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GC–MS characterization and *in silico* evaluation of the reproductive bioactivity of *Amaranthus polygonoides* L. (Amaranthaceae) in bovines

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ABSTRACT

Background: *Amaranthus polygonoides* L., commonly known as "Sirukeerai," is a widely cultivated leafy vegetable in Tamil Nadu, valued for its rapid growth and rich nutritional content. This study investigates the plant's chemical composition using proximate and phytochemical analysis, alongside GC-MS for identifying volatile and semi-volatile compounds. Computational tools like SwissADME and ProTox 3.0 are employed to evaluate the pharmacokinetics and safety profiles of these compounds. Given the key role of GnRH and FSH in bovine reproduction, molecular docking is used to explore the interaction of identified compounds with their respective receptors. The research aims to uncover the plant's potential influence on reproductive health in cattle. **Objective:** To evaluate the nutritional, phytochemical, pharmacokinetic, and molecular docking properties of *Amaranthus polygonoides* leaves to explore their potential influence on reproductive functions in cattle. **Materials and Methods:** *Amaranthus polygonoides* was collected, authenticated, and its dried leaves powdered for analysis. Proximate composition was determined using standard AOAC methods. Methanolic extracts underwent preliminary phytochemical screening and GC-MS analysis to identify bioactive compounds. SwissADME and ProTox-3.0 were used to assess pharmacokinetic and toxicity profiles. Three selected compounds were docked with Gonadotropin-releasing hormone (GnRH) and Follicle-stimulating hormone (FSH) receptors using the PrankWeb tool. **Results:** The leaf powder contained 8.89% moisture, 24.47% crude protein, 8.34% crude fibre, 2.77% ether extract, 13.01% total ash, and 2.10% acid-insoluble ash, with calcium (1.99%), phosphorus (0.51%), salt (2.21%), and a gross energy of 3678 kcal/kg. GC-MS identified ten compounds, of which three—Citronellyl propionate; Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-; and Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid showed favourable ADME properties. Docking revealed stable binding energies with GnRH (-5.968 to -7.146 kcal/mol) and FSH receptors (-5.945 to -7.44 kcal/mol), with the sulfonylated benzene derivative exhibiting the strongest and most stable interactions. **Conclusion:** *Amaranthus polygonoides* demonstrates promising nutritional and bioactive potential. Particularly, the sulfonylated benzene compound shows potential in modulating bovine reproductive hormones, warranting further investigation.

Keywords: *Amaranthus polygonoides*, Phytochemicals, Molecular Docking Simulation, Gonadotropin-Releasing Hormone, Follicle Stimulating Hormone, Pharmacokinetics.

INTRODUCTION

The genus *Amaranthus* encompasses over 75 species globally, grouped into three subgenera. In India alone, more than 18 species have been documented, with *Amaranthus polygonoides* L. commonly known as tropical amaranth or "Sirukeerai" in Tamil, being one of the most widely cultivated leafy vegetables across Tamil Nadu. This plant is favoured for its fast growth, allowing for harvest approximately 25 days after sowing [1]. Notably, *A. polygonoides* is recognized for its rich nutritional profile, offering substantial amounts of protein, dietary fibre, and a variety of essential vitamins and minerals, particularly vitamin B [2].

Understanding the chemical and nutritional composition of this species is critical for evaluating its potential health benefits. Proximate analysis offers insight into the primary nutrients present in the leaf powder, while preliminary phytochemical screening helps to identify the key bioactive compounds. It is important to note that the nutritional and phytochemical content of *Amaranthus* species can vary based on geographic and environmental factors [3].

GC-MS, in particular, is effective for identifying volatile and semi-volatile phytoconstituents [4]. Furthermore, computational tools such as SwissADME and ProTox 3.0 provide insights into the pharmacokinetics, drug-likeness, and toxicity profiles of identified compounds [5].

Reproductive efficiency in cattle is regulated by several hormones, notably gonadotropin-releasing hormone (GnRH) and follicle-stimulating hormone (FSH). GnRH acts on its receptor located on the gonadotrope cells of the anterior pituitary, stimulating the release of FSH and luteinizing hormone (LH)-both vital for reproductive function. FSH, in turn, binds to its receptor on granulosa cells in the ovarian follicles, promoting follicular maturation and estrogen production, thus facilitating the estrous cycle and ovulation [6].

Given the significance of these hormonal pathways, this study aims to identify volatile and semi-volatile compounds in *A. polygonoides* via GC-MS and assess their potential interactions with bovine GnRH and FSH receptors through molecular docking analysis. The findings could offer valuable insights into the plant's potential role in modulating reproductive functions in cattle.

MATERIAL AND METHODS

Collection and Identification of Plant Material

Fresh specimens of *A. polygonoides* were collected from Tirukovilur, located at the Kallakurichi district of Tamil Nadu, India. The plant material was authenticated by the Research and Postgraduate Department of Botany at St. Thomas College, Thrissur, Kerala. A voucher specimen (Herbarium No.04/VPT-CVAS-MTY/2018) was deposited at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala for future reference. The collected leaves were shade-dried and pulverized using an electric pulverizer. The resulting leaf powder was stored in airtight containers at room temperature for subsequent analyses.

Proximate Analysis *of A.polygonoides* Leaf powder

The proximate composition of the *A. polygonoides* leaf powder was determined at the Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Namakkal, Tamil Nadu. The analysis included assessments of moisture content, crude protein, crude fat, crude fibre, ash content, and carbohydrate levels.

Preparation of Methanolic Extract *of A. polygonoides* Leaf powder

A methanolic extract of the *A. polygonoides* leaf powder was prepared using a Soxhlet apparatus. Approximately 50 grams of the dried leaf powder of *A. polygonoides* were placed in a thimble and extracted with 500 mL of methanol for 72 hours, ensuring continuous solvent reflux. The extract was then concentrated by removing the solvent under reduced pressure using a rotary vacuum evaporator. The concentrated extract was stored in a refrigerator at 4°C for further use. The yield percentage of the crude extract was calculated.

Phytochemical Screening of Methanolic Extract *of A. polygonoides* Leaf powder

Preliminary phytochemical screening of the methanolic extract of *A. polygonoides* leaf powder was conducted to identify the presence of various bioactive compounds. Standard qualitative tests, as described by Harborne AJ *et al*, were employed [7].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Methanolic Extract *of A. polygonoides* Leaf powder

The GC-MS analysis of the methanolic extract of *A. polygonoides* leaf powder was performed at the Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala using a Shimadzu GCMS-QP2010S system. The chromatographic separation was achieved on an Rxi-5Sil MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed to increase from an initial 80°C (held for 4 minutes) to 280°C at a rate of 5°C per minute, with a final hold at 280°C for 6 minutes. The injection temperature was set at 260°C, and the ion source temperature was maintained at 200°C. Helium (99.999% purity) was used as the carrier gas at a flow rate of 1 mL/min. The ionizing energy was 70 eV, and mass spectra were recorded in the scan range of 50–500 amu. Compounds were identified by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST 11) and WILEY 8 libraries.

In silico pharmacokinetic and toxicity assessment of identified compounds and molecular docking with GnRH and FSH receptors of *Bovine taurus*

For each identified compound, key chemical properties including IUPAC name, molecular weight, molecular formula, structural class, and SMILES notation were determined. Subsequently, the pharmacokinetic parameters and drug-likeness of these compounds were evaluated using the Swiss ADME online tool. Compounds exhibiting favorable gastrointestinal (GI) absorption, the ability to cross the blood-brain barrier (BBB), and compliance with all five major drug-likeness filters- Lipinski, Ghose, Veber, Egan, and Muegge- were selected for further analysis. To ensure the identified compounds having acceptable safety profiles, the selected compounds were then subjected to toxicity assessment using the ProTox-3.0. For molecular docking studies, the Gonadotropin-Releasing Hormone (GnRH) receptor and the Follicle-Stimulating Hormone (FSH) receptor of *Bos taurus* were chosen as targets. The AlphaFold-predicted structures of these receptors, corresponding to UniProt ID: P32236 and F1MT42, were utilized. Active binding sites on these receptors were predicted using the PrankWeb online tool, which employs the P2Rank algorithm- a machine learning-based method for ligand binding site prediction [8]. Finally, molecular docking was performed for the compounds filtered through SwissADME and ProTox-3.0 with the GnRH and FSH receptors using the same PrankWeb tool.

RESULTS

Plants extraction yield percentage

The *A. polygonoides* L. belonged to the family Amaranthaceae. The methanolic extract of this plant leaves yielded the extract percentage of 21.12 ± 0.29.

Proximate analysis *of A. polygonoides* leaf powder

The proximate analysis of *A. polygonoides* leaf powder reveals its potential as a valuable nutritional resource. The results were given in (Table 1).

Phytochemical Screening of Methanolic Extract of Leaf Powder

Preliminary phytochemical screening of the methanolic extract of *A. polygonoides* leaf powder revealed the presence of several bioactive constituents, including alkaloids, tannins, flavonoids, diterpenes, phenols, flavonoids, steroids, and saponins.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract *of A. polygonoides* leaf powder

The GC-MS analysis of the *A. polygonoides* leaf extract revealed the presence of ten major phytochemical constituents, eluting between 26.69 and 43.25 minutes (Figure 1). The identified compounds represented various classes, including fatty acid esters, terpenoids, saturated and unsaturated fatty acids, and aromatic derivatives. A total of 10 peaks were recorded, with methyl linolenate (49.17%) and phytol (33.26%) identified as the dominant components, indicating high concentration of bioactive lipophilic compounds, particularly a high abundance of unsaturated fatty acid esters and diterpenoids in the extract. The list of GC-MS-identified phytoconstituents from the methanolic extract is presented in (Table 2).

SwissADME Prediction of Pharmacokinetic and Drug-Likeness Properties

The pharmacokinetic and drug-likeness profiles of the phytochemical compounds identified by GC-MS were evaluated using the SwissADME tool. The analysis revealed that all compounds exhibited good predicted gastrointestinal (GI) absorption, with the exception of Phytol, which showed poor absorption. In terms of BBB permeability, all compounds were predicted to cross the BBB except Phytol and Hexadecanoic acid methyl ester, likely due to unfavorable physicochemical properties such as high molecular weight or excessive lipophilicity. When assessed against standard drug-likeness filters, including Lipinski**,** Ghose**,** Veber**,** Egan**,** and Muegge**,** three compounds **(**Citronellyl propionate**,** Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]**,** and Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid**)** successfully passed all five filters, indicating favourable drug-like characteristics (Table 3)

Toxicity Prediction of Selected Phytoactive Compounds

Toxicity assessments of the three compounds filtered through were performed using the *ProTox-II* (v3.0) webserver [9].

The predicted oral LD_{50} for Citronellyl propionate was 5000 mg/kg**,** categorizing it under GHS toxicity class V**,** which indicates relatively low acute toxicity and potential for safe oral administration. No organ-specific toxicity or carcinogenic potential was predicted, with only BBB permeability noted.

The benzene sulfonyl derivative had a predicted LD_{50} of 2500 mg/kg, also placing it in GHS Class V. Although this compound raised ecotoxicity and BBB permeability concerns, no organ toxicity or immunotoxicity was predicted.

Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid exhibited slightly higher predicted toxicity with an LD_{50} of 2000 mg/kg**,** classifying it under GHS Class IV**,** indicating mild to moderate acute toxicity. However, like the other compounds, it showed no predicted organ toxicity and only moderate concerns related to BBB permeability and ecotoxicity (Table 4).

In Silico Molecular Interaction Study

Ligand-Binding Site Prediction:

PrankWeb identified ten potential ligand-binding sites at GnRH receptor**,** with the top-ranked site scoring 9.40. This site involved 13 amino acid residues, had a ligandability probability of 0.43**,** and an evolutionary conservation score of 0.93**,** suggesting functional importance and evolutionary stability. In case of FSH receptor, eight potential ligand-binding pockets were identified. The highest-ranked pocket had a score of 26.11**,** included 29 residue**s**, and demonstrated a high ligandability score of 0.860 along with an AlphaFold confidence score of 73.403, indicating a highly reliable prediction and significant potential for ligand interaction

Molecular Docking:

Molecular docking was conducted between the top binding pockets of the GnRH and FSH receptors and three selected phytochemical compounds identified from GC-MS analysis (Figures 2, 3, 4, 5, 6 and 7), Citronellyl propionate**,** Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy] -2-butenyl] sulfonyl]- and Trans-2-[(1E,5E)-2,6,10- trimethyl-1,5,9-undecatrienyl] cyclopropanecarboxylic acid**.** The docking results were summarized in (Tables 5 and 6).

Table 1: Proximate analysis of dried leaf powder of *Amaranthus polygonoides*

S. No.	Proximate principles	Percentage (%)
1	Moisture	8.89
2	Crude protein	24.47
3	Crude fibre	8.34
4	Ether extract	2.77
5	Total ash	13.01
6	Acid insoluble ash (sand & silica)	2.10
7	Calcium	1.99
8	Phosphorus	0.51
9	Salt	2.21
10	Gross energy	3678 kcal/kg

Table 2: Phytochemical constituents identified in the methanolic extract of *Amaranthus* sp. using Gas chromatography – mass spectrometry

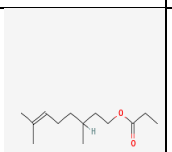
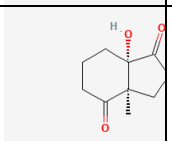
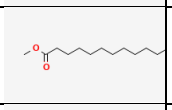
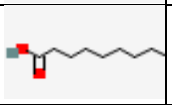
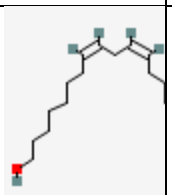
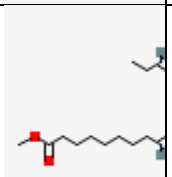
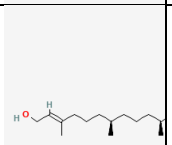
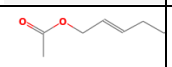
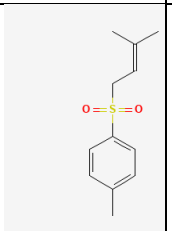
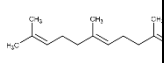
S. No.	Compound Name	RT (min)	IUPAC Name	Mol. Weight (g/mol)	Mol. Formula	Peak Area %	Structure	Structure Class
1	Citronellyl propionate	26.695	3,7-dimethyloct-6-enyl propanoate	212.33	$C_{13}H_{24}O_2$	2.20		Fatty alcohol esters
2	1,6,6-Trimethyl-9-Hydroxybicyclo[4.3.0] Nonan-2-One Hydrazone	28.393	(3*aR*,7*aS*)-7*a*-hydroxy-3*a*-methyl-3,5,6,7-tetrahydro-2*H*-indene-1,4-dione	182.22	$C_{10}H_{14}O_3$	0.85		Alcohols and polyols
3	Heptadecanoic Acid, Methyl Ester	28.521	methyl heptadecanoate	284.5	$C_{18}H_{36}O_2$	8.59		Fatty acid esters
4	Tridecanoic Acid	29.503	tridecanoic acid	214.34	$C_{13}H_{26}O_2$	0.95		Fatty acids and conjugates
5	(Z,Z)-Heptadeca-8,11-Dien-1-Yl Bromide	31.720	(8Z,11Z)-heptadeca-8,11-dien-1-ol	252.4	$C_{17}H_{32}O$	2.90		Fatty alcohols
6	Methyl Linolenate	31.886	methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	292.5	$C_{19}H_{32}O_2$	49.17		Lineolic acids and derivatives
7	Phytol	32.101	(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	296.5	$C_{20}H_{40}O$	33.26		Diterpenoids
8	2,7-Octadieniol Acetate	32.813	2,7-Octadien-1-ol, acetate, (E)-	168.23	$C_{10}H_{16}O_2$	1.28		Fatty alcohol esters
9	Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-	37.998	1-methyl-4-[(3-methyl-2-butenyl)sulfonyl]benzene	224.32	$C_{12}H_{16}O_2S$	0.44		Toluenes
10	Trans-2-[(1e,5e)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid	43.253	ethyl trans-2-((1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl)cyclopropanecarboxylate	304.47	$C_{20}H_{32}O_2$	0.36		Sesquiterpenoids

Table 3: ADME Profiling of Phytochemicals Identified by GC-MS from the Methanolic Extract of *Amaranthus polygonoides*

S. No	Compound name	#Rotatable bonds	#H-bond acceptors	#H-bond donors	TPSA	Consensus Log P	Ali Log S	GI absorption	BBB permeant	No. of violation in drug	Bioavailability
1	Citronellyl propionate	8	2	0	26.3	3.68	-4.5	High	Yes	0	0.55
2	1,6,6-Trimethyl-9-Hydroxybicyclo[4.3.0]Nonan-2-One Hydrazone	0	3	1	54.37	0.93	-0.56	High	Yes	1	0.55
3	Heptadecanoic Acid, Methyl Ester	16	2	0	26.3	5.94	-8.21	High	No	2	0.55
4	Tridecanoic Acid	11	2	1	37.3	4.1	-6.11	High	Yes	1	0.85
5	(Z,Z)-Heptadeca-8,11-Dien-1-Yl Bromide	13	1	1	20.23	4.1	-6.11	High	Yes	1	0.85
6	Methyl Linolenate	14	2	0	26.3	4.1	-6.11	High	Yes	1	0.85
7	Phytol	13	1	1	20.23	6.25	-8.47	Low	No	2	0.55
8	2,7-Octadieniol Acetate	7	2	0	26.3	2.58	-2.73	High	Yes	1	0.55
9	Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-	3	2	0	42.52	2.98	-3.58	High	Yes	0	0.55
10	Trans-2-[(1e,5e)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid	10	2	0	26.3	2.98	-3.58	High	Yes	0	0.55

Table 4: Toxicity prediction of selected compounds using Protox-3.0

Target	Citronellyl propionate		Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]-		Trans-2-[(1e,5e)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid	
	Prediction	Probability	Prediction	Probability	Prediction	Probability
Hepatotoxicity	Inactive	0.73	Inactive	0.69	Inactive	0.69
Neurotoxicity	Inactive	0.81	Inactive	0.71	Inactive	0.77
Nephrotoxicity	Inactive	0.59	Inactive	0.67	Inactive	0.57
Respiratory toxicity	Inactive	0.99	Inactive	0.64	Inactive	0.85
Cardiotoxicity	Inactive	0.71	Inactive	0.72	Inactive	0.78
Carcinogenicity	Inactive	0.74	Inactive	0.69	Inactive	0.67
Immunotoxicity	Inactive	0.96	Inactive	0.99	Inactive	0.99
Mutagenicity	Inactive	0.99	Inactive	0.70	Inactive	0.88
Cytotoxicity	Inactive	0.78	Inactive	0.82	Inactive	0.77
BBB-barrier	Active	0.89	Active	0.93	Active	0.88
Clinical toxicity	Inactive	0.78	Inactive	0.61	Inactive	0.79
Nutritional toxicity	Inactive	0.89	Inactive	0.76	Inactive	0.87

Table 5: Molecular docking of identified compounds with Bovine GnRH receptor

Protein name: Bovine GnRH receptor				
Ligand	**Binding energy**	**Favorable interaction**	**Un favorable interaction**	**Key observation**
Citronellyl Propionate	-5.968	Hydrophobic interactions with ILE A:38 and ILE A:84; Van der Waals contacts with multiple residues	Steric clashes with PHE A:22, LEU A:83, ASP A:115, ASN A:116	Partial compatibility; steric hindrance reduces binding affinity
Benzene, 1-methyl- 4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl) – 2-pentenyl]oxy]-2-butenyl]sulfonyl]-	-6.836	Hydrogen bond and π–cation interaction with LYS A:121; Hydrophobic interactions with LEU A:97, TRP A:107, LEU A:122, PHE A:178; Van der Waals contacts with several residues	None observed	Strong and specific binding; no steric clashes; promising lead compound
Trans-2-[(1E,5E)-2,6, 10-trimethyl-1,5, 9undecatrienyl] cyclopropanecarboxylic acid	-7.146	Hydrophobic interactions with PHE A:309, PHE A:313, TRP A:280; Van der Waals contacts with various residues	Steric clashes with ALA A:127, ALA A:129, ALA A:163, TYR A:126, MET A:125, ALA A:312, PHE A:130	Moderate hydrophobic fit; multiple steric clashes hinder optimal binding

Table 6: Molecular docking of identified compounds with Bovine FSH receptor

Protein name: Bovine FSH receptor				
Ligand	**Binding energy**	**Favorable interaction**	**Un favorable interaction**	**Key observation**
Citronellyl Propionate	-5.945	**Hydrogen bonds**: ASN A:538, TYR A:615, SER A:453 - **Van der Waals**: VAL A:450, LEU A:611, GLU A:454 - **Hydrophobic (Alkyl)**: ALA A:592, LEU A:534, LEU A:535, LEU A:518, VAL A:531, ILE A:588, PRO A:519, MET A:520	None observed	Balanced polar and non-polar interactions. Strong hydrogen bonds support specificity; hydrophobic tail fits well into hydrophobic pocket. Promising for dual binding interactions.
Benzene, 1-methyl- 4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl) – 2-pentenyl]oxy]-2-butenyl]sulfonyl]-	-7.22	**Hydrogen bond**: SER A:607 - **Hydrophobic (Alkyl)**: ILE A:516, LYS A:608, LEU A:611, VAL A:604, ALA A:595, PRO A:600 - **π-Alkyl**: PHE A:353, ALA A:595, VAL A:604	None observed	Predominantly hydrophobic binding. Aromatic and aliphatic moieties enable deep pocket insertion. Limited hydrogen bonding, but π-donor interaction with SER A:607 enhances specificity. Likely strong binder due to non-polar surface engagement.
Trans-2-[(1E,5E)-2,6, 10-trimethyl-1,5, 9undecatrienyl] cyclopropanecarboxylic acid	-7.44	**Hydrogen bonds**: ASN A:538, TYR A:615, SER A:453 - **Hydrophobic (Alkyl/π-Alkyl)**: LEU A:518, ALA A:592, ALA A:595, ILE A:588, VAL A:604, VAL A:514, PHE A:353, MET A:520 - **Van der Waals**: GLU A:454, VAL A:450, LEU A:534, PRO A:519, LEU A:611, PHE A:591, LEU A:535, SER A:607	None observed	Dual strong polar (carboxylic acid) and extensive hydrophobic binding. Cyclopropane ring adds rigidity. High binding affinity and specificity potential. Ideal scaffold for further design.

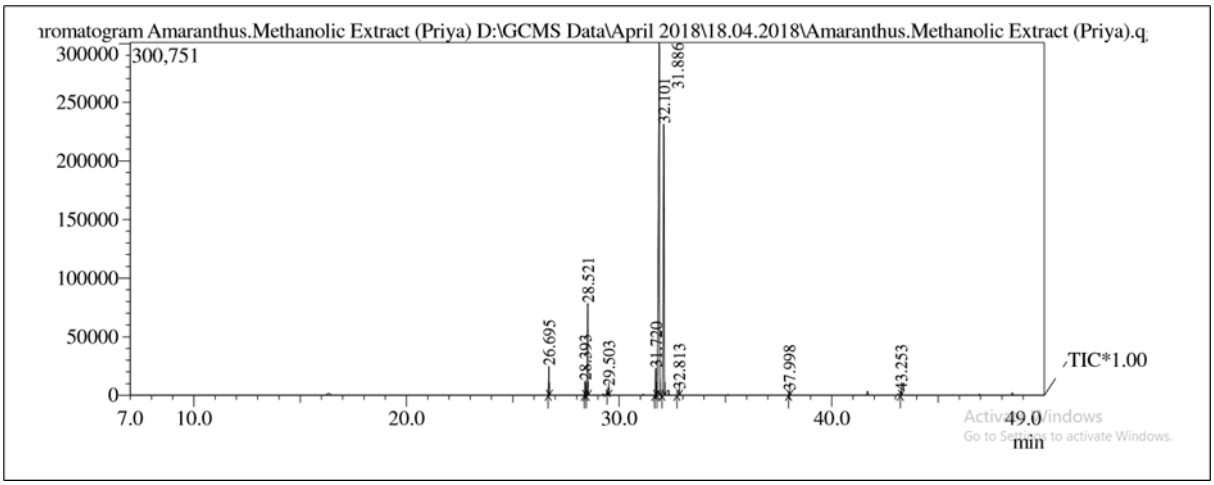


Figure 1: Chromatogram of *A. polygonoides* methanolic leaf extract

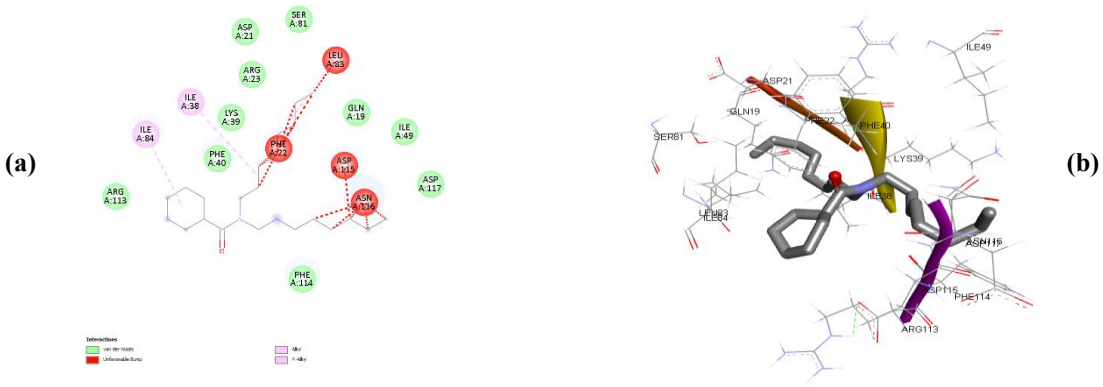


Figure 2: Interaction **(a)** and binding pattern **(b)** of Citronellyl propionate with GnRH receptor

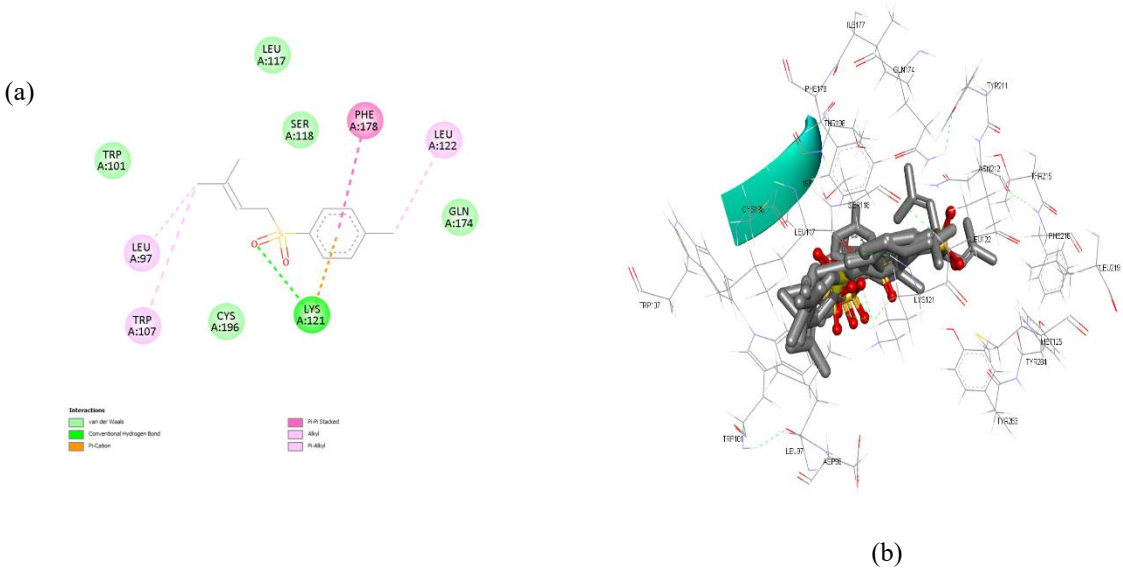


Figure 3: Interaction **(a)** and binding pattern **(b)** of Benzene,1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2 butenyl]sulfonyl]- with GnRH receptor

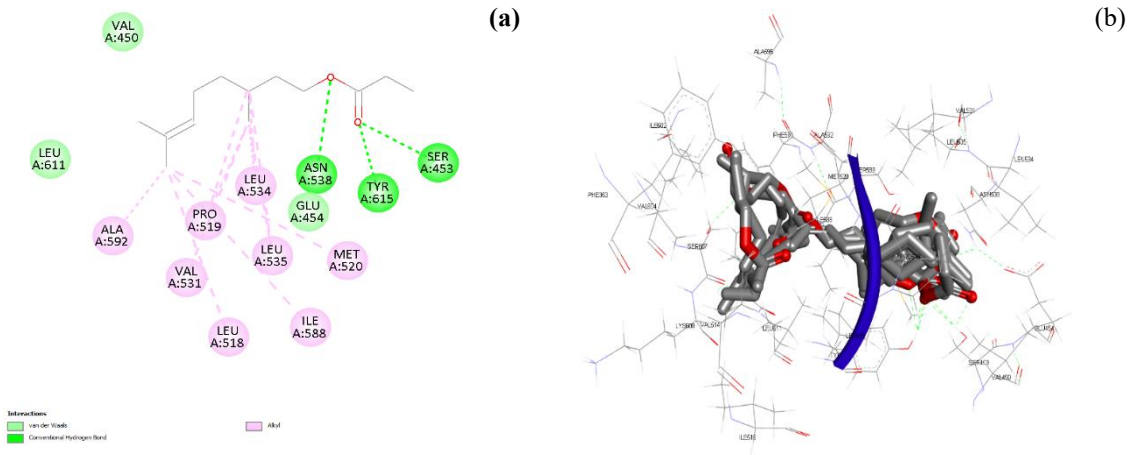


Figure 4: Interaction **(a)** and binding pattern **(b)** of Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl] cyclo propane carboxylic acid with GnH receptor

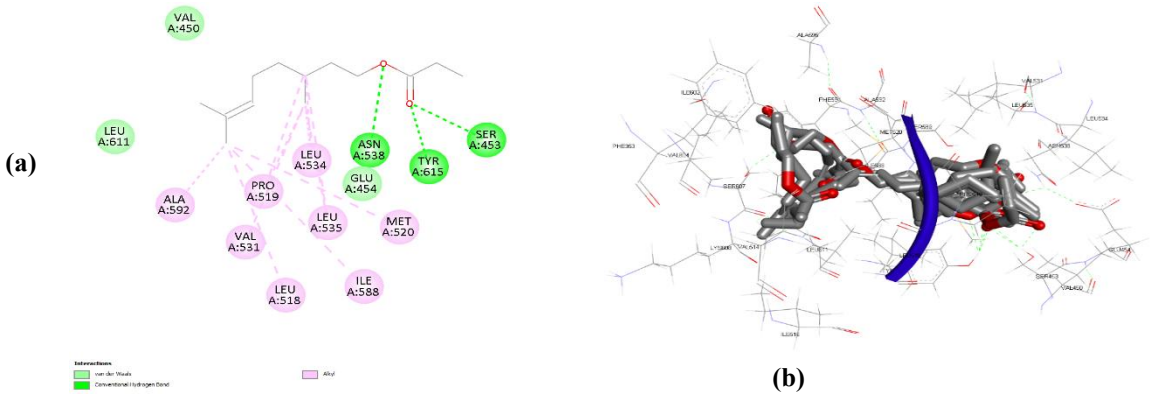


Figure 5: Interaction **(a)** and binding pattern **(b)** of Citronellyl propionate with FSH receptor

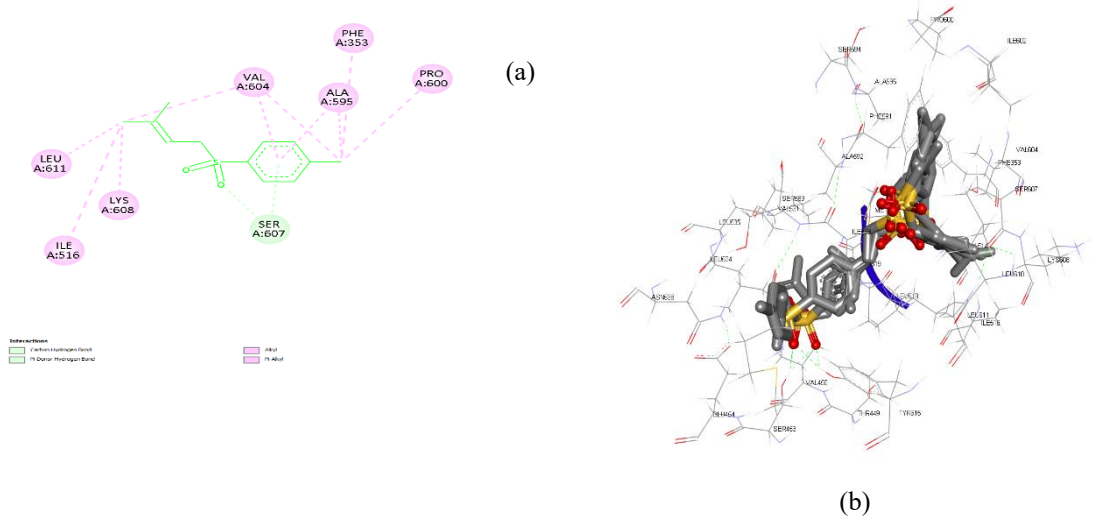


Figure 6: Interaction **(a)** and binding pattern **(b)** of Benzene,1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2 butenyl]sulfonyl]- with FSH receptor

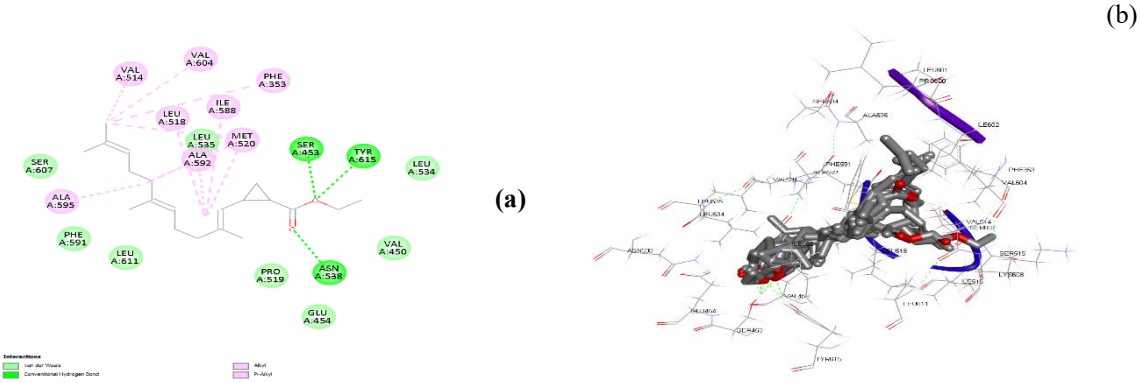


Figure 7: Interaction **(a)** and binding pattern **(b)** of Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl] cyclo propane carboxylic acid with FSH receptor

DISCUSSION

Plants extraction yield percentage

The ethnobotanical data of the plant and its extract percentage yield are presented in Table 1. The extraction yield percentage is consistent with the findings reported by Karamać M *et al*. [10].

Proximate analysis *of A. polygonoides* leaf powder

The high crude protein content of 24.47% positions it as an excellent protein source, crucial for growth, reproduction, tissue repair, and metabolic processes. This aligns with earlier studies on other *Amaranthus* species, which have also been reported to possess high protein levels ranging from 20–27%, supporting their use in animal and human diets [11,12]. The gross energy value of 3678 kcal/kg indicates the leaf powder's capacity to contribute significantly to dietary energy requirements, a trait consistent with reports on other *Amaranthus* species used in feed formulations. The ash content of 13.01% reflects a robust mineral composition, which is a characteristic feature of *Amaranthus* leaves as noted by Alemayehu FR *et al* [12], who emphasized the mineral richness of these leafy vegetables, particularly in calcium, magnesium, and iron.

Moreover, the moderate levels of crude fibre (8.34%) are beneficial for gastrointestinal health, aiding in digestion in livestock. Fibre

content in this range has been shown to enhance gut motility and microbial activity in ruminants [13]. Particularly noteworthy is the calcium (1.99%) and phosphorus (0.51%) content, yielding a calcium to phosphorus (Ca:P) ratio of approximately 3.9:1. This ratio, although higher than the ideal 2:1 for most livestock, may still be advantageous when balanced with other dietary components. Adequate calcium is critical for skeletal development, muscle function, reproductive function and enzymatic activity [14], while phosphorus plays essential roles in energy metabolism and nucleic acid synthesis. The nutrient composition of *A. polygonoides* underscores its potential as a sustainable and cost-effective feed ingredient for improving animal nutrition and productivity.

Phytochemical Screening of Methanolic Extract of Leaf Powder

Preliminary phytochemical screening of the methanolic extract of *A. polygonoides* leaf powder findings are consistent with earlier reports by Shani *et al.,* [15], who documented similar phytochemical profiles in *A. polygonoides*, highlighting its rich secondary metabolite content.

However, qualitative discrepancies were noted when compared with other published studies. Such variations in phytochemical profiles are not uncommon and may be attributed to ecological and environmental factors, including soil composition, geographic location, climate, and harvesting time. These factors significantly influence the biosynthesis and accumulation of phytochemicals in medicinal plants [16]. Moreover, solvent polarity, extraction method, and plant part used can also contribute to differences in phytochemical outcomes, as observed in other phytochemical investigations [17,18].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of methanolic extract *of A. polygonoides* leaf powder

The GC-MS analysis of the *A. polygonoides* leaf extract revealed the high content of unsaturated fatty acids in the extract is of particular interest due to their established role in reproductive physiology. Zeng X *et al* demonstrated that dietary supplementation with unsaturated fatty acids enhances follicular development, oocyte maturation, and embryo viability in cattle [19]. Similarly, Garrel G *et al* reported that unsaturated fatty acids can modulate reproductive function in rats by influencing both basal and GnRH-dependent gonadotrope activity [20]. Zachut M *et al* reported that supplementation of dairy cows with omega-3 fatty acids, including methyl linolenate improved follicle number and increased pregnancy rates [21]. Ranjith D *et al* found that plant rich in linoleneic acid enhanced dominant follicle development and luteal function in dairy cows [22].

Phytol is a diterpene alcohol and a breakdown product of chlorophyll metabolism. It can be metabolized into phytanic acid and retinoids**,** both of which have bioactivity relevant to reproduction. Phytol is a precursor to vitamin A derivatives, which are critical for oocyte maturation, granulosa cell differentiation, regulation of ovulation and luteinization via retinoic acid signalling. Phytol and its metabolites could modulate nuclear receptors like PPARs and RXR**,** which influence steroidogenesis and ovarian function [23]**.**

Although Citronellyl propionate itself lacks specific reproductive studies in cattle, Citronellol has anti-inflammatory and antioxidant properties, which can influence reproductive health by reducing oxidative stress, a known disruptor of ovarian function [24]. Essential oils containing citronellol have shown modulatory effects on sex hormone levels and uterine health in rodents, though not directly in cattle.

The compound Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl] cyclopropane carboxylic acid is a terpenoid-related unsaturated fatty acid derivative**.** Compounds with isoprenoid structures can modulate endocrine functions and are involved in GnRH receptor binding or signal transduction [11].

The compound Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl] appears to be a highly substituted aromatic sulfonyl ether**,** possibly a synthetic or semi-synthetic compound, or a complex natural product derivative. Compounds with lipid tails and aromatic moieties can interact with membrane-bound receptors (e.g., FSH, LH, GnRH receptors**)** or disrupt endocrine pathways.

SwissADME Prediction of Pharmacokinetic and Drug-Likeness Properties

SwissADME predictions have been widely validated and are consistent with established pharmacokinetic behaviors, particularly when applied to phytochemicals identified through GC-MS and *in silico* docking studies. Multiple studies support its reliability as an early-stage screening tool for drug discovery [25].

According to Chen YF *et al*, optimal GI absorption is typically associated with WLOGP values (a measure of lipophilicity) between −0.7 and +5.0 and TPSA (topological polar surface area) values between 20 and 130 Å² [26]. In the current study, most of the GC-MS–identified compounds, fell within these thresholds, supporting their potential for effective GI absorption and passive BBB penetration. Crossing the BBB is particularly important for compounds, especially Methyl linolenate targeting central receptors such as GnRH, which plays a key role in the regulation of reproductive hormones. The identified compounds that exhibited high GI absorption, favourable BBB permeability, and compliance with drug-likeness criteria are promising candidates for further molecular docking and dynamics studies targeting GnRH and FSH receptors to evaluate their potential as modulators of bovine reproductive function.

Toxicity Prediction of Selected Phytoactive Compounds

ProTox-II (v3.0) webserver is *in silico* tool predicts multiple toxicity endpoints, including LD_{50} values, organ toxicity, carcinogenicity, immunotoxicity, and blood-brain barrier (BBB) permeability, based on chemical structure similarity, molecular fingerprints, and machine learning algorithms [27].

The predicted toxicity profile for Citronellyl propionate aligns with previous literature reporting low toxicity and safe use of citronellyl derivatives in cosmetic and food products. Meanwhile, benzene sulfonyl derivative observations are consistent with existing literature where many alkylbenzene derivatives demonstrate moderate to low acute toxicity but may have bioaccumulation potential and persistence in aquatic environments [28].

Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid exhibited slightly higher predicted toxicity with an LD_{50} of 2000 mg/kg**,** classifying it under GHS Class IV**,** indicating mild to moderate acute toxicity. However, like the other compounds, it showed no predicted organ toxicity **a**nd only moderate concerns related to BBB permeability and ecotoxicity.

Importantly, the lack of predicted organ-specific toxicity in all three compounds is a favourable outcome, especially for compounds under consideration for therapeutic use. The consistent prediction of BBB permeability, while not necessarily indicative of neurotoxicity, suggests potential central nervous system (CNS) activity or bioavailability, an important consideration for compounds targeting the GnRH axis and other neuroendocrine functions.

From an environmental perspective, the ecotoxicity alerts for the benzene derivative and the cyclopropane-containing compound underscore the importance of evaluating environmental fate and biodegradability**.** Benzene-based structures, in particular, are known to be persistent organic pollutants with long-term ecological effects [29].

In Silico Molecular Interaction Study

Molecular docking is a critical tool in modern drug discovery and phytochemical screening, enabling the prediction and visualization of interactions between small molecules and their biological targets. This approach provides detailed insights into the binding affinity, structural compatibility, and potential biological activity of candidate compounds. In the present study, PrankWeb**,** a machine learning-based ligand-binding site prediction tool [30], was employed to predict active binding pockets on the bovine gonadotropin-releasing hormone receptor (GnRHR) and the follicle-stimulating hormone receptor (FSHR)**.** These receptors are key regulators in the hypothalamic-pituitary-gonadal axis and are critical targets for modulating reproductive function.

Receptor Selectivity and Binding Insights:

All three ligands demonstrated stronger binding affinitie**s** (more negative binding energies) with the FSH receptor than the GnRH receptor. This suggests that FSHR possesses a more accommodating binding site, possibly due to better shape complementarity and interaction potential. Citronellyl propionate showed moderate and similar affinities toward both receptors, suggesting limited receptor selectivity. Steric hindrance noted in the GnRH receptor was absent in FSHR, leading to more stable interactions. The benzene sulfonyl derivative emerged as a particularly promising ligand for both receptors, especially FSHR. It formed specific hydrogen bonds and π-cation interactions with LYS A:121 in GnRHR and hydrophobic/π-alkyl contacts in FSHR. The presence of π-alkyl interactions significantly stabilizes ligand-receptor complexes by enhancing van der Waals forces and electron cloud overlap, which are well-documented mechanisms in drug-receptor dynamics [9]. Trans-2-[(1E,5E) cyclopropanecarboxylic acid exhibited the strongest binding affinity to FSHR**,** making it a compelling candidate for targeting ovarian function. However, notable steric clashes in the GnRH receptor pocket may reduce its effectiveness in that context. The carboxylic acid functional group likely contributes to strong polar and electrostatic interactions, which are important for ligand stability and specificity in hormone receptor targeting [31].

CONCLUSION

Amaranthus polygonoides, a member of the Amaranthaceae family, has been recognized for its nutritional and medicinal properties. Proximate analysis of its leaf powder indicates significant levels of carbohydrates, proteins, fats, crude fibre, moisture, and ash content, underscoring its potential as a valuable dietary component. Preliminary phytochemical screenings have revealed the presence of various bioactive compounds, including alkaloids, flavonoids, steroids, terpenoids, phenols, saponins, tannins, and reducing sugars, which are known for their health-promoting properties. Further investigation using GC-MS has identified multiple compounds within the methanolic leaf extract of *A. polygonoides*.. *In silico* analyses utilizing SwissADME and ProTox-II tools suggest that this compound Citronellyl propionate, Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]- and Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid possesses high gastrointestinal absorption, the ability to cross the blood-brain barrier, favourable drug-likeness properties, and low predicted toxicity. Molecular docking studies have demonstrated that the predicted binding data offer valuable insights into the potential of these ligands to interact with bovine GnRH and FSH receptors. Benzene, 1-methyl-4-[[3-methyl-2-[[4-methyl-3-(1-methylethyl)-2-pentenyl]oxy]-2-butenyl]sulfonyl]- emerges as a particularly promising compound due to its strong, specific, and clash-free interactions with both receptors, coupled with its low predicted acute toxicity. Trans-2-[(1E,5E)-2,6,10-trimethyl-1,5,9-undecatrienyl]cyclopropanecarboxylic acid also shows excellent affinity for the FSH receptor, making it a strong candidate for FSHR-targeted applications, though its GnRHR interactions are complicated by steric hindrance. This indicates its potential role in modulating reproductive functions in bovine. While these findings are promising, it is essential to conduct comprehensive *in vitro* and *in vivo* studies to validate the therapeutic potential of these compounds. Such research will provide deeper insights into their mechanisms of action, efficacy, and safety profiles, paving the way for potential applications in veterinary medicine and animal husbandry.

Conflict of interest

The authors declared no conflict of interest.

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