounds.

For in-silico molecular docking, CB-Dock was used. Most of the small-molecule binding takes place in protein pockets or cavities because high affinity is

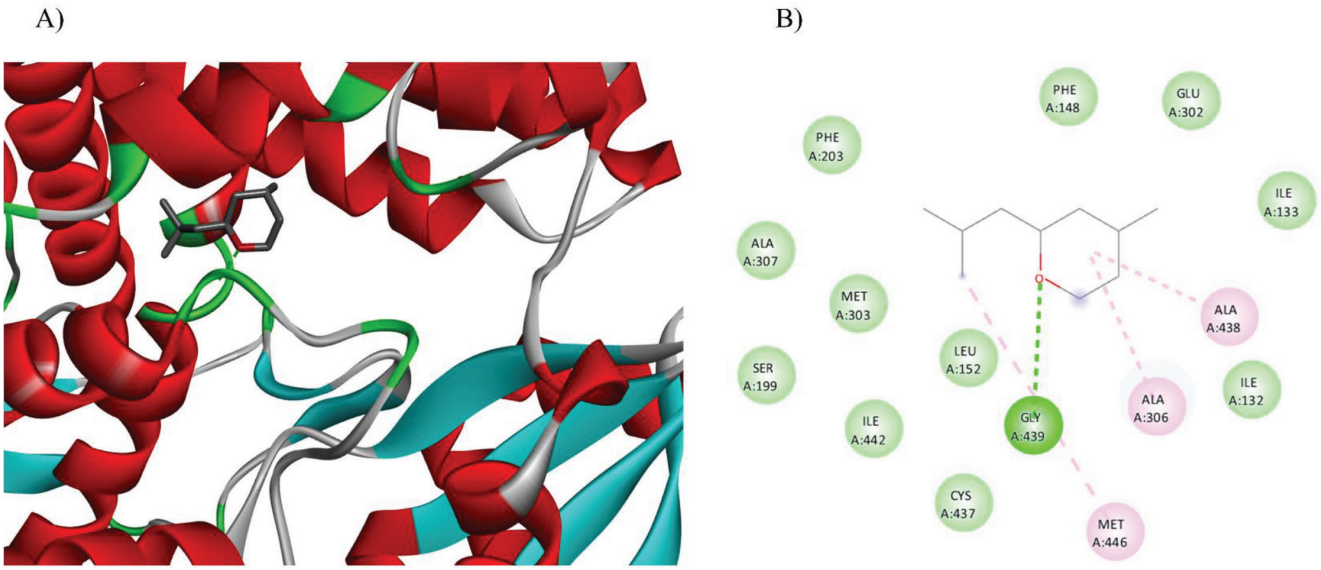


Figure 6. Docking of lavender with aromatase. **(A)** The image on the left side is the structure of the docked complex, obtained from CB-Dock. **(B)** The image on the right side shows the 2D view of the interactions between the ligand and the binding site of the protein.

Table 5. The cavity information and Vina scores of the outcomes

Cavities	Volume	Center x	Center y	Center z	Size x	Size y	Size z	Score
1	2081	84.067	50.405	49.897	23	24	31	-5.5
2	808	72.903	45.146	33.118	17	17	17	-5.3
3	559	98.628	37.872	37.24	17	17	17	-5.2
4	411	94.416	67.135	45.29	17	17	17	-4.3
5	289	69.464	59.317	48.617	17	17	17	-4.3

The largest cavity with a volume of 2081 was selected for flexible docking.
The grid box centre values (centre X =84.067, Y = 50.405, Z = 49.897, size X = 23, Y = 24, Z = 31) were specified for a better conformational pattern in the target protein's active binding site (here, aromatase). The docking score obtained was -5.5 Kcal/mol

Table 6. Comparison of results obtained from docking studies

Drugs docked with Aromatase	Maximum Docking Scores
Danazol	-9
Depo-Provera	-9
Chamomile	-8.1
Curcumin	-8
Lavender	-5.5

achievable only by appropriately large contact interfaces. To detect cavities, CB-Dock looks for concave surfaces. For the next computation, a docking box for a putative cavity needs to be customized by CB-Dock. A decent docking box encloses the native binding posture while excluding the maximum number of extraneous poses. The docking box's centre and size are the most important criteria in this operation. In docking, the more negative the score, the lower the binding free energy and hence the higher docking efficiency[41]. The pose of the docked complex with the lowest binding energy possesses the highest binding affinity and hence is more stable. In recent years, molecular dynamics (MD) simulation has emerged as a significant computational tool used for the research on aromatase inhibition to treat endometriosis. With the aid of MD simulation, the stability of the curcumin-aromatase docked complex was evaluated. Their computational results demonstrated that curcumin is suitable to be proposed for natural-compound-based drug formulations. It has been reported recently that curcumin has therapeutic potential as an anti-cancer, anti-

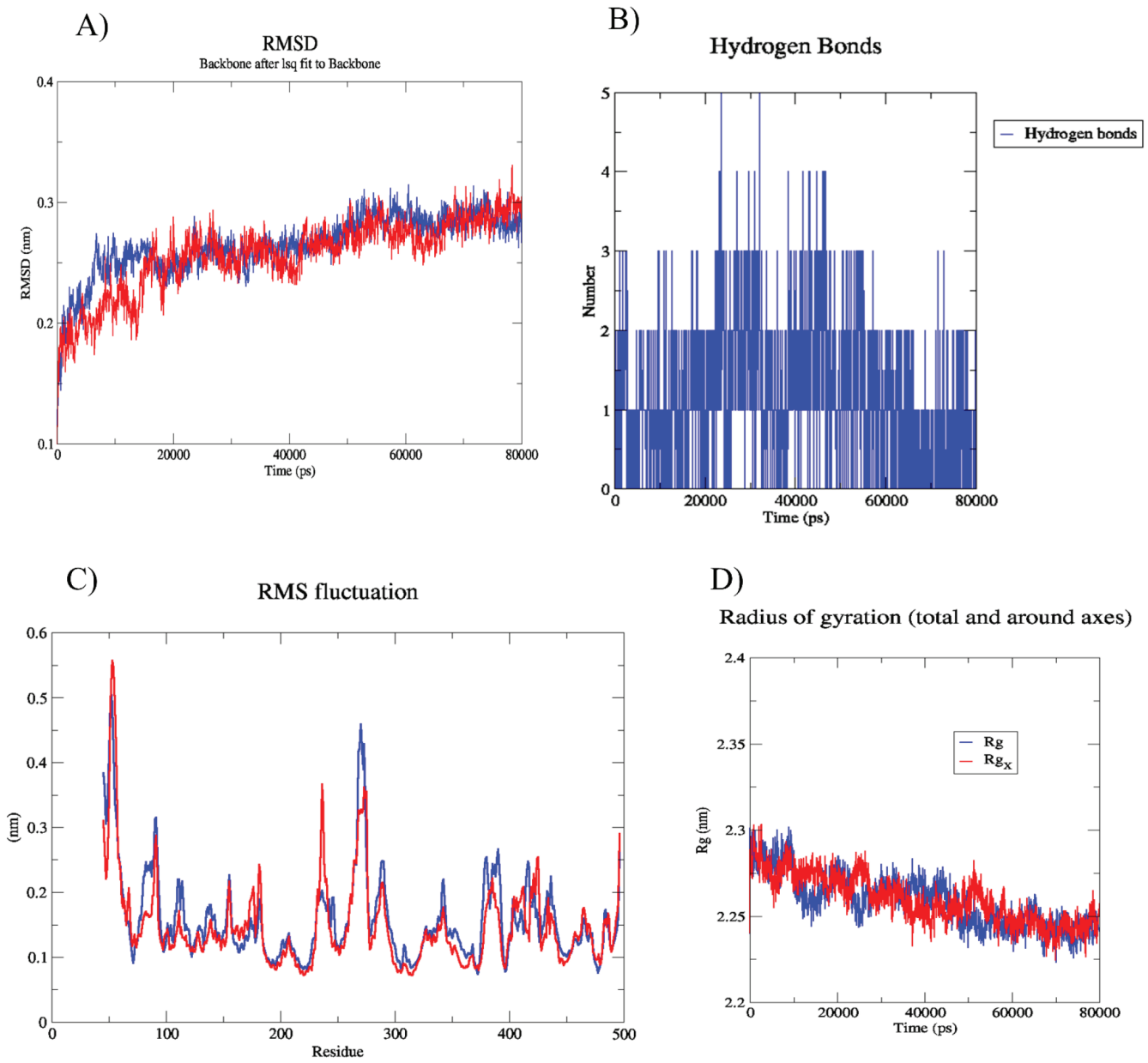


Figure 7. Results obtained during the 80 ns MD simulation studies on the curcumin-aromatase complex. **(A)** Calculation of RMSD of the complex. **(B)** Calculation of several hydrogen bond formation between aromatase and curcumin. **(C)** Calculation of Root-Mean-Square Fluctuation (RMSF). **(D)** Calculation of Rg to probe for the complex's wideness and compactness.

inflammatory, and anti-ageing compound by reducing estradiol synthesis[42]. Curcumin has been shown to reduce inflammation and oxidative stress in endometriosis patients[43,44]. Curcumin can also have a direct effect on endometrial lesions' invasiveness, adhesion, apoptotic death, and angiogenesis[44-46].

Nonetheless, more research into the outcomes of treatment for endometriosis with aromatase inhibitors is required. Furthermore, bigger multi-centre randomized clinical trials involving these inhibitors for the management of endometriosis-associated persistent pelvic discomfort are required. In conclusion, AIs may be useful in the treatment of endometriosis-associated persistent discomfort in the pelvis in reproductive-age as well as post-menopausal women. However, more research and future clinical trials need to be performed to investigate and highlight the therapeutic role of curcumin *in vivo* for treating endometriosis.

5. Conclusions

Endometriosis is a rapidly emerging problem worldwide, affecting women in their reproductive age group. It is associated with excruciating pelvic pain and many other serious gynaecological complications. The treatment options available for endometriosis target the symptoms and not the cause. Many of the medicines used are not effective against all patients, and selective therapy needs to be done. The bioinformatics investigation into the docking efficiency of various drugs on aromatase revealed that Depo-Provera and danazol have the same docking score of -9 and, out of the natural compounds, chamomile, and curcumin both have an almost identical docking score of -8. Lavender has the least negative docking score of -5.5 and hence has the least docking efficiency. Curcumin can be used as an effective treatment for endometriosis since natural compounds are associated with fewer side effects. Several studies have also documented the effects of curcumin in treating endometriosis, and they have demonstrated positive results. In conclusion, docking aromatase with all the subjects of interest reveals that this protein shows propinquity towards curcumin (-8), one of the natural drugs for treating endometriosis. Furthermore, the stability of the curcumin-aromatase complex is being established with the help of MD simulations. Therefore, there is ample scope for research in this area to delineate different kinds of drugs, their sites, and modes of action, and determine their efficacy for treating the disease. Future research agendas ought to tackle further inquiries, on the application of aromatase inhibitors in women with endometriosis, such as the best time for AIs co-treatment, the appropriate dosage, and the definite impact on endometrial receptivity. Thus, more investigations are still required to provide precise and unambiguous evidence that will direct our clinical practice and aid in achieving the greatest outcomes. Further studies are necessary to conclude whether these treatments would be of value for the treatment of endometriosis.

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