



Identification of Fungal Bioactive Compounds Targeting MMP-9 for Endometriosis- An *In silico* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FN and CAD participated in the design of the study. Author FN performed the docking, visualization analysis, wrote the manuscript and managed the literature searches. Authors FN, CAD and MR revised the manuscript to be published. All authors read and approved the final manuscript.

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ABSTRACT

Background: Endometriosis is a chronic inflammatory disease of the female reproductive system characterized by the presence of endometrial tissue outside the uterus that affects 5 to 10% of women of reproductive age, which is approximately 176 million women in the world. The women suffering from endometriosis have been reported to have high levels of matrix metalloproteinase (especially MMP-9) which regulates the inflammatory process. Thus, the aim of this study is to investigate the naturally available anti-inflammatory fungal compounds that can target the MMP-9 by various *in silico* approaches.

Methodology: A wide variety of anti-inflammatory bioactive compounds were screened and five compounds were further selected based on 'Lipinski's rule of five' using the PubChem database. The bioavailability, pharmacokinetics, ADMET properties and biological activity of these compounds were predicted computationally using databases such as SWISS-ADME, PubChem, pkCSM and PASS. The target 1L6J (Crystal structure of human matrix metalloproteinase MMP-9) structure was retrieved from the PDB database. Comparative analysis of the bioactive compounds

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with the target was performed by AutoDock 4.2.6 and further visualisation of the target residues interacting with the compounds was performed using LigPlot v.2. 2. tool.

Results: Based on the docking results, the compounds namely, Ergosterol peroxide, Lovastatin, Javanicin, Asperlin and Ergothioneine exhibited binding energy value of -10.25 kcal/mol, -8.4 kcal/mol, -7.64 kcal/mol, -7.07 kcal/mol and -6.19 kcal/mol respectively whereas Elagolix (control drug) exhibited binding energy value of -4.88 kcal/mol, thus, indicating that the selected bioactive compounds were seen to have better binding energy comparative to the control drug.

Conclusion: Ergosterol peroxide derived from edible mushroom might act as a potential lead compound for designing a therapeutic drug for treating endometriosis and this compound can further be explored to evaluate its level of toxicity and efficacy in the wet laboratory studies by *in vitro* and *in vivo* methods.

Keywords: Endometriosis; matrix metalloproteinase-9; Lipinski rule; Ergosterol peroxide; Elagolix.

ABBREVIATIONS

ASRM	: American Society for Reproductive Medicine
DIE	: Deep Infiltrating Endometriosis
MMP	: Matrix Metalloproteinase
GELB	: Gelatinase B
PTK7	: Protein Tyrosine Kinase 7
MAPK	: Mitogen-Activated Protein Kinase
NF-Kb	: Nuclear Factor Kappa B
Gn-RH	: Gonadotropin-releasing hormone
BMD	: Bone mineral density
PDB	: Protein Data Bank
CASTP	: Computed Atlas of Surface Topography of Proteins
SDF	: Structure Data File
SMILES	: Simplified Molecular-Input Line-Entry System
PASS	: Prediction of Activity Spectra for Substances
HBD	: Hydrogen bond donor
HBA	: Hydrogen bond acceptor
RBC	: Rotatable bond count
TPSA	: Topological polar surface area
ADMET	: Absorption Distribution Metabolism Elimination and Toxicity
VD	: Volume Distribution

1. INTRODUCTION

Endometriosis is a chronic inflammatory disease which occurs due to the presence of endometrial tissue outside the uterine cavity that affects 5 to 10% of women of reproductive age (between the ages of 15 to 49), which is approximately 176 million women in the world. The Endometriosis Society of India had estimated that 26 million Indian women are suffering from endometriosis.

1.1 Symptoms, Stages and Classification of Endometriosis

The most common symptoms of endometriosis include inflammation, dysmenorrhea, abdominal pain, dyspareunia, chronic pelvic pain [1],

infertility, menstrual pain and heavy bleeding [2,3], miscarriage [4] and implantation failure [5,6].

According to the American Society for Reproductive Medicine (r-ASRM) classification [7], endometriosis can be categorized into four stages (Table 1) based upon the location, extent, depth of the endometriosis implants, severity of scar tissue and size of endometrial implants in the ovaries.

On the basis of the location of endometrial tissue, endometriosis can be classified [8] into three main types as: Peritoneal Superficial Endometriosis, Ovarian Endometriomas /chocolate cysts and Deep Infiltrating Endometriosis (DIE).

1.2 Molecular Mechanism of Endometriosis

Though the exact cause of endometriosis remains unclear, several theories and molecular mechanisms have been presented to understand its development. Matrix Metalloproteinases (MMPs) is one such protein involved in the inflammatory process contributes to the progression of endometriosis matrix remodelling process such as reflux, adhesion, proteolysis, proliferation, angiogenesis, and scarring [9]. Among the different types of MMPs, MMP-9 also called as Gelatinase B (GELB) or 92 kDa gelatinase belong to the zinc-metalloproteinase family, which play an important role in tumour formation, angiogenesis-associated diseases and also is known to be involved in the

degradation of the extracellular matrix. The MMP-9 levels are reported to vary with different types of endometriosis and between patients without endometriosis [10]. Therefore, MMP-9 is considered an important biomarker for endometriosis (Fig. 1).

Protein Tyrosine Kinase 7 (PTK7) has been known to activate the mitogen-activated protein kinase (MAPK) and NF-kappaB pathway which have been involved in the upregulation of MMP-9 as seen in the Fig. 1. MAP3K further activates ERK and JNK pathways which in turn activate the AP-1 complex components c-Fos and c-Jun. NF-kB and AP-1 bind to the corresponding binding sites and transactivates the MMP-9 which enhances invasive phenotype of the cells.

Table 1. Stages of endometriosis

Parameters	Stages			
	Stage 1	Stage 2	Stage 3	Stage 4
Severity	Minimal	Mild	Moderate	Severe
Location	Found in organs or tissue lining the pelvis or abdomen	They are located deeper in the tissue	Can spread to one or both ovary or fallopian tubes	Most widespread- to ovary, pelvic lining, fallopian tubes, bowels, intestines
Implants depth	Few small implants/ wounds/lesions and inflammation; no visible scar tissue	More implants than stage 1, presence of scar tissue, total affected area will be less than 2 inches of the abdomen	Many deep implants, small cysts and thick bands of scar tissue (adhesions)	Presence of deep implants, large cysts in ovaries & scar tissues near the intestines and around the ovaries or fallopian tubes
Point Score	1-5	6-10	16-40	>40

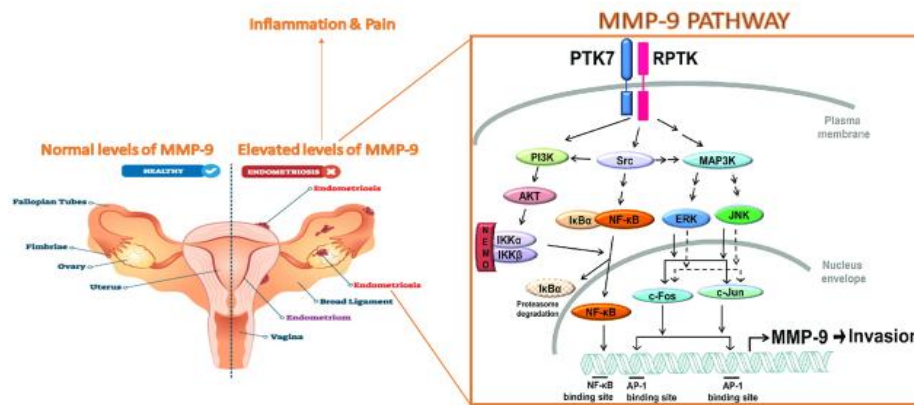


Fig. 1. Normal Healthy endometrium Vs endometriosis condition and MMP-9 pathway involved in endometriosis

1.3 Treatment of Endometriosis

1.3.1 Current treatment options available in endometriosis

The treatment options available for endometriosis include progestin therapy, surgery and using medications such as gonadotropin-releasing hormone (Gn-RH) agonists and aromatase inhibitors. Women who are suffering from endometriosis usually undergo laparoscopic surgery to remove the endometrial tissue. However, there are cases where the endometriosis tissue returns even after surgery, recurrent lesions can occur within a few years [11]. Hence, because of these reasons pain medications are a preferred choice of treatment to reduce the pain and provide relief to women suffering from endometriosis. Elagolix (sold under the brand name Orilissa), a gonadotropin-releasing hormone (GnRH) antagonist was approved for medical use for endometriosis treatment in July 2018 [12] but later was discontinued due to side effects by 5 to 10% of women in clinical trials.

1.3.2 Role of bioactive compounds in endometriosis

Though endometriosis can be treated effectively with these drugs, most of the treatments are not suitable for long-term use due to their side effects [13]. Currently, research needs to be done to find a suitable drug in the treatment of endometriosis which can provide a relief from pain without possessing any side effects. Hence, the natural bioactive compounds can be explored for endometriosis as the bioactive compounds from natural sources are considered to be much safer and non-toxic when compared to the synthetic drugs.

1.4 Current Approach of *In silico* Study

The *in silico* methodologies have been a crucial part in drug development process as it is considered to be a preliminary step in screening the drug compound with least side effects that can aid in the treatment of endometriosis when compared to the conventional methods which involves more cost and time.

Fungal bioactive compounds were screened by literature review, out of which five bioactive compounds such as Ergosterol peroxide, Lovastatin, Javanicin, Asperlin and Ergothioneine were selected.

2. METHODOLOGY

2.1 Preparation of Protein Structure

Protein structure of human matrix metalloproteinase MMP-9 (gelatinase B) with UniProt ID P14780 and PDB ID 1L6J having resolution of 2.5 Å, R-Value Free of 0.230 and R-Value Work of 0.187 was selected from the Protein Data Bank. The 3D structure of the MMP-9 was visualized by Biovia Discovery Studio Visualizer (Table 2). The active sites were predicted by Computed Atlas of Surface Topography of Proteins (CAST-P) (<http://sts.bioe.uic.edu/castp/index.html?3igg>) online tool. The heteroatoms (water molecules) were removed from the 1L6J structure. Before grid preparation, the polar hydrogen atoms and Kollman charge (which was -6.248) were added.

2.2 Ligand

Five bioactive compounds were selected based on literature review. PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) was used to extract the information such as PubChem ID, molecular formula and canonical SMILES. The molecular properties such as molecular weight, xlog P, Hydrogen Bond Donor, Hydrogen Bond Acceptor, Rotatable Bond Count, Topological Polar Surface Area were also predicted computationally using this database. The ligand structure was downloaded in 3D sdf format (.mol). By using the Open Babel software, the ligand file was converted from sdf (.mol) to PDB (.pdb) format and further saved for docking. The molecular properties were predicted computationally by this database. The ADMET, bioavailability and bioactivity of each compound was predicted computationally using the pkCSM (<http://biosig.unimelb.edu.au/pkcsm/prediction>), SWISSADME (<http://www.swissadme.ch/>) and Prediction of Activity Spectra for Substances (PASS) (<http://www.pharmaexpert.ru/passonline/index.php>) tool respectively.

2.3 Molecular Docking and Visualization

Molecular docking is a structure-based drug design approach that identifies the essential amino acid interaction between the target and ligand. Docking of selected bioactive compounds with the target MMP-9 was carried out using AutoDock 4.2.6. The procedure for docking in AutoDock 4.2.6 consisted of six stages, namely protein preparation, ligand preparation, grid preparation, running the grid, docking file

preparation, running AutoDock and analysing docking conformations. It consists of two main programs: *autodock* which performs the docking of the ligand to a set of grids describing the target and *autogrid* which pre-calculates these grids. The binding energy, hydrogen bond, Vander Waal's forces and intermolecular energy were noted. The docking interactions formed via the hydrogen bonds between the receptor and the compound can be analysed using LigPlot v2.2 in a 2D manner from standard pdb file input. It provides the information about the receptor-ligand interaction (hydrogen and hydrophobic interactions) and hydrogen bonds formed. Hydrogen bonds are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spikes radiating towards the ligand atoms they contact.

3. RESULTS AND DISCUSSION

3.1 MMP-9 Role in Various Disorders

The MMP-9 has been linked with several disorders other than endometriosis such as ovarian carcinoma [14], breast cancer [15], lung cancer [16], endometrial cancer [17], cervical cancer [18], neurodegenerative/ neural diseases [19], hypertensive disorder [20], autoimmune

disorder [21], Cardiovascular diseases [22], osteoporosis [23], Arthritis such as rheumatoid arthritis and focal brain ischemia, Pregnancy-associated malaria (Placental malaria), Poly Cystic Ovary Syndrome [24], Uterine fibroids [25]. Also, high levels of MMP-9 were seen in the Covid-19 patients with respiratory failure [26].

3.2 Properties of the Ligand Compounds selected for the study

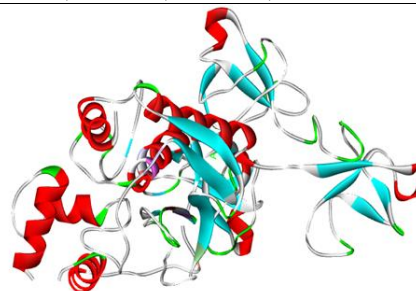
3.2.1 Selection of the ligand compounds based on literature review

During the clinical trial, women suffering from endometriosis were prescribed Elagolix which is a gonadotropin-releasing hormone (GnRH) antagonist [27], was approved for medical use in July 2018 [12], but serious adverse effects were observed such as appendicitis (0.3%), abdominal pain (0.2%), back pain (0.2%), and also other effects such as amenorrhea, decreased bone mineral density (BMD), changes in the blood lipid profile miscarriage, suicidality, and elevated liver enzymes. Because of this reason, Elagolix was discontinued by 5 to 10% of women in clinical trials and thus the natural bioactive compounds could be explored which is of natural origin with less side effects.

Table 2. Target selected for interaction studies

Target	MMP-9
UniProt ID	P14780
PDB ID	1L6J
DESCRIPTION	Crystal structure of human matrix metalloproteinase MMP9 (gelatinase B)
ORGANISMS	<i>Homo sapiens</i>
EXPERIMENTAL METHOD	X-ray diffraction
RESOLUTION	2.50 Å
ACTIVE SITES	ASP34, LEU35, ARG36, ASN38, LEU39, LEU44, GLU47, TYR48, ARG51, TYR52, LYS92, MET94, ARG95, THR96, PRO97, ARG98, ASP182, LYS184, ASP185, GLY186, LEU187, LEU209, GLY213, GLY215, VAL216, PHE232, PRO233, SER242, ASP334, MET338, ARG366, GLY367, ASP368, ARG370, LEU371, PHE387, CYS388, PRO389, ASP390, GLN391, GLY392, TYR393, TYR423, ARG424, PHE425

Visualization of MMP-9 by
Biovia Discovery Studio



Ergosterol peroxide derived from edible mushroom, *Pleurotus ostreatus* (oyster mushroom) possess therapeutic properties such as anti-inflammatory and anticancer. This compound was able to suppress LPS-induced inflammatory responses through inhibition of NF-kappaB and C/EBP β transcriptional activity, and phosphorylation of MAPKs and of cyclooxygenase related with the expression of many inflammatory mediators [28]; also was used to treat gynaecological cancer [29]. Another compound, Lovastatin derived from the same source *Pleurotus ostreatus*- oyster mushroom had shown to inhibit the mechanism of cell proliferation and angiogenesis in an experimental model for the development of endometriosis-like tissue [30]. In addition, it also showed the clinical relevance in treatment of endometriosis [31]. Javanicin, an endophyte derived from *Cloridium* species had shown to possess antibacterial activity and antifungal activity [32]. Asperlin, a fungi marine-derived from *Aspergillus* species

had shown to possess antifungal, anti-inflammatory and anti-atherosclerotic activities [33]. High quantity of ergothioneine is present in different types of edible mushrooms and has been found to have role in cardiovascular disease [34], preeclampsia [35], vascular and neurological disorders.

3.2.2 Structure retrieval of the ligand compounds

The 2D structures of the fungal bioactive compounds and control drug-Elagolix (Fig. 2) were retrieved from the PubChem database.

3.2.3 Prediction of properties-ligand compounds

The PUBCHEM CID, molecular formula and Canonical SMILES of the selected bioactive compounds have been mentioned in the Table 3.

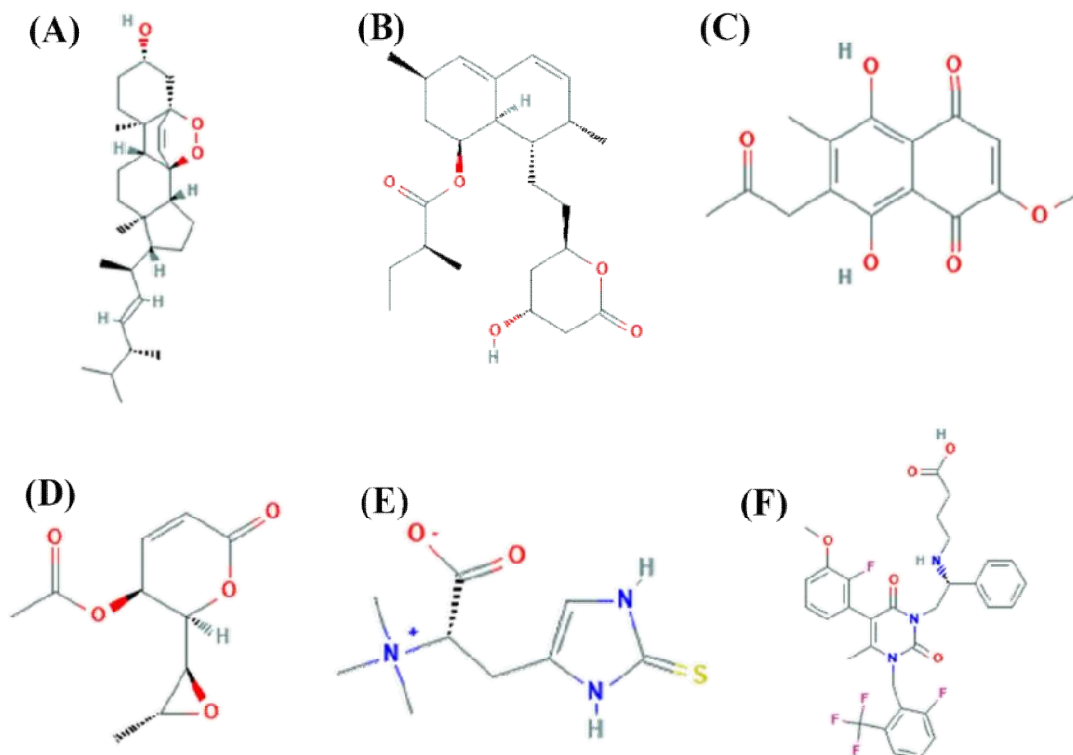


Fig. 2. 2D Structure of the Ligand Compounds Retrieved from PubChem (A) Ergosterol Peroxide, (B) Lovastatin, (C) Javanicin, (D) Asperlin, (E) Ergothioneine and (F) Elagolix

3.2.3.1 Chemical properties of the ligand

The bioactive compounds for docking were selected on the basis of whether the compound followed the 'Lipinski rule of five' as depicted in the Fig. 3, which is considered as a very important criterion in evaluating the efficacy of the drug. Log P commonly used to measure the lipophilicity. The hydrogen bond donor and acceptor are an important criteria to determine the ligand's specificity towards the receptor. Number of Rotatable Bonds (nrotb) is an important parameter to measure the molecular flexibility and oral bioavailability of drugs. An ideal compound should have nrotb less than 10; if it is less than 6, it means that the drug has good bioavailability. Topological Polar Surface Area (TPSA) is the sum of all polar atoms present in the molecule which can help in determining the drugs ability to penetrate the

cells. Molecules with TPSA greater than 140 \AA^2 are poor at penetrating the membrane. A drug is said to be active if there are not more than one violation seen in the Lipinski's rule.

All the compounds selected for the present study adhered to the Lipinski rule of five and showed bioavailability value more than 0.55, TPSA was less than 140 \AA^2 , violation of only one rule maximum was observed in the bioactive compounds. Whereas, the control drug Elagolix, which had a molecular weight of 631.6 g/mol. Log P value of 6, Hydrogen Bond Acceptor (HBA) value was more than 10, bioavailability was low also had two violations seen in Lipinski's rule, indicating that it is not an ideal drug of choice and accentuates the search for novel drugs in treatment of endometriosis.

Table 3. Bioactive compounds-PubChem CID, molecular formula and canonical SMILES

Compound	PubChem CID	Molecular Formula	Canonical SMILES
Ergosterol peroxide	5351516	C ₂₈ H ₄₄ O ₃	CC(C)C(C)C=CC(C)C1CCC2C1(CC3C24C=CC5(C3(CCC(C5)O)C)OO4)C
Lovastatin	53232	C ₂₄ H ₃₆ O ₅	CCC(C)C(=O)OC1CC(C=C2C1C(C=C2)C)CCC3CC(CC(=O)O3)O)C
Javanicin	10149	C ₁₅ H ₁₄ O ₆	CC1=C(C(=C2C(=C1O)C(=O)C=C(C2=O)OC)O)CC(=O)C
Asperlin (6-(1,2-Epoxypropyl)-5,6-dihydro-5-hydroxy-2H-pyran-2-one acetate)	9859172	C ₁₀ H ₁₂ O ₅	CC1C(O1)C2C(C=CC(=O)O2)OC(=O)C
Ergothioneine	5351619	C ₉ H ₁₅ N ₃ O ₂ S	C[N+](C)(C)C(CC1=CNC(=S)N1)C(=O)[O-]
Elagolix	11250647	C ₃₂ H ₃₀ F ₅ N ₃ O ₅	CC1=C(C(=O)N(C(=O)N1CC2=C(C=C2)C(F)(F)F)CC(C3=CC=CC=C3)NCCCC(=O)O)C4=C(C(=CC=C4)OC)F

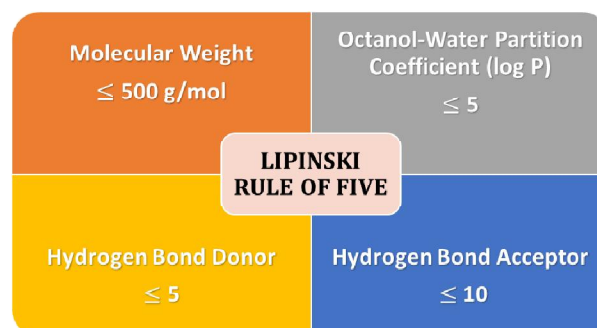


Fig. 3. Lipinski rule of five

The following parameters which were retrieved from the PUBCHEM database have been summarised in the Table 4.

3.2.3.2 ADME properties

The Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) describes the pharmacokinetics and pharmacodynamics properties of the drug that helps to know the drug likeliness of the bioactive compound with medicinal properties and these properties were predicted using pkCSM (Table 5) [36].

3.2.3.3 Absorption

Parameters such Caco-2 permeability and human intestine absorption were tabulated.

Intestinal absorption percentage predicts the proportion of the compounds that were absorbed through the human intestine. If the absorbance is more than 80%, it means that the molecule is well absorbed while if the absorbance value of the molecule is less than 30%, it means that it is poorly absorbed. Most of the compounds showed higher absorption value than compared to the control drug and the compound, Asperlin showed 100% which means it can be highly absorbed. Caco-2 permeability has been used to predict the absorption of orally administered drugs. Most of the compounds showed higher Caco-2 permeability whereas control drug such as Elagolix showed -0.228cm/s .

3.2.3.4 Distribution

The distribution parameter consists of the volume of distribution (Vd). If the volume of distribution

(Vd) value is below $-0.15 \log \text{L/kg}$, it is considered low and considered to be high if Vd is more than $0.45 \log \text{L/kg}$. the Elagolix had the lowest Vd value whereas Ergosterol peroxide had the highest Vd value of $0.552 \log \text{L/kg}$.

3.2.3.5 Metabolism

The cytochrome CYP450 is an important enzyme present in the liver which is responsible for the metabolism of many drugs. There are two isoforms of this enzyme, namely, CYP2D6 and CYP3A4.

3.2.3.6 Excretion

Total clearance of the drugs refers to the bioavailability to determine the dosing rates to achieve steady state concentrations.

3.2.3.7 Toxicity

AMES test determines whether the bioactive compound is mutagenic or not. Asperlin was the only compound that had mutagenic properties.

3.2.4 Bioactivity prediction of biocompounds-PASS database

Pa (probability "to be active") helps in predicting the bioactivity of the compounds. The activities that were having *Pa* value above 0.7 were tabulated which indicates that the compound is highly active to perform the specific activity. The results based on PASS indicated that all the bioactive compounds were showing various activities which had *Pa* above 0.7, whereas the control drug- Elagolix exhibited no activity when *Pa* value was above 0.7 (Table 6).

Table 4. Chemical properties of the ligand

Chemical Properties of the Ligands	Compounds					
	Ergosterol peroxide	Lovastatin	Javanicin	Asperlin	Ergothioneine	Elagolix
Molecular Weight g/mol	428.6	404.5	290.27	212.2	229.3	631.6
xlog P	6.7	4.3	2	0.3	0.3	2.6
Hydrogen bond donor	1	1	2	0	2	2
Hydrogen bond acceptor	3	5	6	5	3	11
Lipinski Rule Violation	1	0	0	0	0	2
Rotatable bond count	4	7	3	3	3	12
Topological polar surface area	38.7 \AA^2	72.8 \AA^2	101 \AA^2	65.1 \AA^2	96.3 \AA^2	99.2 \AA^2
Bioavailability	0.55	0.55	0.56	0.55	0.55	0.17

Table 5. Prediction of ADMET properties from pkCSM

ADMET Properties	Compounds					
	Ergosterol peroxide	Lovastatin	Javanicin	Asperlin	Ergothioneine	Elagolix
Intestinal Absorption (%)	94.682	94.728	70.858	100	93.938	76.963
Caco-2 (log Papp in 10 ⁻⁶ cm/s)	1.177	0.873	0.624	0.892	0.863	-0.228
Volume Distribution (log L/kg)	0.552	0.182	0.14	0.012	-0.451	-1.074
CYP3A4 Substrate	Yes	Yes	No	No	No	Yes
CYP2D6 Substrate & Inhibitor	No	No	No	No	No	No
Total Clearance (log mL/min/kg)	0.592	0.928	0.507	0.671	0.571	-0.035
AMES Toxicity	Non-Mutagen	Non-Mutagen	Non-Mutagen	Mutagen	Non-Mutagen	Non-Mutagen

Table 6. Prediction of compounds- bioactivity using PASS

Compound	Bioactivity (Pa above 0.7)
Ergosterol peroxide	Apoptosis agonist, Caspase 3 stimulant, Chemopreventive, Alcohol O-acetyltransferase inhibitor, Oxidoreductase inhibitor, CYP3A4 substrate, Alkylacetylglycero phosphatase inhibitor, CYP3A substrate, Acylcarnitine hydrolase inhibitor, Alkenylglycerophosphocholine hydrolase inhibitor, Antieczematic, Testosterone 17 β -dehydrogenase (NADP+) inhibitor, Dermatologic, Antipsoriatic, Cholesterol antagonist, Hypolipemic, Immunosuppressant
Lovastatin	Antihypercholesterolemic, Lipid metabolism regulator, Hypolipemic, Vasodilator, coronary, HMOX1 expression enhancer, APOA1 expression enhancer, CYP3A4 substrate, CYP3A5 substrate, CYP3A substrate, HMG CoA reductase inhibitor, Antifungal, Reductase inhibitor, Cholesterol synthesis inhibitor, Immunosuppressant, Antieczematic, CYP2D substrate, CYP2D6 substrate, Angiogenesis stimulant
Javanicin	Gluconate 2-dehydrogenase (acceptor) inhibitor, UGT1A9 substrate, UGT1A6 substrate, Ubiquinol-cytochrome-c reductase inhibitor, Chlordecone reductase inhibitor, Reductant, UGT1A3 substrate, Antiseborrheic, Aspulvinone dimethylallyltransferase inhibitor, UDP-glucuronosyltransferase substrate, UGT1A substrate, TP53 expression enhancer, Antimutagenic, Apoptosis agonist, HIF1A expression inhibitor
Asperlin	Antineoplastic, β glucuronidase inhibitor, Antihelminthic (Nematodes), CYP2A11 substrate, Antifungal, Phosphatase inhibitor, H ⁺ -exporting ATPase inhibitor, HIF1A expression inhibitor, Membrane integrity antagonist, TP53 expression enhancer, CYP2H substrate, CYP2C12 substrate
Ergothioneine	Dimethylhistidine N-methyltransferase inhibitor, Chloride peroxidase inhibitor
Elagolix	-

3.3 Docking

Docking was performed with the Crystal structure of human matrix metalloproteinase MMP-9 (gelatinase B) [PDB 1L6J [37]] and bioactive compounds.

Molecular docking and comparative analysis of the selected fungal bioactive compounds and the control drug was performed using the software AutoDock 4.2.6. Prior to docking, the solvent molecules were removed from the structure of the target and the active sites

residues in the target were retrieved using CASTp.

Each compound was docked individually and the results obtained from each docking provided information on the following parameters such as the Binding energy, Ligand efficiency, Intermolecular energy, Vander Waal's dissolved energy, Electrostatic energy and Torsional energy (Table 7).

3.3.1 Analysis of docking results and visualization

The selected bioactive compounds and control drug were individually docked against the MMP-9. All the selected bioactive compounds were shown to have binding energy more than the control drug-Elagolix, indicating that more research work is needed to study the efficacy of the drug and bring it to the clinical trial stage for the treatment of endometriosis.

The bioactive compounds such as Ergosterol peroxide, Lovastatin, Javanicin, Asperlin and Ergothioneine exhibited binding energy value of -10.25 kcal/mol, -8.4 kcal/mol, -7.64 kcal/mol, -7.07 kcal/mol and -6.19 kcal/mol respectively whereas Elagolix (control drug) exhibited binding energy value of -4.88 kcal/mol, which indicates that the selected compounds were seen to have better binding energy comparative to the control drug.

The hydrophobic interactions are the most common type of interaction seen in the protein-ligand complex and are said to be stronger than hydrogen bond, Vander Waal's forces and intermolecular energy. The MMP-9 target residues interacting with the bioactive metabolites and the control drugs has been visualized (Fig. 4)

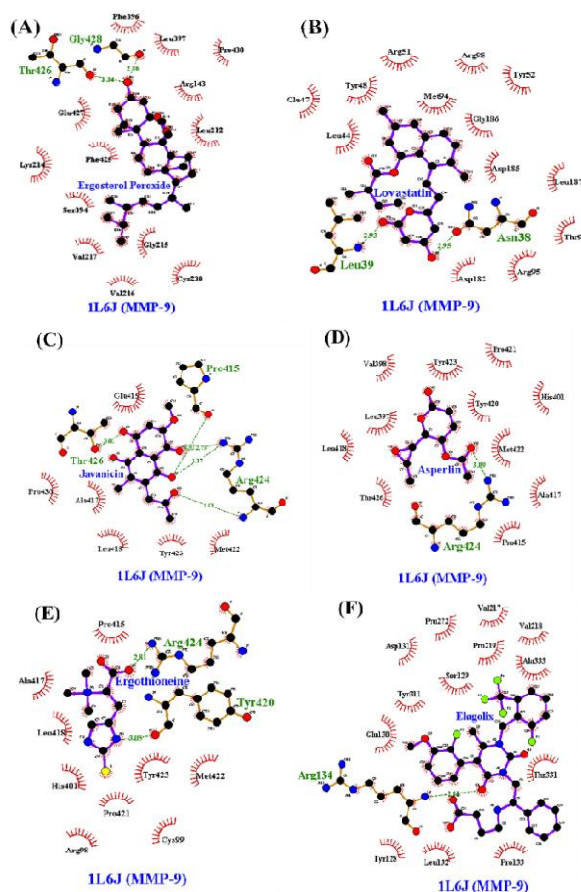


Fig. 4. Visualization by LigPlot + v.2.2 of MMP-9 with (A) Ergosterol Peroxide, (B) Lovastatin, (C) Javanicin, (D) Asperlin, (E) Ergothioneine and (F) Elagolix

Table 7. Summary of top ranked fungal bioactive compounds screened against MMP-9 with their respective binding energies, ligand efficiency, inter molecular energy, electrostatic energy, torsional energy, total internal unbound energy and interacting residues

M.D. of MMP-9 with	No. of H bond	Binding Eg	Ligand efficiency	Intermolecular Eg	vdW + hb + desolve Eg	Electrostatic Eg	Torsional Eg	Total internal unbound	1L6J (MMP-9) Residues Interacting with Metabolites
Ergosterol peroxide	2	-10.25	-0.33	-11.75	-11.48	-0.26	1.49	0.78	Thr426, Gly428 , Arg143, Leu313, Gly315, Val316, Val317, Cys330, Lys384, Ser394, Phe396, Leu397, Pro430, Phe435, Glu437
Lovastatin	2	-8.4	-0.29	-10.79	-10.78	-0.01	2.39	-2.61	Asn38, Leu39 , Leu44, Glu47, Tyr48, Arg51, Tyr52, Met94, Arg95, Thr96, Arg98, Asp182, Asp185, Gly186, Asp186, Leu187
Javanicin	3	-7.64	-0.36	-9.13	-8.78	-0.35	1.49	-1.73	Pro415, Arg424, Thr426 , Glu416, Ala417, Leu418, Met422, Tyr423, Pro430
Asperlin	4	-7.07	-0.47	-7.96	-7.72	-0.25	0.89	-0.27	Arg424 , Leu397, Val398, His401, Pro415, Ala417, Leu418, Tyr420, Pro421, Met422, Tyr423, Thr426
Ergothioneine	2	-6.19	-0.41	-7.38	-6.87	-0.51	-1.19	-1.05	Tyr420, Arg424 , Arg98, Cys99, His401, Pro415, Ala417, Leu418, Pro421, Met422, Tyr423
Elagolix	1	-4.88	-0.11	-9.06	-8.69	-0.37	4.18	-5.56	Arg143 , Glu130, Asp131, Leu133, Pro133, Tyr138, Ser139, Val307, Tyr311, Ala333, Thr334, Pro373, Val383, Pro389

4. CONCLUSION AND FUTURE PERSPECTIVE

Based on the properties prediction, it can be concluded that all the selected bioactive compounds did not violate more than one Lipinski's rule, only the control drug was seen to have more than one violations. When computationally predicted using PASS database it was shown that the bioactive compounds exhibited several activities when the Pa value was above 0.7, while the control drug exhibited no activity. The bioavailability of the compounds was predicted by SWISSADME and it was seen that the bioavailability of control drug was very low, which indicated that the control drug has poor absorption where all the bioactive compounds showed bioavailability higher than 0.55.

The molecular docking was performed using AutoDock 4.2.6 and visualization was done using LigPlot v2.2. Based on the results Ergosterol peroxide, Lovastatin, Javanicin, Asperlin and Ergothioneine exhibited binding energy value of -10.25 kcal/mol, -8.4 kcal/mol, -7.64 kcal/mol, -7.07 kcal/mol and -6.19 kcal/mol respectively whereas Elagolix (control drug) exhibited binding energy value of -4.88 kcal/mol.

Collectively, these results encourage further research of new drug from the fungal source which will effectively act against the MMP-9 receptor with the ultimate goal in the treatment of endometriosis and Ergosterol peroxide (source-edible mushroom) can be further investigated for its role in the wet laboratory studies by *in vitro* and *in vivo* study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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