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SHORT COMMUNICATION article

# Polycystic ovary syndrome: Molecular modeling study on potential *Lepidium sativum* bioactive compounds in modulating kiss-1 gene function

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**Abstract**: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder in women of reproductive age, characterized by hormonal imbalances, insulin resistance, and metabolic disturbances. The KiSS-1 gene, which encodes the kisspeptin neuropeptide, is crucial for regulating reproductive hormones through the hypothalamic-pituitary-gonadal axis. Genetic polymorphisms in KiSS1 diminish kisspeptin activity and exacerbate PCOS symptoms. This study investigates the potential of bioactive compounds from *Lepidium sativum* (garden cress) to modulate KiSS-1 gene function and address PCOS-related dysfunctions. A set of eight bioactive compounds from *Lepidium sativum* was screened using molecular docking with Virtual Screening software for Computational Drug Discovery to assess their interactions with wild-type and polymorphic forms of the KiSS-1 gene. The chemical structures of the compounds were designed using ChemSketch and are visualized for molecular interactions using BIOVIA Discovery Studio. Sequence data for the wild-type and polymorphic variants of the KiSS-1 gene were obtained from the Protein Data Bank and the National Center for Biotechnology Information. Pharmacokinetic properties and drug-likeness of the selected compounds were evaluated using SwissADME, with particular reference to Lipinski's Rule of Five criteria. Alpha-linolenic acid, oleic acid methyl ester, stigmasterol, and α-D-glucopyranoside exhibited strong binding affinities and established stable interactions with the wild-type and polymorphic forms of the KiSS-1 gene. Among them, alpha-linolenic acid and stigmasterol showed the most favorable binding profiles, characterized by stable hydrogen bonding and high binding energy values, indicating strong potential as modulators of KiSS-1 activity. Notably, the binding affinity of alpha-linolenic acid was reduced in the polymorphic variant compared to the wild-type, supporting the hypothesis of diminished gene function associated with PCOS-related polymorphisms. SwissADME analysis confirmed that these top candidates possess favorable pharmacokinetic properties and comply with Lipinski's Rule of Five, suggesting good oral bioavailability and drug-likeness. This computational study suggests that bioactive compounds from *Lepidium sativum* have the potential to interact effectively with both wild-type and polymorphic forms of the KiSS-1 gene. Their strong binding affinities indicate a possible role in restoring gene function, which may contribute to alleviate symptoms of POCS. The study considers epigenetic mechanisms such as DNA methylation and histone modification through which these compounds may enhance KiSS-1 gene expression. This dual mechanism positions *Lepidium sativum* as a promising plant-based therapeutic candidate for PCOS.

## Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, affecting about 4.0%-20.0% of women of reproductive age worldwide, depending on the diagnostic criteria used [1, 2]. PCOS is characterized by a combination of symptoms, including irregular menstrual cycles, hyperandrogenism, and the presence of polycystic ovaries as observed through imaging [3, 4]. Beyond its reproductive symptoms, PCOS is associated with metabolic disturbances, including insulin resistance, obesity, and an increased risk of type 2 diabetes and cardiovascular diseases [4, 5]. These complexities make PCOS a multifaceted disorder that requires a deeper understanding of its underlying mechanisms. The KiSS-1 gene encodes the kisspeptin neuropeptide, which plays a key role in regulating reproductive hormones through the hypothalamic-pituitary-gonadal (HPG) axis [6, 7]. Kisspeptin binds to its receptor (KISS1R) on GnRH neurons, stimulating the release of GnRH, which in turn triggers the secretion of LH and FSH from the pituitary [6]. Genetic variations in KiSS-1, such as those studied by Daghestani and others, can reduce kisspeptin activity, disrupt hormone balance, and worsen PCOS symptoms [8-10]. **Figure 1** illustrates the Hypothalamic-Pituitary-Gonadal (HPG) axis, where kisspeptin, secreted by KiSS-1 neurons, stimulates the release of GnRH, subsequently triggering the secretion of LH and FSH. Dysregulation of this pathway plays a key role in the hormonal imbalances observed in PCOS.

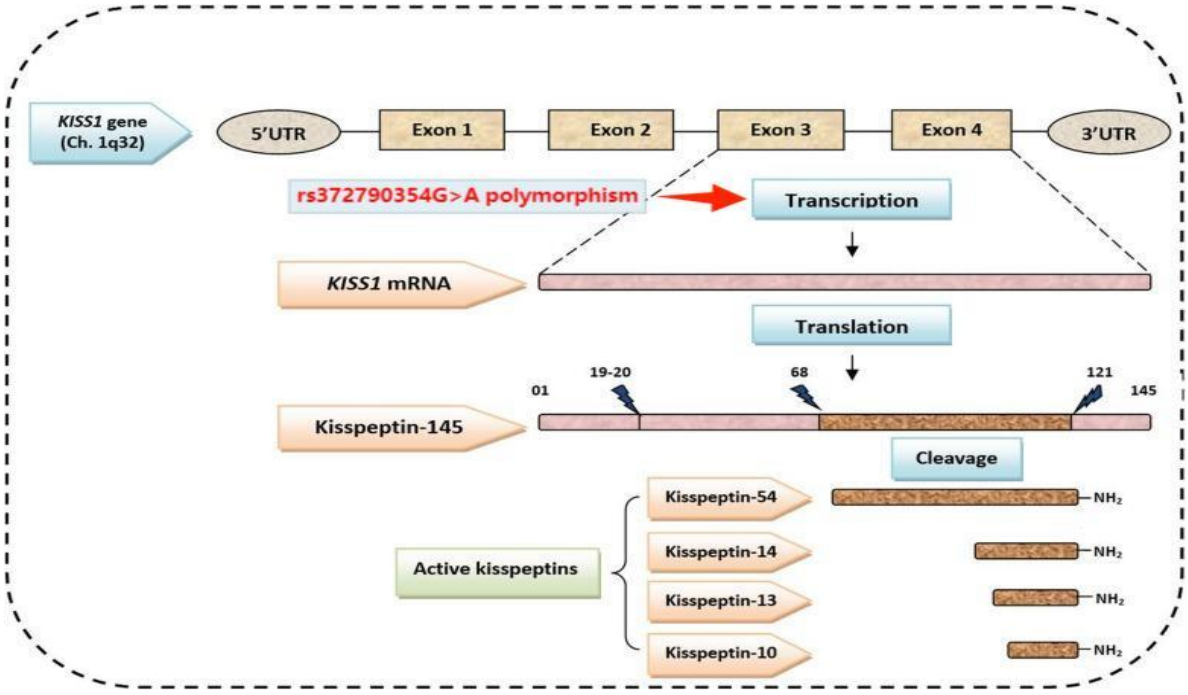


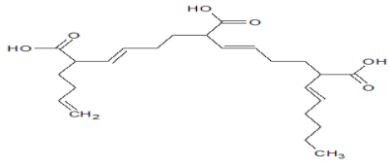
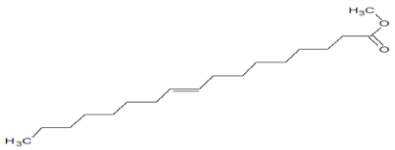
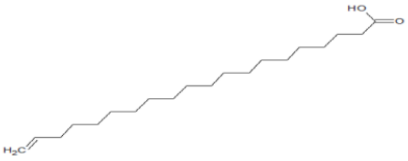
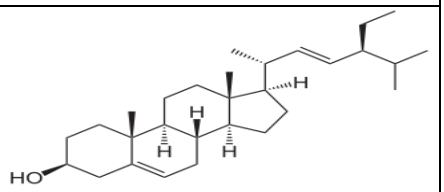
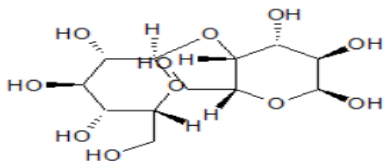

**Figure 1:** Hypothalamic-Pituitary-Gonadal Axis

Kisspeptin secreted by KiSS-1 neurons stimulates GnRH release, triggering LH and FSH secretion. Disruptions in this pathway contribute to hormonal imbalances in PCOS [11].

Current PCOS management includes lifestyle modification and pharmacological interventions [12], but these approaches do not address underlying genetic or epigenetic dysfunctions [13, 14]. Plant-based bioactive compounds, such as those from *Lepidium sativum,* have shown potential in modulating gene expression and restoring metabolic balance [15-18]. The bioactive Constituents of *Lepidium sativum* seeds (Garden Cress) include fatty acids (Alpha-linolenic acid (an omega-3 fatty acid), oleic acid, and linoleic acid. Sterols including: stigmasterol and β-sitosterol. Glucosides, including: α-D-glucopyranoside derivatives. In addition to the presence of alkaloids, flavonoids, and phenolic compounds that are known for antioxidant and anti-inflammatory activities.

The seeds also consist of vitamins and minerals, including: vitamins A, C, E, calcium, and iron [15]. *Lepidium sativum* seeds have many biological activities including antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory effects [15, 17]. Their presence supports *Lepidium sativum* 's traditional use in managing reproductive disorders, including PCOS, by modulating hormone balance and oxidative stress [17]. **Table 1** summarizes the IUPAC names, functional groups, and chemical structures of the major constituents found in *Lepidium sativum* [16].

**Table 1:** IUPAC names, functional groups, and chemical structures of *Lepidium sativum* constituents

| Name | IUPAC Name | Main Functional Group | Structure |
|---|---|---|---|
| Alpha-linolenic acid (ALA) | (9*Z*,12*Z*,15*Z*)-Octadeca-9,12,15-trienoic acid | Carboxylic acid group (-COOH)<br>Alkene group (C=C) |  |
| Oleic acid methyl ester | Methyl (9Z)-octadec-9-enoate | Methyl Ester group (-COOCH3)<br>Alkene group (C=C) |  |
| 11-Eicosenoic acid | (11Z)-Eicos-11-enoic acid | Carboxylic acid group (-COOH)<br>Alkene group (C=C) |  |
| cis-9-eicosenoic acid | (9Z)-Eicos-9-enoic acid | Carboxylic acid group (-COOH)<br>Alkene group (C=C) |  |
| Palmitic acid | Hexadecanoic acid | Carboxylic acid group (-COOH) |  |
| Methyl stearate | Methyl octadecanoate | Methyl Ester group (-COOCH3) |  |
| Stigmasterol | Stigmasta-5,22-dien-3β-ol | Hydroxyl group (-OH)<br>Alkene group (C=C) |  |
| Sucrose | β-D-Fructofuranosyl α-D-glucopyranoside | Hydroxyl group (-OH) |  |

Molecular modeling is an essential computational approach used to investigate and predict how bioactive compounds from plants interact with specific biological targets at the molecular level. By simulating the binding interactions between plant-derived molecules and proteins or genes of interest, molecular modeling helps to elucidate the mechanisms of action, binding affinities, and stability of these interactions. This approach accelerates drug discovery by identifying promising candidates for therapeutic development, optimizing lead compounds, and reducing the need for extensive laboratory experiments. In the context of studying plant constituents, molecular modeling provides valuable insights into how natural compounds may modulate biological pathways involved in diseases, enabling a rational design of plant-based treatments [19]. The aim of this study is to identify compounds from *Lepidium sativum* that can interact with both wild-type and polymorphic forms of the KiSS-1 gene, potentially restoring its function and reducing symptoms associated with PCOS.

## Materials and methods

*Molecular modeling tools:* Chemical structures of *Lepidium sativum* constituents were drawn using ChemSketch [20] and visualized with BIOVIA Discovery Studio [21]. The KiSS-1 gene sequences (wild-type and polymorphic variants) were obtained from the Protein Data Bank (PDB) [22] and NCBI [7].

*Molecular docking:* Ligands and receptor structures were prepared and energy-minimized. Docking was performed using AutoDock 4 [23] and PyRx [24], targeting both wild-type and polymorphic KiSS-1 gene-related proteins. Binding affinities and hydrogen bond interactions were recorded.

*Pharmacokinetic profiling:* SwissADME [25] was used to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. Drug-likeness was evaluated based on Lipinski's rule of five [26] and related guidelines.

*Epigenetic assessment:* Potential for modulation of gene expression via DNA methylation and histone modification was considered based on the literature and computational predictions [13, 14, 27, 28].

## Results and discussion

Molecular docking analysis combined with pharmacokinetic profiling of eight bioactive compounds from *Lepidium sativum* demonstrated favorable binding affinities and stable interactions with both wild-type and polymorphic variants of the KiSS-1 gene. Notably, compounds such as alpha-linolenic acid and stigmasterol showed the strongest binding, suggesting potential efficacy in modulating gene function. Pharmacokinetic evaluation further indicated that these compounds possess suitable absorption, distribution, metabolism, and excretion properties, complying with drug-likeness criteria [29]. The comprehensive results, including binding energies, interaction residues, and pharmacokinetic parameters, are summarized and analyzed in **Table 2**, highlighting their potential as therapeutic agents for PCOS. **Table 2** provides key physicochemical and pharmacokinetic properties of the selected compounds from *Lepidium sativum*. For each compound, the molecular weight (in g/mol) reflects its size, which influences absorption and membrane permeability. The consensus LogP (octanol/water partition coefficient) indicates the compound’s lipophilicity, affecting solubility and cell membrane crossing ability. The number of hydrogen bond donors and acceptors relates to the compound’s potential to form hydrogen bonds, impacting binding interactions and solubility. The number of rotatable bonds is a measure of molecular flexibility, which can influence binding affinity and pharmacokinetics. The bioavailability score predicts the likelihood of the compound to be orally bioavailable, while the synthetic accessibility score estimates the ease of chemical synthesis, guiding the feasibility of drug development.

**Table 2:** Pharmacokinetic properties from SwissADME

| Compound's Name | Molecular Weight (g/mol) | Consensus Log Po/w | Num. H-bond Donors | Num. H-bond Acceptors | Num. Rotatable Bonds | Bioavailability Score | Synthetic Accessibility |
|---|---|---|---|---|---|---|---|
| 9-Octadecenoic acid methyl ester (Oleic acid methyl ester) | 296.49 | 5.65 | 0 | 2 | 15 | 0.55 | 3.05 |
| 9,12,15-Octadecatrienoic acid (alpha-linolenic acid) | 278.44 | 5.84 | 1 | 2 | 14 | 0.85 | 4.21 |
| 11-Eicosenoic acid | 310.51 | 6.81 | 1 | 2 | 18 | 0.85 | 3.42 |
| cis-9-eicosenoic acid | 310.51 | 8.03 | 1 | 1 | 21 | 0.55 | 3.96 |
| Hexadecanoic acid (Palmitic acid) | 256.42 | 5.20 | 1 | 2 | 14 | 0.85 | 2.31 |
| Methyl stearate | 298.50 | 5.54 | 0 | 2 | 15 | 0.55 | 2.53 |
| Stigmasterol | 412.71 | 7.38 | 1 | 1 | 4 | 0.55 | 5.73 |
| α-D-glucopyranoside, O-α-D-glucopyranosyl-(1→)-ß-D-fruc | 342.30 | -3.30 | 8 | 10 | 4 | 0.55 | 5.33 |

The eight selected bioactive compounds from *Lepidium sativum* display diverse functional groups that likely influence their molecular interactions and biological efficacy. Alpha-linolenic acid (ALA) and oleic acid methyl ester contain unsaturated hydrocarbon chains, which may enhance lipophilicity and facilitate membrane permeation as well as receptor binding. Carboxylic acid moieties present in ALA, 11-eicosenoic acid, cis-9-eicosenoic acid, and palmitic acid serve as hydrogen bond donors and acceptors, promoting strong and specific interactions with amino acid residues within the active sites of target proteins. Stigmasterol's steroidal scaffold, characterized by hydroxyl and alkene functionalities, is conducive to hydrophobic and hydrogen bonding interactions with membrane-bound receptors and may also influence epigenetic pathways through modulation of receptor-mediated signaling [28, 30, 31].

Pharmacokinetic evaluation shows that the majority of the compounds possess favorable drug-like properties based on Lipinski's Rule of Five. All compounds have molecular weights under 500 g/mol, indicating good membrane permeability potential. The consensus LogP values vary, with most compounds exhibiting moderate to high lipophilicity (LogP between 5.20 and 8.03), while α-D-glucopyranoside is distinctly hydrophilic (LogP of -3.30). This variation suggests different pathways for cellular uptake and distribution. Bioavailability scores range from 0.55 to 0.85, with alpha-linolenic acid, 11-eicosenoic acid, and palmitic acid showing the highest predicted oral bioavailability (0.85). Synthetic accessibility assessments indicate that most compounds are relatively easy to synthesize, with palmitic acid and methyl stearate being the most synthetically accessible, scoring 2.31 and 2.53, respectively.

Detailed analysis of two key compounds, alpha-linolenic acid and stigmasterol, revealed promising pharmacokinetic profiles. Alpha-linolenic acid demonstrated good oral bioavailability, moderate lipophilicity, and low synthetic complexity, and has been previously reported to possess anti-inflammatory effects [18, 32]. Stigmasterol exhibited favorable lipophilicity, moderate bioavailability, and satisfactory drug-likeness, with studies indicating that phytosterols like stigmasterol may influence epigenetic regulation [33]. **Table 3** summarizes the molecular docking results for each ligand, detailing their binding affinities, hydrogen bonding interactions, and binding site characteristics with both the wild-type and polymorphic KiSS-1 gene variants. For each ligand, the Vina binding affinity (expressed in kcal/mol) indicates the strength of the interaction, with more negative values representing stronger binding. The number of hydrogen bonds formed between the ligand and the target provides insight into interaction stability. Additionally, the groove binding and interaction types describe the specific binding site regions and nature of molecular interactions, such as hydrophobic contacts, hydrogen

bonding, or *Van der Waals* forces, observed in the docking simulations. **Table 3** shows the molecular docking analysis highlighted distinct binding profiles between the wild-type and polymorphic variants of the KiSS-1 gene. Stigmasterol demonstrated the strongest binding affinity across both variants, with a binding energy of -7.5 kcal/mol, suggesting its strong potential as a lead compound for therapeutic applications targeting KiSS-1-related pathways. Notably, alpha-linolenic acid exhibited a higher binding affinity for the polymorphic variant (-4.6 kcal/mol) than for the wild-type (-4.0 kcal/mol), which may indicate a compensatory interaction that could help restore or enhance the impaired function of the polymorphic KiSS-1 gene in PCOS patients. These differential binding patterns suggest that specific bioactive compounds from *Lepidium sativum* might be selectively effective against genetic variants associated with PCOS, warranting further experimental validation to explore their therapeutic benefits.

**Table 3:** Binding affinities and hydrogen bond interactions of tested compounds with normal and variated KiSS-1 gene receptors

| | Normal Gene | | | Variated Gene | | |
|---|---|---|---|---|---|---|
| Ligand | Vina Binding affinity (kcal/mol) | Number of hydrogen Bonds | Groove binding and Interaction Type | Vina Binding affinity (kcal/mol) | Number of hydrogen Bonds | Groove binding and interaction Type |
| 11-Eicosenoic Acid | -3.9 | 4 | Major Groove<br>Hydrogen bonds<br>Hydrophobic bonds<br>*Van der Waals* | -4.1 | 2 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds |
| 9,12,15-Octadecatrienoic Acid (Alpha-Linolenic Acid) | -4.0 | 4 | Major Groove<br>Hydrogen bonds<br>Hydrophobic bonds<br>*Van der Waals* | -4.6 | 1 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds |
| Cis-9-Eicosenoic Acid | -4.0 | 1 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds | -4.0 | 1 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds |
| Methyl Stearate | -3.7 | 2 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds | -3.7 | 0 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals* |
| 9-Octadecenoic Acid Methyl Ester (Oleic Acid Methyl Ester) | -4.0 | 1 | Major Groove<br>Hydrophobic bonds<br>*Van der Waals*<br>Hydrogen bonds | -4.1 | 2 | Major Groove<br>Hydrogen bonds<br>Hydrophobic bonds<br>*Van der Waals* |
| Hexadecanoic Acid (Palmitic Acid) | -3.8 | 2 | Major Groove<br>Hydrogen bonds<br>Hydrophobic bonds | -3.6 | 2 | Major Groove<br>Hydrogen bonds<br>Hydrophobic bonds |
| Stigmasterol | -7.5 | 0 | Major Groove<br>Hydrophobic bonds | -7.5 | 0 | Major Groove<br>Hydrophobic bonds |
| A-D-Glucopyranoside, O-A-D-Glucopyranosyl-(1, FWDAWR)-SS-D-Fruc | -6.8 | 12 | Major Groove<br>Hydrogen bonds | -6.7 | 12 | Major Groove<br>Hydrogen bonds |

The molecular docking analysis of eight bioactive compounds from *Lepidium sativum* revealed notable differences in their interactions with both wild-type and polymorphic KiSS-1 gene variants. All compounds consistently bound within the major groove of the gene targets, but their binding affinities, hydrogen bonding profiles, and interaction types varied, indicating different modes of interaction and potential biological implications. Stigmasterol demonstrated the strongest binding affinity among all compounds, with a docking score of -7.5 kcal/mol for both wild-type and polymorphic forms. Interestingly, despite forming no hydrogen bonds, its high binding energy was attributed to strong hydrophobic interactions, indicating that it may serve as a

robust lead compound capable of stabilizing KiSS-1 regardless of genetic variation. Similarly, α-D-glucopyranoside, a disaccharide derivative, also showed strong binding to both gene forms (-6.8 and -6.7 kcal/mol) and formed 12 hydrogen bonds in each case. This suggests a highly stable interaction driven by polar contacts, although its strong hydrophilicity may limit membrane permeability and oral bioavailability. Alpha-linolenic acid (ALA) presented a notable case of variant-specific interaction. It exhibited stronger binding to the polymorphic variant (-4.6 kcal/mol) compared to the wild-type (-4.0 kcal/mol), though with fewer hydrogen bonds (1 vs. 4, respectively). This suggests that ALA may exert a compensatory effect in PCOS patients carrying the KiSS-1 polymorphism, possibly through favorable structural complementarity or enhanced hydrophobic interactions in the altered gene variant.11-Eicosenoic acid also showed a slightly stronger binding affinity for the polymorphic form (-4.1 kcal/mol) than for the wild-type (-3.9 kcal/mol), although the number of hydrogen bonds decreased from four to two. This shift implies a greater reliance on non-polar interactions in the polymorphic form. In contrast, cis-9-eicosenoic acid exhibited equal binding affinities (-4.0 kcal/mol) and formed one hydrogen bond with both gene variants, indicating non-selective and consistent binding behavior likely driven by hydrophobic and *Van der Waals* forces. Methyl stearate and palmitic acid showed relatively low binding affinities across both gene variants. Methyl stearate had identical scores (-3.7 kcal/mol) with a reduction in hydrogen bonds in the polymorphic form (from 2 to 0), suggesting reduced interaction stability. Palmitic acid displayed slightly better binding to the wild-type (-3.8 kcal/mol) than to the polymorphic gene (-3.6 kcal/mol), with two hydrogen bonds in both cases, indicating weak and non-selective binding. Oleic acid methyl ester (9-octadecenoic acid methyl ester) showed slightly improved binding to the polymorphic gene (-4.1 kcal/mol) compared to the wild-type (-4.0 kcal/mol), with an increase in hydrogen bonds (2 vs. 1), suggesting a slightly more favorable interaction with the mutated form of KiSS-1.In summary, these results indicate that while most *Lepidium sativum* compounds bind consistently to both gene forms via the major groove, stigmasterol and alpha-linolenic acid emerge as the most promising candidates. Stigmasterol's strong hydrophobic binding and alpha-linolenic acid's variant-specific affinity position them as potential therapeutic agents, especially in the context of PCOS-related KiSS-1 gene polymorphisms. Further experimental validation is needed to confirm these computational findings and assess their functional relevance in biological systems [30, 31, 34, 35].

Molecular docking analysis revealed distinct binding patterns between the wild-type and polymorphic variants of the KiSS-1 gene. Among the tested compounds, stigmasterol exhibited the highest binding affinity for both variants (-7.5 kcal/mol), highlighting its potential as a promising lead compound for further therapeutic development. Alpha-linolenic acid displayed a notable difference in binding behavior, with a stronger affinity for the polymorphic variant (-4.6 kcal/mol) than for the wild-type (-4.0 kcal/mol). This suggests a possible compensatory mechanism, wherein alpha-linolenic acid may enhance the function of the polymorphic KiSS-1 variant, offering therapeutic value in managing PCOS-related gene dysfunction.

**Table 4** presents the molecular docking results for the reference standard compound (1), including its Vina binding affinity, number of hydrogen bonds, and the nature of its interaction with the target site. The Vina binding affinity (expressed in kcal/mol) reflects the strength of the interaction between the compound and the KiSS-1 gene target, with more negative values indicating stronger binding. The number of hydrogen bonds formed provides insight into the stability and specificity of the ligand–receptor interaction. Additionally, the groove binding and interaction type describes the specific binding region on the target molecule-such as major or minor grooves-and the types of molecular interactions involved, such as hydrogen bonding, hydrophobic interactions, or *Van der Waals* forces. The molecular docking analysis of two reference standards-Taxol and Spermine-provides a benchmark for evaluating the binding behavior of *Lepidium sativum* compounds against both wild-type and polymorphic KiSS-1 gene targets.

**Table 1:** Binding affinities and interactions of Taxol with β-tubulin (reference standard 1) and Spermine with normal vs. Polymorphic KISS-1 gene (reference standard 2).

| Reference standard (1) | Vina binding affinity (kcal/mol) | Number of hydrogen bonds | Groove binding and interaction Type |
|---|---|---|---|
| Taxol | -9.1 | 1 | Minor groove hydrophobic bonds hydrogen bonds, *Van der Waals* electrostatic Interactions |
| Reference Standard (2) | Vina binding affinity (kcal/mol) with normal vs. polymorphic gene | Number of hydrogen bonds with normal vs. polymorphic gene | Groove Binding and Interaction Type with normal & polymorphic gene |
| Spermine | For normal gene (-4.1) For polymorphic gene (-4.0) | For normal gene (6) For polymorphic gene (5) | Major groove Hydrogen bonds electrostatic interactions hydrophobic bonds *Van der Waals* |

Taxol exhibited a strong binding affinity of -9.1 kcal/mol, indicating a highly stable interaction with the target. It formed one hydrogen bond and bound within the minor groove of the target structure. The interaction profile included hydrophobic interactions, hydrogen bonding, *Van der Waals* forces, and electrostatic interactions, suggesting a well-balanced and robust binding mode. This diverse interaction pattern contributes to the high affinity, reinforcing Taxol's effectiveness as a standard compound in docking studies. Spermine demonstrated moderate binding affinities for both the normal (-4.1 kcal/mol) and polymorphic (-4.0 kcal/mol) KiSS-1 gene variants. It formed six hydrogen bonds with the normal gene and five with the polymorphic variant, indicating relatively strong and stable polar interactions in both cases. Spermine interacted predominantly within the major groove, engaging in hydrogen bonding, electrostatic interactions, hydrophobic interactions, and *Van der Waals* forces. The consistency in binding affinity and interaction types across both gene variants suggests that spermine may maintain stable interaction regardless of genetic variation, although its moderate binding strength limits its potential as a therapeutic agent compared to stronger binders like Taxol.

The comparative Insight shows that Taxol exhibits a much stronger binding affinity and a broad interaction profile, its limited hydrogen bonding (only one) contrasts with the multiple hydrogen bonds formed by Spermine. However, the significantly stronger energy value of Taxol likely results from the synergy of various non-covalent interactions, particularly hydrophobic and electrostatic forces within the minor groove. In contrast, Spermine's interaction appears to rely more heavily on polar interactions (hydrogen bonds and electrostatics) within the major groove. These reference results provide a valuable context for assessing the binding efficiency and interaction quality of bioactive compounds from *Lepidium sativum*, particularly in differentiating between general binders and those with variant-specific therapeutic potential [36, 37].

All eight phytochemicals derived from *Lepidium sativum* successfully docked to both the wild-type and polymorphic forms of the KiSS-1 gene product, demonstrating measurable binding affinities and stable interactions within the major groove of the receptor domain. Notably, alpha-linolenic acid and stigmasterol exhibited the most favorable binding profiles. Stigmasterol showed the highest docking score (-7.5 kcal/mol) for both gene variants, suggesting strong and consistent affinity primarily mediated via hydrophobic interactions. Alpha-linolenic acid displayed moderate binding energies (-4.0 kcal/mol for wild-type; -4.6 kcal/mol for polymorphic), with binding stabilization facilitated by a combination of hydrogen bonding and *Van der Waals* interactions. The reduced binding affinity of alpha-linolenic acid to the polymorphic variant, compared to the wild-type, is consistent with the structural and functional perturbations typically associated with PCOS-linked

KiSS-1 polymorphisms [35, 38-41]. This differential interaction suggests that alpha-linolenic acid may exert a functional compensatory effect, potentially restoring partial gene activity in the polymorphic context through ligand-mediated stabilization of the receptor conformation [42, 43]. Beyond classical ligand-receptor interactions, the structural characteristics of select compounds, particularly stigmasterol, imply potential involvement in epigenetic modulation. Phytosterols such as stigmasterol have been implicated in the regulation of gene expression *via* modulation of DNA methylation patterns, histone acetylation states, and chromatin accessibility. This dual mechanism of action-combining direct binding to gene targets with possible epigenetic influence, positions *Lepidium sativum* compounds as promising candidates for further investigation in the therapeutic management of PCOS-related KiSS-1 dysregulation [9, 44, 45].

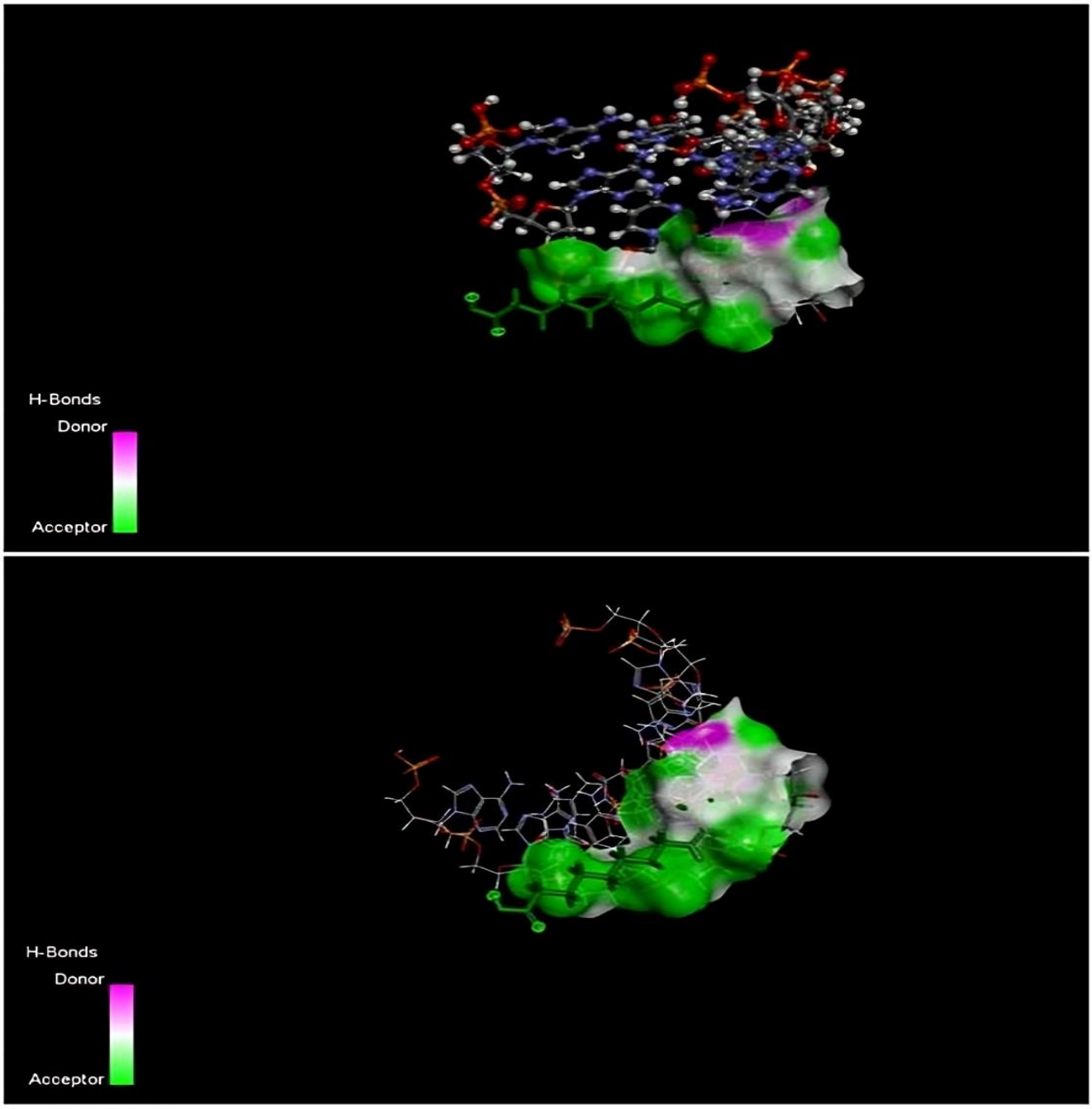



**Figure 2:** Binding of α-Linolenic Acid to KiSS-1 Gene Variants. Reduced hydrogen bonding in the polymorphic variant (top image) compared to the normal gene (bottom image) correlates with impaired functionality in PCOS

The influence of fatty acids and plant compounds on epigenetic regulation has been well documented [13, 27]. Epigenetic analysis suggests that *Lepidium sativum* compounds may further enhance KiSS-1 expression via modulation of methylation and histone acetylation [17, 18]. These mechanisms are particularly relevant in PCOS, where epigenetic dysregulation has been implicated in its pathogenesis [14].

*Conclusion*: This molecular modeling study highlights the therapeutic potential of bioactive compounds from *Lepidium sativum* in modulating the function of both wild-type and polymorphic KiSS-1 gene variants implicated in PCOS. Alpha-linolenic acid and stigmasterol emerged as the most promising candidates, exhibiting strong binding affinities and stable interaction profiles that may compensate for the functional deficits caused by KiSS-1 polymorphisms. Moreover, the capacity of these compounds, particularly stigmasterol, to potentially influence epigenetic regulatory mechanisms further enhances their relevance as plant-based modulators of gene expression. These findings provide a compelling rationale for the integration of *Lepidium sativum* constituents into future experimental and clinical research aimed at developing novel therapeutic strategies targeting the molecular and epigenetic dysregulation of KiSS-1 in PCOS.

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