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Original Article

In silico analysis of *Nigella sativa* bioactive compounds as fertility potential in folliculogenesis disorders

[Análisis *in silico* de compuestos bioactivos de *Nigella sativa* como potencial de fertilidad en trastornos de la foliculogénesis]

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Abstract

Context: The capacity to get pregnant is known as fertility. Every woman has reproductive rights to be fertile. In reality, there are still many cases of infertility. Infertility is due to impaired folliculogenesis. Low GDF-9 and estrogen receptors can be used as biomarkers in folliculogenesis disorders. *Nigella sativa* is a plant with medicinal properties as an antidote and protects against toxicity in the lungs, heart, liver, digestive tract, and reproductive system.

Aims: To identify the potential of the bioactive compounds *N. sativa* as activators of GDF-9 and estrogen receptor proteins on fertility in an *in silico* study approach.

Methods: All ligands from *N. sativa* extract were from previous studies, and protein preparations were taken from PubChem database. The conversion of the *sdf* file to *pdb* on the ligand was carried out using the OpenBabel v2.3.1 software. The drug-like molecule properties of chemical compounds from *N. sativa* were identified through a drug-likeness test on the SwissADME server and using PyRx v9.9.0 software, LigPlot+ v.2.2 software, and PyMol v2.5 software to analyze and visualize the potential of *N. sativa* compounds against GDF-9 and estrogen receptor.

Results: Compounds cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- have the lowest bond affinity, hydrogen, and hydrophobic interactions, as well as the same position of amino acid interactions to control.

Conclusions: Cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- can act as candidates for activating GDF-9 and estrogen receptors for fertility.

Keywords: estrogen receptor; GDF-9; molecular docking; *Nigella sativa;* reproduction rights.

Resumen

Contexto: La capacidad de quedar embarazada se conoce como fertilidad. Toda mujer tiene derechos reproductivos para ser fértil. En realidad, todavía hay muchos casos de infertilidad. La infertilidad se debe a una foliculogénesis alterada. Los bajos GDF-9 y los receptores de estrógeno pueden usarse como biomarcadores en los trastornos de la foliculogénesis. *Nigella sativa* es una planta con propiedades medicinales como antídoto y protege contra la toxicidad en los pulmones, corazón, hígado, tracto digestivo y sistema reproductivo.

Objetivos: Identificar el potencial de los compuestos bioactivos *N. sativa* como activadores de GDF-9 y proteínas receptoras de estrógenos sobre la fertilidad en un enfoque de estudio *in silico*.

Métodos: Todos los ligandos del extracto de *N. sativa* procedían de estudios previos y las preparaciones de proteínas se tomaron de la base de datos PubChem. La conversión del archivo sdf a pdb en el ligando se realizó utilizando el software OpenBabel v2.3.1. Las propiedades de las moléculas similares a fármacos de los compuestos químicos de *N. sativa* se identificaron a través de una prueba de similitud de fármacos en el servidor SwissADME y utilizando el software PyRx v9.9.0, el software LigPlot+ v.2.2 y el software PyMol v2.5 para analizar y visualizar el potencial. de compuestos de *N. sativa* contra GDF-9 y receptor de estrógenos.

Resultados: Los compuestos cis-13,16-ácido docasadienoico y colestan-3-ol, 2-metileno-, (3β,5α)- tienen la menor afinidad de enlace, interacciones de hidrógeno e hidrofóbicas, así como la misma posición de las interacciones de aminoácidos al control.

Conclusiones: El ácido cis-13,16-docasadienoico y el colestan-3-ol, 2-metileno-, (3β,5α)- pueden actuar como candidatos para activar los receptores de GDF-9 y estrógenos para la fertilidad.

Palabras Clave: acoplamiento molecular; derechos de reproducción; GDF-9; *Nigella sativa*; receptor de estrógeno.

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INTRODUCTION

Fertility is the ability to get pregnant (Zegers-Hochschild et al., 2017). Every couple must have the desire to get pregnant and have children. However, many couples still cannot get pregnant after marriage. Failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse is called infertility (Datta et al., 2016). Infertile couples of childbearing age worldwide are estimated at around 50-80 million. Large-scale studies have shown that about half of all cases of infertility occur due to female factors, 20 to 30% male factors, and 20 to 30% due to common causes of both genders (Babakhanzadeh et al., 2020). Infertility is a reproductive health problem that affects human rights. Infertility causes deep human suffering, especially on the part of women. For women, the effects of infertility can destroy a marriage. Therefore, reproductive rights include the right to overcome infertility problems (Inhorn, 2009). One of the causes of infertility is folliculogenesis disorders (Patel et al., 2015).

The ovaries contain numerous primordial follicles. Some of these follicles enter the growth phase and progress through the primary and secondary follicle stages. Follicular granulosa cells proliferate to develop a multi-layered structure during follicular development. Then, a fluid-filled cavity forms within the follicle during the antral follicle stage. This fluid-filled cavity is called the antrum. In the antral follicle, the granulosa cells separate to form cumulus cells, which envelop the oocyte (Wigglesworth et al., 2015). Two-way communication between the oocyte and granulosa cells is necessary for ovarian function and fertility (Alam et al., 2018). Granulosa cells supply nutrients, metabolites, and molecular signals to oocytes, while oocytes promote granulosa cell proliferation, differentiation, and function. Oocytes regulate follicular development (Matzuk et al., 2002). Oocytes are required for early primordial follicle formation and regulate the specific characteristics and functions of cumulus and mural granulosa cells. During maturation, the oocyte secretes a factor that induces cumulus expansion, namely GDF-9 (Soyal et al., 2000).

GDF-9 is an important oocyte-derived factor that regulates ovarian function (Emori and Sugiura, 2014). GDF-9 stimulates granulosa cell differentiation, including inducing LH receptors and steroidogenesis (Durán-Pastén and Fiordelisio, 2013). According to Ito's research, mice with GDF-9 deficiency were found to be infertile because follicular development could not proceed beyond the primordial follicle stage (Ito et al., 2022). Besides GDF-9, there is a protein essential in the process of folliculogenesis, namely Estrogen Receptor (ESR 1). It plays a crucial role in reproduction by functioning as a mediator of the hormone estrogen. Previous research findings suggested that abnormalities in ESR 1 could lead to infertility (Arnal et al., 2017; Rumi et al., 2017).

Nigella sativa L. (family *Ranunculaceae*) is an annual herb with many pharmacological properties (Tavakkoli et al., 2017). Muslims call this plant *Habbatus Sauda, Alhabahat lsawda* and *Alkamoun Alaswad* (Sahak et al., 2016). It also has medicinal properties as an antidote, protecting against toxicity in several organs, including the brain, kidneys, lungs, liver, heart, digestive tract, and reproductive system (Hannan et al., 2021). Based on previous research, administration of *N. sativa* extract can increase reproductive potential in male rats by increasing testosterone and LH levels in male rats (Sayed, 2019).

So far, research studies on the potential of *N. sativa* extract as a fertility drug in women, especially increasing estrogen receptors and GDF-9 have not been found in the literature review. This research will predict the potential of *N. sativa* extract as a fertility drug by increasing two fertility biomarkers, namely GDF-9 and ESR 1. The binding of certain compounds in *N. sativa* extract is expected to increase the activation of these two target proteins.

MATERIAL AND METHODS

Ligand preparation

The ligands used in this study were based on *N. sativa* compounds obtained in previous studies, namely 5-hydroxymethylfurfural, thymoquinone, thymol, 2-isopropylidene-5-methylhex-4-enal, phenol, 3-(1,1-dimethylethyl)-4-methoxy-, p-tert-butyl catechol, tetradecanoic acid, tetradecanoic acid, ethyl ester, hexadecanoic acid, methyl ester, hexadecanoic acid, ethyl ester, 9,12-octadecadienoic acid, methyl ester, oleic acid, 9,12-octadecadienoic acid (Z,Z)-, eicosanoic acid, cis-13,16-docasadienoic acid, cis-11,14-eicosadienoic acid, methyl ester, methyl 19-hexacosenoate, cholestan-3-ol, 2-methylene-, (3β,5α)-, 9,12,15-octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-, and palmitic acid β -monoglyceride (Nivetha and Prasanna, 2016). As for the control, use clomiphene fertility therapy, which is known to increase fertility through increasing levels of endogenous gonadotropins and to stimulate the ovaries to increase the number of follicles (Haas and Casper, 2017). CID information and 3D structure of target and control compounds with structure data format (sdf) files were obtained from Pub-

Chem (https://pubchem.ncbi.nlm.nih.gov/). OpeBabel 2.3.1 Software used for energy minimization and conversion of sdf files to protein databank format (pdb) (Kim et al., 2021).

Protein preparation

GDF-9 and ESR 1 act as targets for *N. sativa* compounds. 3D structures with pdb files for both targets were obtained from Uniprot database (https://www.uniprot.org/). Water molecules and contaminant ligands on the target were removed via PyMol v2.5 software to optimize and prepare molecular docking (Rigsby and Parker, 2016).

Druglikeness identification

The drug-like molecule in chemical compounds from *N. sativa* was identified through a drug-likeness test on the SwissADME server (http://www.swissadme.ch/). Parameters such as Lipinski, Ghose, Veber, Eggan, and Bioavailability scores are used to determine drug-like molecules in candidate compounds. The drug-likeness test aims to identify the similarity of the properties of the query compound with the drug molecule (Daina et al., 2017).

Molecular docking

In this study, the ligand interaction from *N. sativa* with GDF-9 and ESR 1 was simulated through molecular docking simulations on PyRx v9.9.0 software. Docking simulation aims to identify the ability of ligand binding activity and interaction patterns on the target. Binding affinity is the negative energy that is formed when the ligand and target interact to form a stable molecular complex (Tripathi et al., 2021; Wijaya et al., 2021).

Chemical interaction

The LigPlot+ v.2.2 program was used to identify the chemical interactions of the ligand-protein complex in this investigation. Hydrogen and hydrophobic chemical bond interactions are shown in the software. This type of interaction in the ligand-protein complex is facilitated by weak binding to trigger specific biological responses, such as the level of activation and the stability of the molecular complex (Dibha et al., 2022).

Structural visualization

Ligand-target complexes are displayed in 3D structures using PyMol v2.5 software with color and structure selection methods. The colors shown are based on the ligand atomic composition and single protein structure. Structural types consisting of cartoons, sticks, and transparent surfaces with standard publications are used in this study (Rigsby and Parker, 2016).

Data analysis

The data analysis process was carried out in stages, including (1) drug-likeness, analysis of the similarity of all compounds using the Lipinski, Ghose, Veber, Eggan, Muegge methods, and the Bioavailability score was used to determine the drug in the candidate compound, (2) to determine the molecular docking of the compound with the lowest binding affinity, (3) after molecular docking, the compound with the lowest affinity was simulated to observe the molecular interactions between the compound and the two target proteins.

RESULTS

Preparation of ligands, visualization of ligands, and protein target

The chemical compounds of *N. sativa* consist of 5-hydroxymethylfurfural, thymoquinone, thymol, 2-isopropylidene-5-methylhex-4-enal, phenol, 3-(1,1-dimethylethyl)-4-methoxy-, p-tert-butyl catechol, tetradecanoic acid, tetradecanoic acid, ethyl ester, hexadecanoic acid, methyl ester, hexadecanoic acid, ethyl ester, 9,12-octadecadienoic acid, methyl ester, oleic acid, 9,12-octadecadienoic acid (Z,Z)-, eicosanoic acid, cis-13,16-docasadienoic acid, cis-11,14-eicosadienoic acid, methyl ester, methyl 19-hexacosenoate, cholestan-3-ol, 2-methylene-, (3β, 5α)-, 9,12,15-octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)-methyl]ethyl ester, (Z,Z,Z)-, palmitic acid β-monoglyceride, and clomifene (control) from PubChem with information consisting of CID number and cite (Table 1), 3D structures of the ligands are shown as sticks (Fig. 1). This study aims to identify the activity of compounds from *N. sativa* for GDF-9 and ESR 1 activation. GDF-9 (Uniprot ID: O60383) with a 454-mer sequence length, ESR 1 (Uniprot ID: P03372) 595-mer. Target structures are displayed as transparent surfaces and cartoons with a single color (Fig. 2).

Drug-likeness

The identification of drug-likenes indicates that all compounds from *N. sativa* can be used for further analysis of their potential as fertility activators (Table 2).

Molecular docking

Molecular docking in this study aims to identify patterns of molecular interactions and assess the level

of binding activity between compounds of *N. sativa* on target proteins, namely GDF-9 and ESR 1. The docking results show that the compounds from *N. sativa* consist of cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- showed a more negative binding affinity than the other compounds, and the control (clomifene) (Table 3). The ligand-protein complexes from the docking simulation results with the most negative binding affinity are displayed with stick structures, transparent surfaces, and cartoons with single staining (Fig. 3).

Molecular interactions between ligand-target proteins

The analysis of molecular interactions shows that cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- can form weak bond interactions consisting of hydrogen and hydrophobic (Fig. 4). Cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- exhibited the same interaction position through Leu354, Glu380, Leu379, Trp383, Ile358, Val376 in the ESR 1 and GDF-9 domains via Tyr275, Leu62, Ser271, the yellow color is the ESR 1 domain and the blue color is the GDF-9 domain (Table 4).

DISCUSSION

GDF-9 and ESR 1 are important proteins in the process of forming follicles in the ovaries. GDF-9 stimulates granulosa cell differentiation, including inducing LH receptors and stereoidogenesis (Durán-Pastén and Fiordelisio, 2013). While ESR 1 functions as a mediator of the hormone estrogen, which plays an important role in growth hormone in the reproductive system, triggers for ovulation, and also functions to maintain oocyte development. The ovarian follicles' hormone estrogen is secreted by the granulosa cells (Hamilton et al., 2017; Rumi et al., 2017).

Table 1. *N. sativa* ligands sample preparation from PubChem database.

No	Compound	CID	SMILE Canonical
1.	5-Hydroxymethylfurfural	237332	C1=C(OC(=C1)C=O)CO
2.	Thymoquinone	10281	CC1=CC(=O)C(=CC1=O)C(C)C
3.	Thymol	6989	CC1=CC(=C(C=C1)C(C)C)O
4.	2-Isopropylidene-5-methylhex-4-enal	534886	CC(=CCC(=C(C)C)C=O)C
5.	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	6932	CC(C)(C)C1=C(C=CC(=C1)O)OC
6.	p-tert-Butyl catechol	7381	CC(C)(C)C1=CC(=C(C=C1)O)O
7.	Tetradecanoic acid	11005	CCCCCCCCCCCCCC(=O)O
8.	Tetradecanoic acid, ethyl ester	31283	CCCCCCCCCCCCCC(=O)OCC
9.	Hexadecanoic acid, methyl ester	8181	CCCCCCCCCCCCCCCC(=O)OC
10.	Hexadecanoic acid, ethyl ester	12366	CCCCCCCCCCCCCCCC(=O)OCC
11.	9,12-Octadecadienoic acid, methyl Ester	8203	CCCCCC=CCC=CCCCCCCCC(=O)OC
12.	Oleic acid	445639	CCCCCCCCC=CCCCCCCCC(=O)O
13.	9,12-Octadecadienoic acid (Z,Z)-	3931	CCCCCC=CCC=CCCCCCCCC(=O)O
14.	Eicosanoic acid	10467	CCCCCCCCCCCCCCCCCCCC(=O)O
15.	cis-13,16-Docasadienoic acid	5312554	CCCCCC=CCC=CCCCCCCCCCCCC(=O)O
16	cis-11,14-Eicosadienoic acid, methyl ester	6430995	CCCCCC=CCC=CCCCCCCCCCC(=O)OC
17.	Methyl 19-hexacosenoate	91692796	CCCCCCC=CCCCCCCCCCCCCCCCCCC(=O)OC
18.	Cholestan-3-ol, 2-methylene-, (3β,5α)-	22213932	CC(C)CCCC(C)C1CCC2C1(CCC3C2CCC4C3(CC(=C)C(C4)O)C)C
19.	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	21159744	CCC=CCC=CCC=CCCCCCCCC(=O)OC(COC(=O)C)COC(=O)C
20.	Palmitic acid β –monoglyceride	123409	CCCCCCCCCCCCCCCC(=O)OC(CO)CO
21.	Clomifene (Control)	2800	CCN(CC)CCOC1=CC=C(C=C1)C(=C(C2=CC=CC=C2)Cl)C3=CC=CC=C3

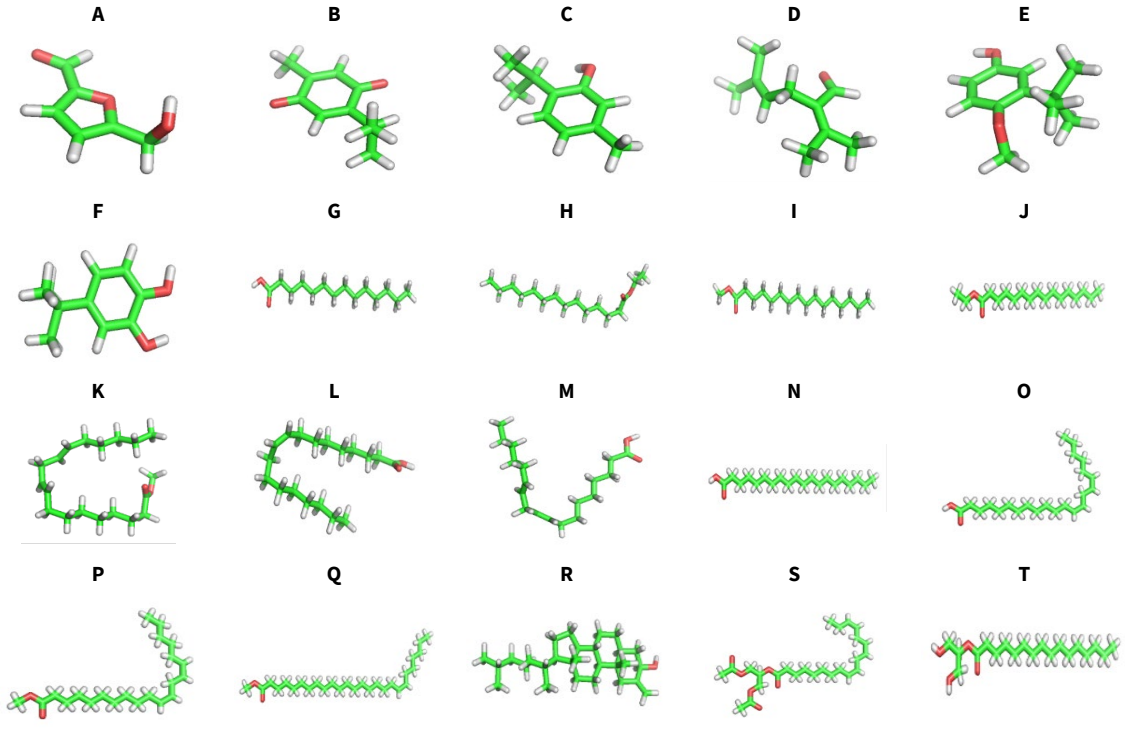

Figure 1. Ligands structural visualization.

(A) 5-Hydroxymethylfurfural; **(B)** Thymoquinone; **(C)** Thymol; **(D)** 2-Isopropylidene-5-methylhex-4-enal; **(E)** Phenol, 3-(1,1-dimethylethyl)-4-methoxy-; **(F)** p-tert-Butyl catechol; **(G)** Tetradecanoic acid; **(H)** Tetradecanoic acid, ethyl ester; **(I)** Hexadecanoic acid, methyl ester; **(J)** Hexadecanoic acid, ethyl ester; **(K)** 9,12-Octadecadienoic acid, methyl ester; **(L)** Oleic acid; **(M)** 9,12-Octadecadienoic acid (Z,Z)-; **(N)** Eicosanoic acid; **(O)** cis-13,16-Docasadienoic acid; **(P)** cis-11,14-Eicosadienoic acid, methyl ester; **(Q)** Methyl 19-hexacosenoate; **(R)** Cholestan-3-ol, 2-methylene-, (3β,5α)-; **(S)** 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-; **(T)** Palmitic acid β -monoglyceride; **(U)** Clomifene (control).

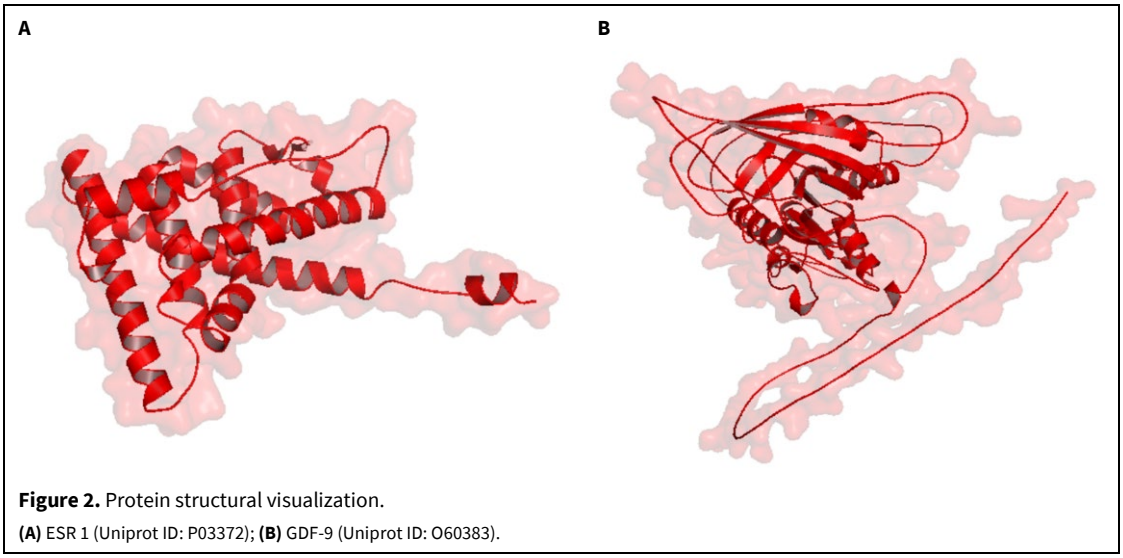

Figure 2. Protein structural visualization.

(A) ESR 1 (Uniprot ID: P03372); **(B)** GDF-9 (Uniprot ID: O60383).

Table 2. The result of drug-likeness analysis.

No	Compound	Lipinski	Ghose	Veber	Eggan	Bioavailability score
1.	5-Hydroxymethylfurfural	Yes	No	Yes	Yes	0.55
2.	Thymoquinone	Yes	Yes	Yes	Yes	0.55
3.	Thymol	Yes	No	Yes	Yes	0.55
4.	2-Isopropylidene-5-methylhex-4-enal	Yes	No	Yes	Yes	0.55
5.	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	Yes	Yes	Yes	Yes	0.55
6.	p-tert-Butyl catechol	Yes	Yes	Yes	Yes	0.55
7.	Tetradecanoic acid	Yes	Yes	No	Yes	0.85
8.	Tetradecanoic acid, ethyl ester	Yes	Yes	No	Yes	0.55
9.	Hexadecanoic acid, methyl ester	Yes	No	No	Yes	0.55
10.	Hexadecanoic acid, ethyl ester	Yes	No	No	No	0.55
11.	9,12-Octadecadienoic acid, methyl ester	Yes	No	No	No	0.55
12.	Oleic acid	Yes	No	No	No	0.85
13.	9,12-Octadecadienoic acid (Z,Z)-	Yes	No	No	No	0.85
14.	Eicosanoic acid	Yes	No	No	No	0.85
15.	cis-13,16-Docasadienoic acid	Yes	No	No	No	0.85
16	cis-11,14-Eicosadienoic acid, methyl ester	Yes	No	No	No	0.55
17.	Methyl 19-hexacosenoate	Yes	No	No	No	0.55
18.	Cholestan-3-ol, 2-methylene-, (3β,5α)-	Yes	No	Yes	No	0.55
19.	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	Yes	No	No	Yes	0.55
20.	Palmitic acid β-monoglyceride	Yes	Yes	No	Yes	0.55
21.	Clomifene (control)	Yes	No	Yes	No	0.55

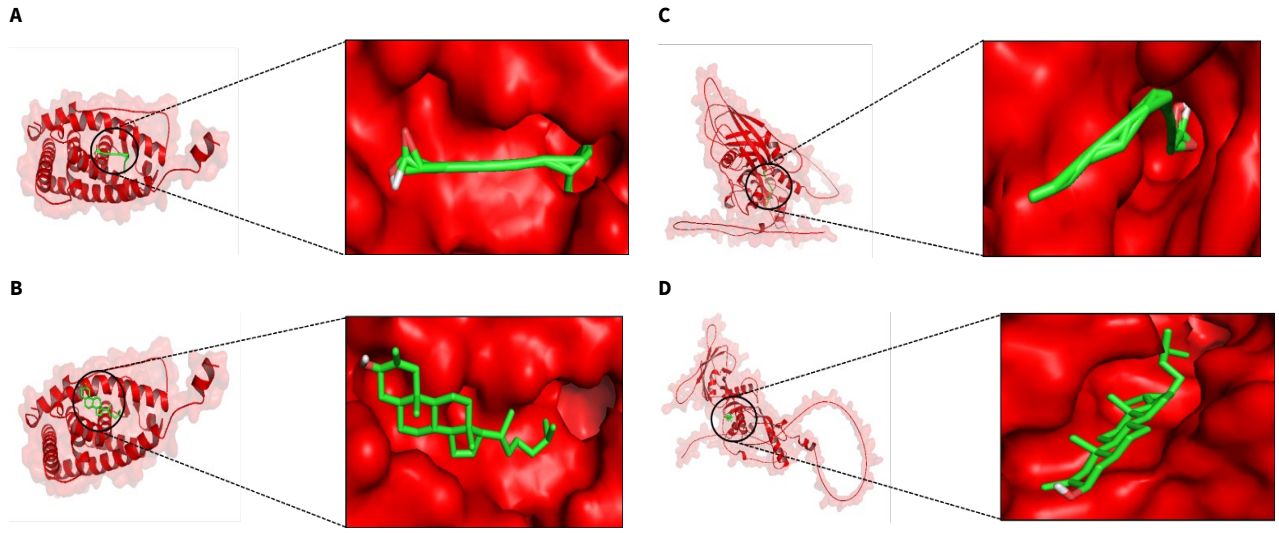

Figure 3. Targets-ligand structural visualization from the docking simulation.

(A) cis-13,16-Docasadienoic acid_ESR 1; **(B)** Cholestan-3-ol, 2-methylene-, (3β,5α)-_ESR 1; **(C)** cis-13,16-Docasadienoic acid_GDF-9; **(D)** Cholestan-3-ol, 2-methylene-, (3β,5α)-_GDF-9.

Table 3. The docking result of *N. sativa* compound-target.

No	Compound	CID	Binding affinity (kcal/mol)	
			ESR 1	GDF-9
1.	5-Hydroxymethylfurfural	237332	-6.4	-5.3
2.	Thymoquinone	10281	-6.2	-6.3
3.	Thymol	6989	-6.1	-5.9
4.	2-Isopropylidene-5-methylhex-4-enal	534886	-5.9	-5.3
5.	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	6932	-6.2	-5.2
6.	p-tert-Butyl catechol	7381	-6.4	-5.7
7.	Tetradecanoic acid	11005	-5.8	-5.2
8.	Tetradecanoic acid, ethyl ester	31283	-6.1	-4.7
9.	Hexadecanoic acid, methyl ester	8181	-6.1	-4.5
10.	Hexadecanoic acid, ethyl ester	12366	-6.1	-5.3
11.	9,12-Octadecadienoic acid, methyl ester	8203	-4.9	-5.2
12.	Oleic acid	445639	-4.7	-5.2
13.	9,12-Octadecadienoic acid (Z,Z)-	3931	-6.4	-5.0
14.	Eicosanoic acid	10467	-6.4	-5.0
15.	cis-13,16-Docasadienoic acid	5312554	**-7.6**	**-7.3**
16	cis-11,14-Eicosadienoic acid, methyl ester	6430995	-6.3	-4.2
17.	Methyl 19-hexacosenoate	91692796	-6.5	-4.1
18.	Cholestan-3-ol, 2-methylene-, (3β,5α)-	22213932	**-7.1**	**-8.0**
19.	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	21159744	-6.7	-5.4
20.	Palmitic acid β –monoglyceride	123409	-4.6	-5.4
21.	Clomifene (control)	2800	**-6.8**	**-6.4**

Table 3. The results of chemical interaction analysis.

Ligan-protein	Chemical interaction
cis-13,16-Docasadienoic acid_ESR 1	**Hydrophobic:** Leu525, Thr347, Ala350, Leu384, Gly521, Leu354, Glu380, Leu379, Trp383, Ile358, Lys529, Asp351 **Hydrogen:** Val376
Cholestan-3-ol, 2-methylene-, (3β,5α)-_ESR 1	**Hydrophobic:** Leu354, Glu380, Ile358, Lys362, Leu372, Gln375, Val376, Leu379, Trp383
cis-13,16-Docasadienoic acid_GDF-9	**Hydrophobic:** Glu442, Pro63, Tyr279, Glu444, Tyr275, Leu62, Phe66 **Hydrogen:** Asn112, Ser271, Asp269, Ala272
Cholestan-3-ol, 2-methylene-, (3β,5α)-_GDF-9	**Hydrophobic:** Lys67, Thr148, Lys441, Val150, Ser271, Gly60, Trp278, Ala274, Tyr275, Leu62, Ala64

In this study, we carried out molecular docking between compounds (ligands) from *N. sativa* and target proteins, namely GDF-9 and ESR 1 proteins, to see the potential of these ligands to affect the target protein. Identification of drug-likeness is used to determine the similarity of the properties of the query compound to the drug molecule with reference to physicochemical properties through parameters such as Lipinski, Ghose, Veber, and Eggan in SwissADME, compounds must meet at least one rule to be called a drug-like molecule. The ability of a medicine to circulate in the body is measured by its drug bioavailability, with an ideal score of >0.25 (Martin, 2005; Daina et al., 2017). According to the results of drug similarity identification, all of the *N. sativa* compound's ligands met the requirements for drug similarity parameters by satisfying at least one rule with a bioavailability score of 0.55 (Table 2).

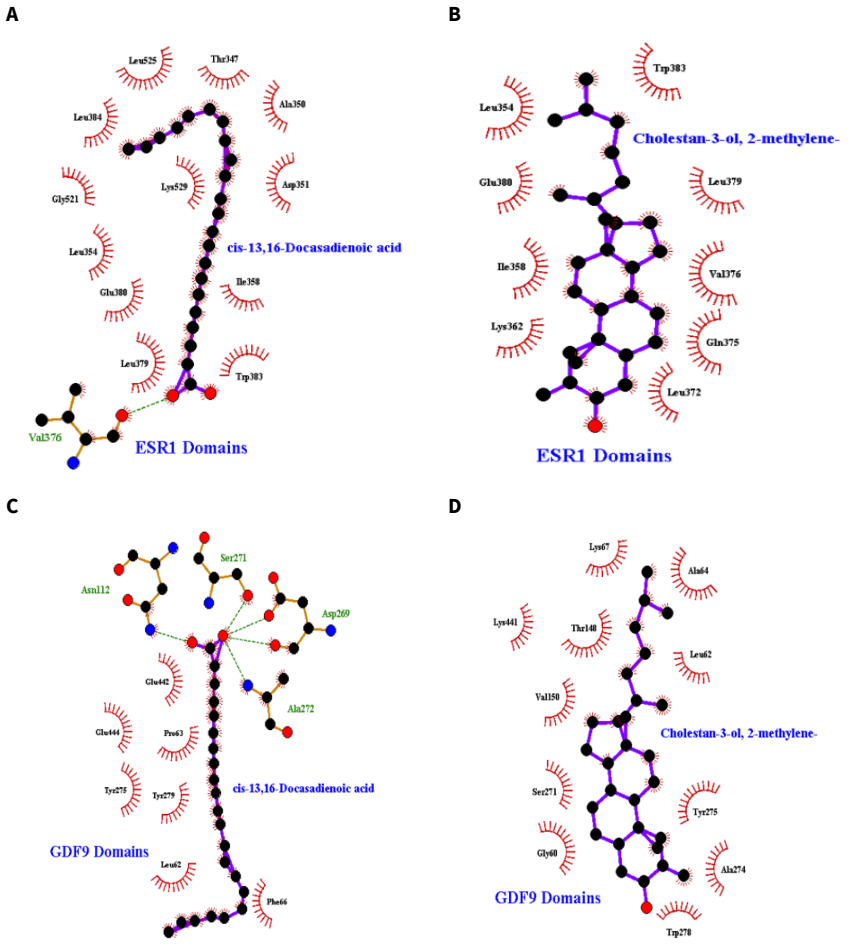

Figure 4. The visualization of molecular interaction.

(A) cis-13,16-Docasadienoic acid_ESR 1; **(B)** Cholestan-3-ol, 2-methylene-, (3β, 5α)-_ESR 1, **(C)** cis-13,16-Docasadienoic acid_GDF-9; **(D)** Cholestan-3-ol, 2-methylene-, (3β,5α)-_GDF-9.

Molecular docking can evaluate the degree of ligand binding activity on the target and reveal patterns of molecular interactions. The ability of ligand activity refers to the level of the binding energy score. Binding affinity is the negative energy created when the ligand attaches to the target (Aini et al., 2022). The activity level of the ligand is determined by the value of the binding affinity that is formed when it interacts with the target. Negative values of binding affinity determine bond strength, more negative values indicate the strongest ligand bonds (Wijaya et al., 2021). Candidate compounds with more negative binding affinity values and binding to functional domains can act as activators on the target protein. In this study, cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- showed more negative binding affinity than the other compounds and clomifene (control). These results indicate that cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- in *N. sativa* extract have the potential to affect the physiological properties of the target protein because they have a higher binding ability than clomiphene. Both of these compounds are predicted to trigger the activation of GDF-9 and ESR 1.

Docking visualization shows how the docking results interact between the ligand and the target protein. The interaction between ligands and amino acid residues can be visualized using this method. The interactions can occur in the form of non-covalent bonds, such as electrostatics, Van der Waals interactions, hydrogen bonds, and hydrophobic bonds. Hydrogen bonds develop in molecules due to the attraction between hydrogen atoms and other atoms with high electronegativity. Hydrogen bonds are the strongest bonds among other bonds. Meanwhile, hydrophobic bonds have an important role in the stability of the ligand to the receptor. Hydrophobic bonds tend to avoid watery environments and cluster on the inside of protein structures To reduce interactions with water, which could harm the protein structure (Lins and Brasseur, 1995; Dibha et al., 2022). Based on the research results, the molecular interactions showed that cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- could form weak bond

interactions consisting of hydrogen and hydrophobic. Cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- showed the same interaction position through Leu354, Glu380, Leu379, Trp383, Ile358, Val376 in the ESR 1 and GDF-9 domains through Tyr275, Leu62, Ser271. The interaction position on the same target of the two query compounds is predicted as a practical side that triggers target activation.

The limitation of this study is based on prediction or virtual screening. For this reason, it is necessary to study both *in vitro* and *in vivo* to obtain drug molecule candidates from *N. sativa*.

CONCLUSION

Chemical compounds from *Nigella sativa* consisting of cis-13,16-docasadienoic acid and cholestan-3-ol, 2-methylene-, (3β,5α)- can act as candidates for GDF-9 and ESR 1 activators because these two compounds have the binding affinity value is more negative than the control (clomifene) and forms weak bond interactions such as hydrogen and hydrophobic. Weak bonds can trigger ligand activity to affect the activation of both targets and then trigger a response in increasing fertility.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Amalia A	Hendarto H	Mustika A
Concepts or ideas	x	x	x
Design	x	x	
Definition of intellectual content	x	x	x
Literature search	x	x	
Experimental studies	x	x	x
Data acquisition	x		x
Data analysis	x	x	x
Statistical analysis	x		x
Manuscript preparation	x		
Manuscript editing	x		x
Manuscript review	x	x	x

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