Trop J Nat Prod Res, December 2025; 9(12): 6119 - 6127 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In-Silico Screening of Seahorse-Derived Compounds Targeting Kisspeptin, GnRH, FSH, and LH Receptors for Reproductive Health Applications

Trisnawati Mundijo[1]*, Yurnadi H Midoen[2], Putra Santoso[3], Aldi T Rahman[3]

[1]*Department of Histology and Cellular Biology, Faculty of Medicine, Universitas Muhammadiyah Palembang, South Sumatra, Indonesia.*
[2]*Department of Medical Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, West Sumatra, Indonesia*

ARTICLE INFO

Article history:
Received 25 August 2025
Revised 18 November 2025
Accepted 04 December 2025
Published online 01 January 2026

ABSTRACT

Natural ingredients derived from plants and animals, such as those associated with seahorses (*Hippocampus* sp.), exhibit numerous benefits, including overcoming health problems. The seahorse is a marine teleost fish with many reproductive health benefits, according to the literature, 20 active substances in seahorses are thought to be beneficial in the reproductive system. *In silico* studies are necessary as an initial step in determining how the bioactivity of active substances in seahorses affects the reproductive system, via investigations of kisspeptin (KISS1), gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) receptors. In this study, target protein structures ware obtained from the Protein Data Bank (https://www.rcsb.org/), molecular docking analysis was carried out using MOE.2022.02 software, and a drug-likeness test was performed using Lipinski's rule of five via the server at https://www.scfbio-iitd.res.in/. Additionally, a toxicity test was conducted using the pKCSM server at https://biosig.lab.uq.edu.au/pkcsm/. The analytical results show that oleic acid is the most effective compound, with good bioavailability, non-toxicity, and the ability to bind to KISS1, GnRH, FSH, and LH receptors. The study reveals the potential of seahorses in the isolation of natural products with medicinal value.

Keywords: Seahorse, Reproductive System, Kisspeptin, Gonadotropin Releasing Hormone, Follicle Stimulating Hormone, Luteinizing Hormone.

Introduction

Infertility is a reproductive health issue a particular concern, especially for married couples unable to bear children after 12 months of unprotected sexual intercourse. Infertility is approximately 85% identifiable, and 15% unexplained. The infertility of couples worldwide is projected to rise due to lifestyle and environmental factors, and impacts the psychosocial functioning of spouses.[1,2,3,4] Recent years have seen rapid growth in the development of natural ingredients to overcome health problems. Natural products derived from plants and animals exhibit numerous benefits, such as the ability to address reproductive health issues. Indonesia, with a notably high biodiversity, displays significant potential in regard to the exploration of natural ingredients, such as compounds derived from seahorses (*Hippocampus* sp.). According to the literature, seahorses contain a variety of essential elements, including trace elements, amino acids, progesterone, steroids, minerals, cholesterol, vitamins, carbohydrates, fatty acids, fibre, and taurine.[5,6,7,8,9]Previous studies have reported the reproductive heath benefits of seahorses,[10,11] both *in vivo* and *in vitro*.[12,13,14,15] According to Mundijo et al.,[16] administering seahorse extract can reduce the expression of caspase-3 and FasL in hypogonadal mice. In addition, Mundijo et al., [17] reported that seahorse extract is safe in regard to the body weight, haematological profiles, and blood chemistry levels of mice.

*Corresponding author.Email: trisna.akbar911@gmail.com
Tel: +6281367543331

Citation: Mundijo T, Midoen YH, Santoso P, Rahman AT. *In-Silico* Screening of Seahorse-Derived Compounds Targeting Kisspeptin, GNRH, FSH, and LH Receptors for Reproductive Health Applications. Trop J Nat Prod Res. 2025; 9(12): 6119 – 6127 https://doi.org/10.26538/tjnpr/v9i12.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

However, studies on how the mechanisms in the body are influenced by the active ingredients in seahorse extract are lacking. According to the literature, 20 active ingredients in seahorse extract are predicted to have benefits for the reproductive system.[18,19] Therefore, *in-silico* investigations are necessary as an initial step in identifying the potential effect and role of active substances in seahorse extract in relation to reproductive system. This study, employs as an *in-silico* approach to explore the mechanism by which active substances in seahorse extract affects the reproductive system, focusing on the kisspeptin (KISS1), gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) receptors.

Materials and Methods

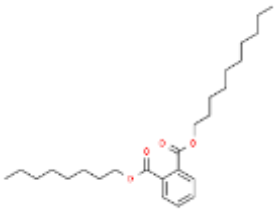
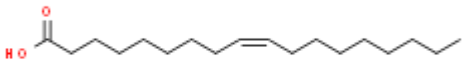
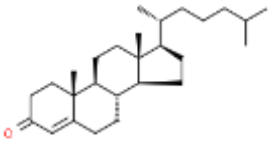
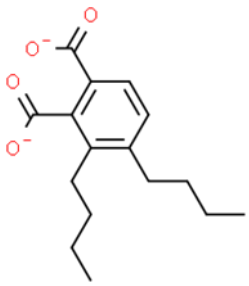
Ligand Preparation

Twenty bioactive compounds in seahorses were selected: bis(2-ethylhexyl)phthalate, 9-octadecenoic acid, cholest-4-en-3-one, dibutyl phthalate, hentriacontanol, pentacosane, hypoxanthine, coprostane, oxacycloheptadecan-2-one, chrysophanol, cholesteryl benzoate, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), bis(2-ethyl heptyl) phthalate, hexahydropyrrolo [1,2a] pyrazine-1,4- dione, 3β-hydroxycholest-5-en-7-one, cholest-5-ene-3β,7β-diol, cholest-5-ene-3β,7α-diol, 5(E), 8(E), 11(E),14(E)-escosatetraienoic acid, and methyl eicosa5,8,11,14,17-pentaenoate. The ligand compound structure were taken from the PubChem database, with the 2D structures displayed in Table 1.

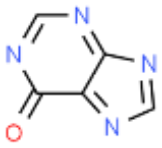
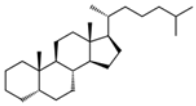
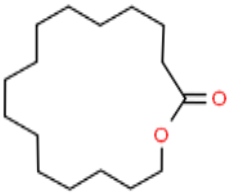
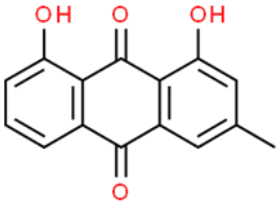
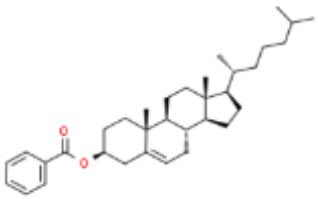
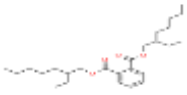
In addition, native ligands/standard drugs were selected for each receptor. Based on the literature, using the standard drug of KISS1 receptor (MVT-02),[20] standard drug of GnRH receptor (Buserelin),[21] synthetic analogue of GnRH (Goserelin),[22] standard drug of the LH receptor (leuprolide),[23] native ligand of the LH receptor,[24] standard drug of the FSH receptor (thiazolidinedione), and native ligand of the FSH receptor (urofollitropin).[25]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Bioactive compound of seahorses

No	Bioactive compound	ChemSpider ID	2D Structure of compound
1	Bis(2-ethyl hexyl)phthalate	8077	
2	9-Octadecenoic Acid	393217	
3	Cholest-4-en-3-one	2224119	
4	3β-Hydroxycholest-5-en-7-one	-	
5	Cholest-5-ene-3β,7β-diol	-	
6	Cholest-5-ene-3β,7α-diol	-	
7	Dibutyl phthalate	11238937	
8	Hentriacontanol	61640	
9	Pentacosane	11900	

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

10	Hypoxanthine	11205977	
11	Hexahydropyrrolo [1,2a] pyrazine-1,4- dione	-	
12	Coprostane	4446724	
13	Oxacycloheptadecan-2-one	7695	
14	Chrysophanol	9793	
15	Cholesteryl benzoate	2005815	
16	Eicosapentaenoic acid (EPA)	393682	
17	Docosahexaenoic acid (DHA)	393183	
18	5(E), 8(E), 11(E),14(E)-escosatetraienoic acid	-	
19	Methyl eicosa5,8,11,14,17- pentaenoate	-	
20	Bis(2-ethyl heptyl) phthalate	2285613	

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Protein Preparation
Target protein structures were retrieved from the Protein Data Bank at https://www.rcsb.org/: KISS1R (PDB ID: KISSIR model), GnRH receptor (7BR3), LH receptor (7FIH), and FSH receptor (4ay9). The positive controls included: MVT-02 (KISS1R), buserelin and goserelin (GnRH), leuprolide and native ligand (LHR), thiazolidinedione and urofollitropin (FSHR).

Molecular Docking
Molecular docking was conducted using the Molecular Operating Environment (MOE) software, version 2019.0102, released in 2019; by the Chemical Computing Group (CCG), Canada. A drug-likeness test using the Lipinski's rule of five was conducted via the server at https://www.scfbio-iitd.res.in/, and toxicity testing was carried out using the pKCSM server at https://biosig.lab.uq.edu.au/pkcsm/.

Results and Discussion

Seahorses-Derived Compounds as Drug-Like Molecules
Data on 14 of the 20 identified compounds in seahorses (Table 1.) are available. In the PubChem database; further analysis was, therefoer, performed on these 14 compounds. As shown in Table 2., the drug similarity analysis was carried out based on Lipinski's rules, where a molecule is considered a promising drug candidate if it meets at least three of the five Lipinski rules; molecular mass <500 Da, lipophilicity (LOGP) <5, hydrogen bond donors (HBD) <5, hydrogen bond acceptors (HBA) <10, and molar refractivity (MR)=40-130.[26] Most of the compounds contained in seahorse extract meet Lipinski's rules, though several compounds do not comply with these rules. Of the 14 analysed compounds, two violate two Lipinski rules: 1-hentriacontanol and cholesterol benzoate. The compatibility of a compound in following Lipinski's rules indicates its oral bioavailability after consumption.[26] A compound could exhibit good bioavailability if it meets at least three of the five Lipinski rules.[27] According to the results of this analysis, the compounds contained in seahorse extract are predicted to be well absorbed by the body when consumed orally.

Table 2: The drug similarity analysis of seahorse extract compounds based on Lipinski's rules

No	Compound (Ligan)	Lipinski's Rules					
		MW (<500 Dalton)	**LogP (<5)**	**Hydrogen Donor (<5)**	**Hydrogen *Acceptor* (<10)**	***Molar Refractivity* (40-130)**	**Violation (≤1)**
1	1-Hentriacontanol	452	11.31	1	1	146.65	2
2	6-Oxopurine (Hypoxanthine)	136	-0.18	2	4	34.2	0
3	Bis(2-ethyl heptyl) phthalate	418.6	6.9	0	4	121.91	1
4	Cholest-4-en-3-one	384	7.596	0	1	118.05	1
5	Cholesterol Benzoate	490	9.25	0	2	148.69	2
6	Chrysophanic acid	254	2.18	2	2	67.81	0
7	Coprostane	372	8.49	0	0	117.68	1
8	Decyl octyl phthalate	390	6.2	0	4	112.68	1
9	Dibutyl phthalate	278	3.6	0	4	76.82	0
10	Docosahexaenoic acid	328	6.54	1	2	105.08	1
11	Eicosapentanoic acid	302	5.99	1	2	95.94	1
12	Oleic Acid	282	6.108	1	2	87.08	1
13	Oxacycloheptadecan-2-one	254	5	0	2	75.59	1
14	Pentacosane	352	9.99	0	0	117.53	1

Prediction of Compound Safety in Seahorse Extract
The toxicity analysis aimed to determine the safety profile of the compounds contained in seahorse extract. As shown in Table 3., several compounds are predicted to show toxicity issues. However, safe compounds were also classified based on the parameters used in this study: 6-oxopurine (hypoxanthine), dibutyl phthalate, oleic acid, and oxacycloheptadecan-2-one.

AMES Toxicity is an indicator of a mutagenic compound, and the human ether-a-go-go-related gene (hERG) chanel is a potassium channel that plays a role in the electrical activity of the heart; compounds able to block the channel (inhibitors) are categorised as cardiotoxic.[28]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: The prediction of bioactive compound toxicity of seahorse extract

No	Compound (Ligan)	Criteria of Toxicity				
		AMES *Toxicity*	**hERG 2 Inhibitor**	***Oral Rat Acute Toxicity* (LD50)**	***Hepatotoxicity***	**Minnow *Toxicity***
1	1-Hentriacontanol	No	Yes	1.936	No	-4.542
2	6-Oxopurine (Hypoxanthine)	No	No	2.152	No	3.046
3	Bis(2-ethyl heptyl) phthalate	No	Yes	1.335	No	-3.072
4	Cholest-4-en-3-one	No	Yes	2.287	No	-2.098
5	Cholesterol Benzoate	No	Yes	2.411	No	-2.726
6	Chrysophanic acid	Yes	No	2.275	No	1.603
7	Coprostane	No	Yes	2.542	No	-2.617
8	Decyl octyl phthalate	No	Yes	1.273	No	-3.54
9	Dibutyl phthalate	No	No	1.624	No	0.499
10	Docosahexaenoic acid	No	No	1.459	Yes	-1.765
11	Eicosapentanoic acid	No	No	1.449	Yes	-1.41
12	Oleic Acid	No	No	1.417	No	-1.438
13	Oxacycloheptadecan-2-one	No	No	2.171	No	1.052
14	Pentacosane	No	Yes	1.716	No	-3.576

The lethal dose (LD50; oral rat acute toxicity) represents the amount of an orally administered compound that causes the death of 50% of a group of test animals; an LD50>0.025 mol/kg is categorised as safe. Thus, based on LD50 values, all seahorse-derived compounds in this study were included in the safe category.[29]

The Hepatoxicity parameter indicates the potential of a compound to induce liver damage. [30] Minnow toxicity measurements indicate that at toxicity potential of <-0.3 for a compound in an aquatic environment categorised as high acute toxicity.[31] Based on this parameter, several compounds can be categorised as toxic; however, it is essential to note that minnow toxicity primarily focuses on the potential ecological impacts that at compound may have on aquatic organisms; rather than the direct impact on humans. Therefore, even though a compound may be considered toxic based on this parameter, it does not always reflect a direct human health hazard. [32]

Molecular Docking Analysis of Ligand Capability

Molecular docking is a computational method used to predict the binding activity in receptor ligand complexes. This approach helps identify the most stable ligand conformation in terms of binding energy in the active site of receptor proteins by considering its binding mode.[33] As presented in Table 4. and Figure 1-4., several compounds show potential binding affinities when compared to the control compounds. The lower the binding affinity, the more stable the bond between the ligand and the receptor protein.[34] Comparative docking analysis revealed that the binding affinity of oleic acid is comparable to that of the control ligands, especially regarding GnRH and LHR. The molecular docking analysis indicated that most compounds found in seahorse extract have a binding affinity close to or below that of the control ligands (marked in red).

This study revealed, that oleic acid has the potential to serve as a primary compound for reproductive modulation. This is likely due to its direct regulation of the synthesis and activity of antioxidant enzymes.[35] Previous studies have reported that oleic acid, an anti-inflammatory molecule, causes a decrease in oxidative stress-triggering mediators.[36,37] Furthermore, other studies have indicated that oleic acid affects the production of lipid mediators and membrane protein interactions, thereby affecting signal transduction mechanisms. [38,39]

Figure 1: Visualization of the interaction between ligand and KISS1R (kisspeptin receptor). The interaction between KISS1R protein with: (A) 1-Hentriacontanol, (B) Bis(2-ethylheptyl) phthalate, (C) Cholest-4-en-3-one, (D) Cholesteryl Benzoate, (E) Chrysophanic acid, (F) Coprostane, (G) Decyl octyl phthalate, (H) Dibutyl phthalate, (I) Docosahexaenoic acid, (J) Eicosapentanoic acid, (K) Hypoxanthine, (L) Oleic acid, (M) Oxacycloheptadecan-2-one, (N) Pentacosane, (O) MVT-02 (standard drug of Kisspeptin receptor).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

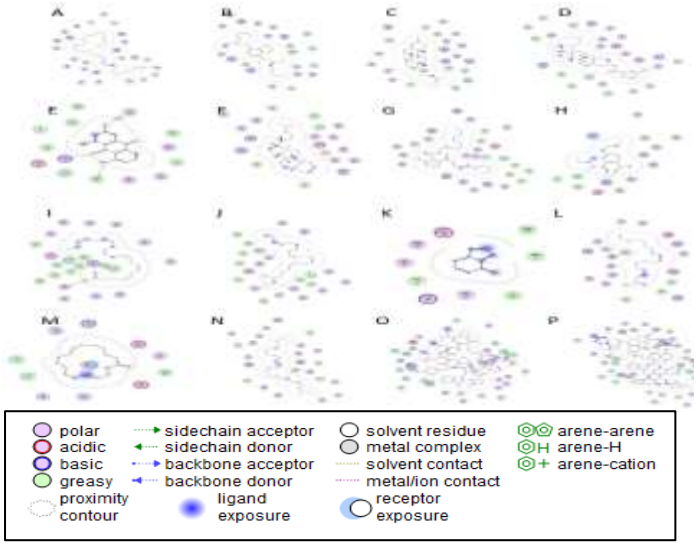


Figure 2: Visualization of the interaction between ligand and GnRH receptor (7BR3). The interaction between GnRH protein with: (A) 1-Hentriacontanol, (B) Bis(2-ethylheptyl) phthalate, (C) Cholest-4-en-3-one, (D) Cholesteryl Benzoate, (E) Chrysophanic acid, (F) Coprostane, (G) Decyl octyl phthalate, (H) Dibutyl phthalate, (I) Docosahexaenoic acid, (J) Eicosapentanoic acid, (K) Hypoxanthine, (L) Oleic acid, (M) Oxacycloheptadecan-2-one, (N) Pentacosane, (O) Buserelin (standard drug of GnRH receptor), (P) Goserelin (synthetic analogue of GnRH).

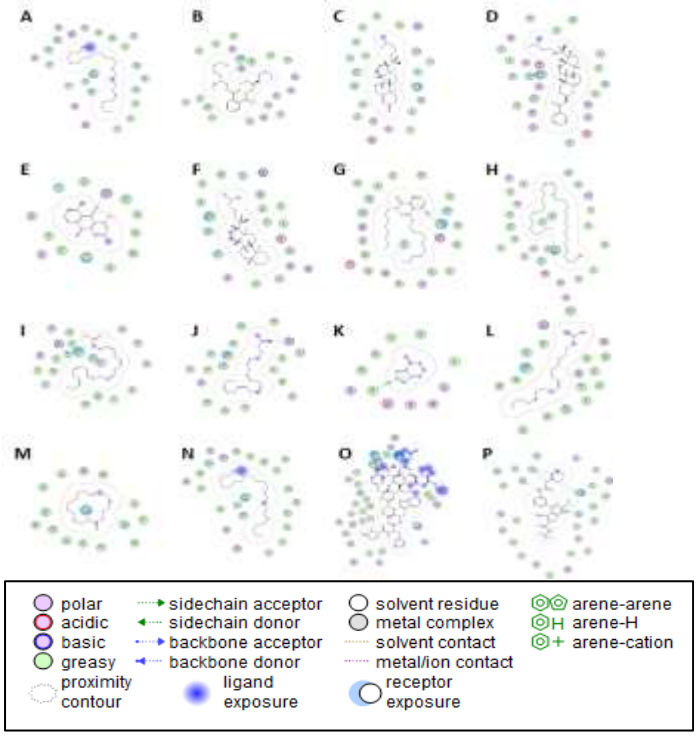


Figure 3: Visualization of the interaction between ligand and LH receptor (7FIH). The interaction between LH protein with: (A) 1-Hentriacontanol, (B) Bis(2-ethylheptyl) phthalate, (C) Cholest-4-en-3-one, (D) Cholesteryl Benzoate, (E) Chrysophanic acid, (F) Coprostane, (G) Decyl octyl phthalate, (H) Dibutyl phthalate, (I) Docosahexaenoic acid, (J) Eicosapentanoic acid, (K) Hypoxanthine, (L) Oleic acid, (M) Oxacycloheptadecan-2-one, (N) Pentacosane, (O) Leuprolide (standard drug for LH receptor), (P) Native ligand of LH receptor.

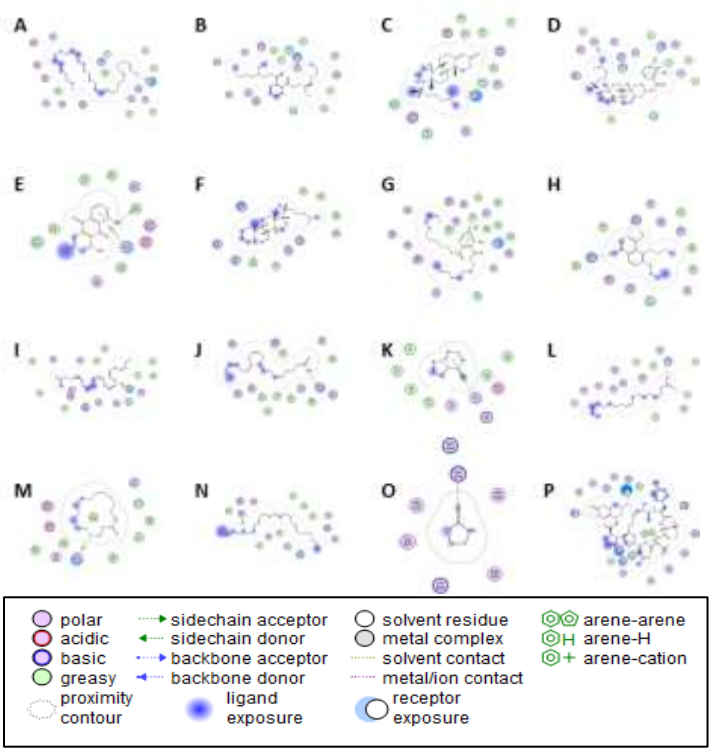


Figure 4: Visualization of the interaction between ligand and FSH receptor (4ay9). The interaction between FSH protein with: (A) 1-Hentriacontanol, (B) Bis(2-ethylheptyl) phthalate, (C) Cholest-4-en-3-one, (D) Cholesteryl Benzoate, (E) Chrysophanic acid, (F) Coprostane, (G) Decyl octyl phthalate, (H) Dibutyl phthalate, (I) Docosahexaenoic acid, (J) Eicosapentanoic acid, (K) Hypoxanthine, (L) Oleic acid, (M) Oxacycloheptadecan-2-one, (N) Pentacosane, (O) Thiazolidinone (standard drug for FSH receptor), (P) Urofollitropin (native ligand for FSH receptor).

The KISS1 receptor is a G-protein-coupled receptor (GPCR) that could activate the signalling of phospholipase C-protein kinase C-extracellular signal-regulated kinase (PLC-PKC-ERK). Increasing the activation of this protein through specific molecules could prevent hypogonadotropic hypogonadism.[40] GnRH receptors belong to the family of GPCRs, playing an essential role in initiating the reproductive hormone cascade and the release of gonadotropins, FSH and LH.[41] Additionally, increasing the activation of this protein has shown beneficial effects on enhancing oocyte maturity and preserving ovarian function.[42,43]

The protein of the LH receptor, a member of GPCR family, plays an essential role in the reproductive system. Activation of this protein may occur through the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway, which can trigger steroidogenesis, closely related to the production of testosterone and progesterone. Moreover, this protein is closely related to the extracellular signal-regulated kinase 1/2 (ERK1/2) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signalling pathways, which are related to gamete maturation.[44] A similar process was also demonstrated for the protein of the FSH receptor through the cAMP/PKA channel, related to steroidogenesis and progesterone production, and folliculogenesis through the ERK/PI3K/AKT pathway. This is related to the growth process of gametes and the improvement of gametes quality.[45,46] Thus, the study reveals the potential of seahorse-extracted compounds in improving reproductive functioning by targeting these proteins.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: The Results of molecular docking of compounds in Seahorse Extract toward receptor proteins

No	Compound (ligand)	*Binding affinity* (Kcal/mol)			
		KISS1R (Kisspeptin receptor)	**7BR3 (GnRH receptor)**	**7FIH (LH receptor)**	**4ay9 (FSH receptor)**
1	1-Hentriacontanol	-7.6667[1]	-11.3049[1]	-10.196[1]	-8.862[1]
2	Bis(2-ethyl heptyl) phthalate	-6.4676	-9.3303[1]	-7.811[1]	-6.729
3	Cholest-4-en-3-one	-5.6473	-8.1282[1]	-7.910[1]	-7.191[1]
4	Cholesteryl Benzoate	-6.5653	-9.4863[1]	-7.046[1]	-6.237
5	Chrysophanic acid	-4.781	-6.0135	-11.450[1]	-8.184[1]
6	Coprostane	-5.7057	-8.4816[1]	-9.113[1]	-7.967[1]
7	Decyl octyl phthalate	-6.8067	-9.54[1]	-4.421	-4.679
8	Dibutyl phthalate	-5.5324	-6.2098	-7.432[1]	-6.151
9	Docosahexaenoic acid	-6.1469	-8.5344[1]	-7.290[1]	-5.798
10	Eicosapentanoic acid	-6.0678	-7.6213	-5.950	-5.847
11	Hypoxanthine	-4.0177	-4.2061	-8.310[1]	-7.996[1]
12	Oleic acid	-5.9479	-7.7739[1]	-7.996[1]	-7.037[1]
13	Oxacycloheptadecan-2-one	-5.2524	-6.6959	-8.238[1]	-7.101[1]
14	Pentacosane	-6.871	-9.4188[1]	-9.483[1]	-7.506[1]
15	*Standard drug of Kisspeptin receptor (MVT-02)*	-9.3937			
16	*Standard drug of GnRH receptor (Buserelin)*		-8.9092		
17	*A synthetic analog of GnRH (Goserelin)*		-12.5599		
18	*Standard drug for LH receptor (Leuprolide)*			-10.171	
19	*The native ligand of the LH receptor*			-9.7944	
20	*Standard drug of FSH receptor (Thiazolidinedione)*				-3.684
21	*Native ligand for FSH receptor (Urofollitropin)*				-10.327

[1]: Binding affinity in seahorse extract close to the control ligand or below the control ligand

Conclusion

Oleic acid demonstrated favourable binding affinities towards KISS1R, GnRH, FSHR, and LHR, along with acceptable pharmacokinetic and toxicity profiles, suggesting its potential as a lead compound for reproductive modulation. These findings provide a rationale for future *in vitro* and *in vivo* validation studies, establishing seahorse-derivered compounds as potentially beneficial natural products for the reproductive system.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to thank supporting institutions for data access and technical support during computational analysis and Mrs. Dina Khairani for the suggestions in this manuscript.

References

1. Carson SA, Kallen AN. Diagnosis and management of infertility: A Review. J the Am Med Assoc. 2021; 326(1):65-67.
2. Braverman AM, Davoudian T, Levin IK, Bocage A, Wodoslawsky S. Depression, anxiety, quality of life, and infertility: a global lens on the last decade of research. Fertil Steril. 2024; 121(3):379-383.
3. Bai CF, Sun JW, Li J, Jing WH, Zhang XK, Zhang X, Ma LL, Yue R, Cao FL. Gender differences in factorsassociated with depression in infertility patients. J Adv Nurs. 2019; 75(12):3515-3524.
4. Glazer CH, Eisenberg ML, Tøttenborg SS, Giwercman A, Flachs EM, Bräuner EV, Vassard D, Pinbong A, Schmidt L, Bonde JP. Male factor infertility and risk of death: A nationwide record-linkage study. Human Reprod. 2019; 34(11):2266-2273.
5. Sari EM, Nurilmala M, Abdullah A. Amino acid profile and bioactive compound in seahorse (*Hippocampus comes)*. J Trop Mar and Sci Technol. 2017; 9(2):605-617.
6. Safryna DA. 2020. Characteristics of the Seahorse (*Hippocampus comes*) and Hidrolyzate. Thesis. Bogor Agricultural Institute. Bogor.
7. Sun J, Xia S, Xie S, Yang Y, Cui P, Shao P, Xu Y, Shuang L.Biochemical composition of wild and cultured seahorses (*Hippocampus kuda* Bleeker). Aqua Res. 2020; 51(4):1-9.
8. Scobell SK, Mackenzie DS. Reproductive endocrinology of Syngnathidae. J Fish Biol. 2011; 78:1662-1680.
9. Mundijo T, Suyatna FD, Wibowo AE, Supriyono A, Midoen YH. Characterization of seahorse (*Hippocampus comes* L.) extracts originating from culture and nature in Pesawaran, Lampung, Indonesia. J Adv Vet Anim Res. 2022; 9(4):610–616.
10. Mundijo T, Suyatna FD, Wibowo AE, Lestari SW, Yusra Y, Midoen YH. The seahorse (*Hippocampus comes* L.) extract ameliorates sperm qualities, testosterone level, and serum biochemistry in rats induced by depo medroxyprogesterone acetate. J Adv Vet Anim Res. 2023; 10(1):126-131.
11. Dianty RS, Midoen YH, Lestari SW, Mundijo T, Kusmardi K. Effect of seahorse (*Hippocampus comes* L.) extract on population and apoptotic of spermatogenic and leydig cells in rats after depot medroxyprogesterone acetate induction. Trop J Nat Prod Res. 2024; 8(2):6272-6278.
12. Kumaravela KS, Balasubramaniana RT, Sonneschein L. Review Article: Seahorses – A source of traditional medicine. Nat Prod Res. 2012; 26(24):2330-2334.
13. Chen L, Wang X, Huang B. The genus *Hippocampus*-a review on traditional medicinal uses, chemical constituents and pharmacological properties. J Ethnopharm. 2014; 13(162):104-111.
14. Kim MY, Jeon YJ, Huh JS, Kim SD, Park KK, Cho M. Effect of enzymatic hydrolysate from seahorse (*Hippocampus abdominalis*) on testosterone secretion from TM3 leydig cells and in male mice. Appl Biol Chem. 2016; 59(6):869-879.
15. Martos SG, Nuez MZ, Pérez JAC, Blanco TM, Pérez-Pé R, Casao A. Involvement of progesterone and estrogen receptors in the ram sperm acrosome reaction. Domest and Endocrinol. 2021; 74:106527.
16. Mundijo T, Midoen YH, Suyatna FD, Wibowo AE, Kusmardi K. Effect of seahorse extract (*Hippocampus comes* L.) on caspase-3 and TUNEL assay in rats after depot medroxyprogesterone acetate induction. Pharmacogn J. 2022; 14(4):253–258.
17. Mundijo T, Suyatna FD, Wibowo AE, Yusra Y, Midoen YH. Safety and effectiveness of seahorse extract (*Hippocampus comes* L.) on the hematological profile and body weight of male rats induced by depo medroxyprogesterone acetate. J Adv Vet Anim Res. 2024; 11(3):717-721.
18. Wu X, Zhang Q, Hu J. QSAR study of the acute toxicity to fathead minnow based on a large dataset. SAR and QSAR in Environmental Research. 2016; 27(2):147–164.
19. Li K, Yan L, Zhang Y, Yang Z, Zhang C, Li Y, Kalueff AV, Li W, Song C. Seahorse treatment improves depression-like behavior in mice exposed to CUMS through reducing inflammation/Oxidants and restoring neurotransmitter and neurotrophic function. J Ethnopharm. 2020; 250:112487.
20. Abbara A, Ufer M, Voors-Pette C, Berman L, Ezzati M, Wu R, Lee TY, Ferreira JCA, Migoya E, Dhillo WS. Endocrine profile of the kisspeptin receptor agonist MVT-602 in healthy premenopausal women with and without ovarian stimulation: results from 2 randomized, placebo-controlled clinical trials. Fertil Steril. 2024; 121(1):95-106.
21. Parker KL, Schimmer BP. Chapter: Buserelin. In: Reference Module in Biomedical Sciences. Elsevier. 2001.
22. National Center for Biotechnology Information (NCBI). LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases. 2012; Goserelin.
23. Sidhu G, Tripp J. Leuprolide. StatPearls Publishing; 2025.
24. DrugBank. Buserelin: Targets. 2025; https://go.drugbank.com/drugs/DB06719
25. Sriraman V, Denis D, De Matos D, Yu H, Palmer S, Nataraja S. Investigation of a thiazolidinone derivative as an allosteric modulator of follicle stimulating hormone receptor: Evidence for its ability to support follicular development and ovulation. Biochem Pharmacol. 2014; 89(2):266-275.
26. Kharisma VD, Agatha A, Ansori ANM, Widyananda MH, Rizky WC, Dings TGA, Derkho M, Lykasova I, Antonius Y, Rosadi I, Zainul R. Herbal combination from *Moringa oleifera* Lam. and *Curcuma longa* L. as SARS-CoV-2 antiviral via dual inhibitor pathway: A bioinformatics approach. J Pharm and Pharmacog Res. 2022; 10(1):138–146.
27. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the Rule of 5 and Drugability. Adv Drug Deliv Rev. 2016; 101:89.
28. Ahmad L, Kuznetsov AE, Pirzada AS, Alsharif KF, Daglia M, Khan H. Computational pharmacology and computational chemistry of 4-hydroxyisoleucine: Physicochemical, pharmacokinetic, and DFT-based approaches. Front Chem. 2023; 11:1145974.
29. Onguéné PA, Simoben CV, Fotso GW, Andrae-Marobela K, Khalid SA, Ngadjui BT, Mbaze LM, Ntie-Kang F. *In silico* toxicity profiling of natural product compound libraries from African flora with anti-malarial and anti-HIV properties. Comput Biol Chem. 2018; 72:136–149.
30. Kumar R, Rani R, Narang SK, Rai S, Hajam YA. Hepatotoxicity: Its physiological pathways and control measures using phyto-polyphenols. Phytomedicine: A Treasure of pharm active prod from plants. 2021:621–653.
31. Wu J, Liu Z, Su J, Lu H, Liao D, Song Q. Chemical constituents of the seahorse *Hippocampus trimaculatus* from the east China Sea. Chem Nat Comp. 2017; 53(5):982-983.
32. Erickson RJ, Mount DR, Highland TL, Hockett JR, Hoff DJ, Jenson CT, Norberg-King TJ, Forsman B. Acute toxicity of major geochemical ions to fat head minnows (*Pimephales promelas*). Part A: Observed relationships for individual salts and salt mixtures. Environ Toxicol Chem. 2022; 41(9):2078.
33. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in disease management. Sci Rep. 2023; 13(1):1–18.
34. Rahman AT, Rafia, Jethro A, Santoso P, Kharisma VD, Murtadlo AAA, Purnamasari D, Soekamto NH, Ansori, ANM, Kuswati, Mandeli RS, Aledresi KAMS, Yusof NFM, Jakhmola V, Rebezov M, Zainul R, Dobhal K, Parashar T, Ghifari MA, Sari DAP. *In silico* study of the potential of endemic sumatra wild turmeric rhizomes (*Curcuma*

sumatrana: Zingiberaceae) As anti-cancer. Pharmacog J. 2022; 14(6):806–812.
35. Bhattacharjee B, Pal PK, & Chattopadhyay A, Bandyopadhyay D. Oleic Acid protects against cadmium induced cardiac and hepatic tissue injury in male wistar rats: A Mechanistic study. Life Sci. 2020; 1(244):117324.
36. Oh YT, Lee JY, Lee J, Kim H, Yoon KS, Choe W, Kang I. Oleic acid reduces lipopolysaccharide-induced expression of inos and COX-2 in BV2 murine microglial cells: possible involvement of reactive oxygen species, p38 MAPK, and IKK/NF-KappaB signaling pathways. Neurosci. Lett. 2009; 466(2):93–97.
37. Harvey KA, Walker CL, Xu Z, Whitley P, Pavlina TM, Hise M, Zaloga GP, Siddiqui RA. Oleic acid inhibits stearic acid-induced inhibition of cell growth and pro-inflammatory responses in human aortic endothelial cells. J Lip Res. 2010; 51(12):3470–3480.
38. Speizer LA, Watson MJ, Brunton LL. Differential effects of omega-3 fish oils on protein kinase activities in vitro. Am J Physiol. 1991; 261(1 Pt 1): E109–114.
39. Ponnappan S, Ponnappan U. Aging and immune function: Molecular mechanisms to interventions. Antioxid Redox Sig. 2011; 14(8):1551–1585.
40. Hu KL, Zhao H, Chang HM, Yu Y, Qiao J. Kisspeptin/kisspeptin receptor system in the ovary. Front Endocrinol. 2018; 8:331073.
41. Yan W, Cheng L, Wang W, Wu C, Yang X, Du X, Ma L, Qi S, Wei Y, Lu Z, Yang S, Shao Z. Structure of the human gonadotropin-releasing hormone receptor GnRH1R reveals an unusual ligand binding mode. Nat Comm. 2020; 11(1):1–10.
42. Blumenfeld Z. Fertility preservation using GNRH agonists: rationale, possible mechanisms, and explanation of controversy. Clin med insights. Reprod Health. 2019; 13:1-13.
43. Merkison J, Malcom C, Decherney A. Use of gonadotropin-releasing hormone (GnRH) agonist trigger in fertility preservation for patients with inherited genetic disorders. Front Endocrinol 2022; 13:826419.
44. Narayan P. Genetic models for the study of Luteinizing hormone receptor function. Front Endocrinol 2015; 6: 162212.
45. Casarini L, Crépieux P. Molecular mechanisms of action of FSH. Front Endocrinol 2019; 10:305.
46. Hunzicker-Dunn ME, Lopez-Biladeau B, Law NC, Fiedler SE, Carr DW, Maizels ET. PKA and GAB2 play central roles in the FSH signaling pathway to PI3K and AKT in ovarian granulosa cells. Proc Natl Acad Sci U S A. 2012; 109(44):2979-2988.