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Original article

Bioactive Metabolites of *Asparagus racemosus* with Dual Effects on Insulin Modulation and Estrogen Regulation Pathways for Hormonal Balance

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ABSTRACT:

Background and purpose: *Asparagus racemosus* or Shatavari is a well-known Ayurvedic herb recognized for its benefits in hormone balancing, lactation enhancement, menstrual pain relief, and fertility support. The present study aimed to investigate the bioactive potential of Shatavari roots to understand the molecular mechanisms underlying its reproductive and metabolic benefits.

Experimental approach: Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed to identify the chemical constituents of Shatavari root extract. Based on the GC–MS profile, in silico molecular docking to explore its interactions with key protein targets, including Cytochrome P450 1A2 (CYP1A2) and the insulin receptor tyrosine kinase domain (INSR). Docking simulations were carried out using AutoDock, and binding affinities, inhibition constants, and interaction patterns were analysed to elucidate potential mechanisms of action.

Results: GC-MS analysis in this study highlighted that some of the major bioactive compounds such as tridecanoic acid derivatives, tetradecanoic acid, and n-hexadecanoic acid, which are predicted to inhibit CYP1A2 and which could be helpful in regulating estrogen. Additionally, compounds demonstrated antioxidant, anti-inflammatory, and insulin-sensitizing properties. Docking studies showed that tetradecanoic acid binds stably to CYP1A2, suggesting competitive or mixed-type inhibition, and interacts weakly with INSR, indicating a modulatory role in insulin signaling.

Conclusion: This study highlights the potential for the formulation of novel plant-derived therapeutics targeting reproductive and metabolic dysfunctions, while emphasizing the need for further clinical validation to establish their efficacy, safety, and mechanisms of action in human subjects.

Keywords: *Asparagus racemosus*, Tetradecanoic acid; Metabolite analysis

INTRODUCTION:

Asparagus racemosus, also locally known as shatavari, is a traditional Ayurvedic plant valued for its many therapeutic uses, particularly in improving the health of female reproductive systems. All parts of the plant exhibit diverse pharmacological effects and is revered as the "queen of herbs"[1]. In addition to its role in reproductive physiology, research demonstrates that Shatavari possesses antioxidant and anti-stress properties by modulating the hypothalamic-pituitary-adrenal axis and monoaminergic systems[2]. The metabolic advantages, such as α-amylase/α-glucosidase inhibition and enhanced glycaemic indicators in diabetic animal models, correspond with conventional antidiabetic assertions, while data in humans is scarce[3].

The pharmacological properties of the root are associated with specific phytochemicals, particularly steroidal saponins (shatavarins I–V), fructo-oligosaccharides, mucilage, racemosol glycosides, and isoflavone-like compounds, which together facilitate its estrogen-mimetic, galactagogue, and adaptogenic effects[4]. Experimental and clinical evidence indicates that shatavarins have uterotrophic and prolactin-modulating effects, which enhance mammary lobulo-alveolar development and postpartum lactation with Shatavari supplementation[5]. Preclinical studies elucidate phytoestrogenic mechanisms via modulation of oestrogen receptor expression[2]. The root is one of the most important parts, yet it has not been studied as extensively as others. The accumulating evidence from phytochemical, preclinical, and emerging clinical studies substantiates numerous traditional applications of Shatavari, while highlighting the necessity for comprehensive human research addressing root dose standardization, long-term safety, and interactions with standard hormonal therapies.

MATERIALS & METHODS

Study Design

The present study was designed to investigate the bioactive potential of *A. racemosus* roots by integrating experimental phytochemical profiling with computational molecular docking. First, GC–MS analysis was performed to identify the constituent compounds. Based on the GC–MS profile, a prominent metabolite was selected for in silico docking.

Plant Material Extraction and GC–MS Analysis

A. racemosus root powder was dried and subjected to Soxhlet extraction with methanol as the solvent to achieve efficient recovery of polar and semi-polar phytoconstituents and concentrated under rotary evaporator. GC–MS analysis (Agilent system, DB-5MS column) and compound identification were carried out under standard conditions, including helium as the carrier gas, splitless injection, programmed oven temperature and hold time, and electron ionisation, with spectra matched against NIST and Wiley libraries, following previously reported methods[6,7].

In Silico Molecular Docking Studies

Tetradecanoic acid was selected based on its prominence in the GC–MS profile to investigate its interaction with metabolic and signaling targets. The two-dimensional (2D) chemical structure of tetradecanoic acid was retrieved from the PubChem database (CID: 2756016). The ligand was converted into a three-dimensional (3D) conformation and subjected to geometry optimization using Avogadro software (version 1.2.0). Energy minimization was performed employing the MMFF94 force field to eliminate steric clashes and achieve a stable, low-energy conformation suitable for docking analysis[8]. The three-dimensional crystal structures of the target proteins were retrieved from the Protein Data Bank (PDB). The selected protein targets included Cytochrome P450 1A2 (CYP1A2) in complex with α-naphthoflavone (PDB ID: 2HI4) and the insulin receptor tyrosine kinase domain (INSR) in complex with a small-molecule inhibitor (PDB ID: 4IBM). For CYP1A2, the heme prosthetic group was retained due to its essential role in catalytic activity. Polar hydrogen atoms were added, and Kollman united-atom charges were assigned to all protein atoms to enhance docking accuracy[9]. All co-crystallized ligands, non-essential water molecules, and heteroatoms were removed prior to docking. The optimized ligand structure was saved in PDB format and subsequently both

target and ligand converted to PDBQT format using AutoDock Tools (ADT) 4.2.6[10], during which Gasteiger partial charges were assigned and all rotatable bonds were defined to allow full ligand flexibility during docking simulations[11].

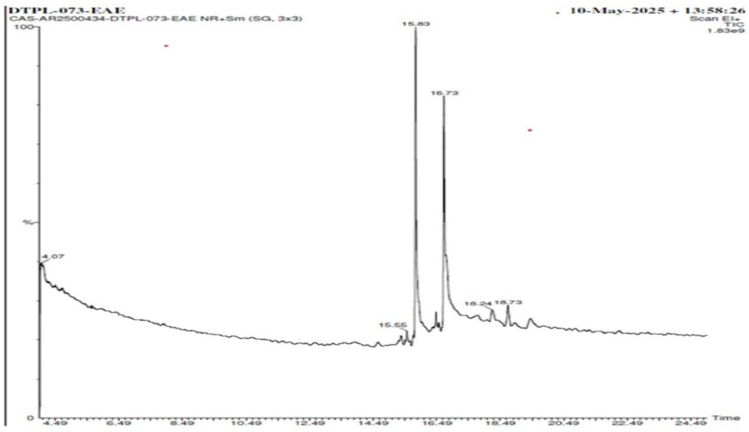
Docking grid boxes were generated using AutoGrid 4.2 to define the active binding regions of the target proteins. For CYP1A2, the grid box was centered on the heme catalytic site and for INSR, the grid box was positioned around the ATP-binding pocket of the kinase domain. For both targets, grid box dimensions were set to 40 × 40 × 40 points along the x, y, and z axes with a grid spacing of 0.375 Å[12]. The grid center coordinates were defined as (2.674, 18.041, 19.672) for CYP1A2 and (3.925, −9.753, 6.836) for INSR. Grid parameters were kept identical across docking runs to ensure reproducibility.

Molecular docking was performed using AutoDock 4.2.6, employing the Lamarckian Genetic Algorithm (LGA)[10]. A total of 100 independent docking runs were carried out for each protein–ligand system to ensure adequate conformational sampling. Docking parameters were maintained at default settings, including a population size of 150, a maximum number of 2.5 × 10^6 energy evaluations, and a mutation rate of 0.02. Docked conformations were clustered based on a root-mean-square deviation (RMSD) tolerance of 2.0 Å.

RESULTS:

The GC–MS chromatogram, as shown in Figure 1, indicated the presence of various bioactive compounds, such as fatty acids, esters, and heterocyclic molecules, many of which are documented to exhibit antioxidant, anti-inflammatory, endocrine-modulating, and metabolic regulatory properties. The identification of these compounds corroborates the phytochemical diversity of *A. racemosus* roots and offers analytical validation for its traditional medicinal uses. The diversity of identified metabolites indicates a synergistic interaction of Shatavari in reproductive, metabolic, and hormonal health contexts.

Figure 1. Soxhlet extraction with methanol *A. racemosus* roots & identification of active compounds by using GC-MS



Tridecanoic acid, methyl ester Fatty acid ester, n-Hexadecanoic acid Saturated fatty acid, Tetradecanoic acid Saturated fatty acid, 1-Ethyl-3-[2- (octadecylthio)ethyl]thiourea Organosulfur compound have CYP1A2 Inhibitor roles which helps in Estrogen metabolism. n-Hexadecanoic acid Saturated fatty acid, Tetradecanoic acid Saturated fatty acid, 9,12-Octadecadienoic acid (Z,Z) Polyunsaturated fatty acid and 3,7-Diacetamido-7H-striazolo[5,1-c]-s-tr are anti-inflammatory and antioxidants roles. Octanoic acid, 2-methyl Branched-chain fatty acid is known to regulate lipid metabolism and insulin signalling thus help in PCOD treatment. The total medical roles are tabulated in Table 1.

Table 1. Shows the various molecules present in the root extract of *A. racemosus* along with their molecular formula, molecular weight and their possible medicinal roles.

Sl. No	Name of the Molecule	Mol. Formula	Mol. Wt.	Possible medicinal Role
1	Tridecanoic acid, methyl ester	C14H28O2	228.37	CYP1A2 Inhibitor, Catechol-O-Methyl Transferase Inhibitor, Antimicrobial Activity, Arachidonic Acid Inhibitor, Increased Aromatic Amino Acid Decarboxylase Activity, and Uric Acid Production Inhibition
2	Octanoic acid, 2-methyl	C9H18O2	158.24	Catechol-O-Methyl Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, Acidifier, Urine-Acidifier, Anti-inflammatory and Antimicrobial properties, improves insulin metabolism
3	n-Hexa decanoic acid	C21H46O2 Si2	386.76	CYP1A2 Inhibitor, Antioxidant, Antimicrobial, and Anti-inflammatory activities
4	Tetradecanoic acid	C14H28O2	228.37	CYP1A2 Inhibitor, Acidulant, Arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity, inhibit uric acid production, Hemolytic and Anti fibrinolytic effects, Anticancer potential, Antioxidant activity, Anti-inflammatory properties
5	Tridecanoic acid	C13H26O2	214.34	CYP1A2 Inhibitor, Catechol-O-Methyl Transferase Inhibitor, Arachidonic Acid Inhibitor, Increased Aromatic Amino Acid Decarboxylase Activity, Uric Acid Production Inhibition, Antifungal and Antibacterial Activities
6	9,12-Octadecadienoic acid (Z,Z)	C18H32O2	280.45	Antibacterial properties, Anti-inflammatory, Antioxidant, and Anticancer effects.
7	2-Methyl-Z,Z-3,13-octa decadienol l	C19H36O	280.49	Antimicrobial Activity, Insecticidal Potential
8	1-Ethyl-3-[2-(octadecylthio)ethy l]thiourea	C23H48N2 S2	416.77	CYP1A2 Inhibitor, Antimicrobial Activity, Potential Anticancer Properties
9	Imidazole, 2-amino-5-[(2-carboxy)vinyl]	C6H7N3O 2	153.14	Antibacterial Activity, Antifungal Properties
10	Pterin-6-carboxylic acid	C7H5N5O 3	207.15	Acidifier, Acidulant, Arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity, Inhibits production of uric acid, Cofactor function, Anticancer, Neuroprotective,

				Antioxidant, Antiprotozoal, Antiviral Properties.
11	Dibutyl phthalate	C7H9N7O 2	223.19	Allelopathic activity, antimicrobial activity, Insecticidal activity
12	Diisooctyl phthalate	C24H38O4	390.56	Allelopathic activity
13	3,7-Di acetamido-7H-striazolo[5,1-c]-s-tr	C7H9N7O 2	223.19	Antifungal, Antibacterial, Anticancer, Anti-inflammatory, Anticonvulsant, Antiparasitic, Antiviral, Enzyme inhibitors

Docked complexes were ranked based on their binding free energy (ΔGbind) and predicted inhibition constant (Ki). Protein–ligand interactions were visualized and analyzed using BIOVIA Discovery Studio Visualizer. Docking analysis demonstrated that tetradecanoic acid binds stably within the substrate-binding cavity of CYP1A2, exhibiting a favourable binding free energy (ΔGbind = −6.35 kcal/mol; Ki = 22.18 μM). Notably, the ligand was positioned in close proximity to the heme prosthetic group (HEM900), forming a metal–acceptor interaction, a characteristic feature of potential CYP inhibitors. The catalytic efficiency of CYP1A2 critically depends on appropriate substrate positioning near the heme iron center, which facilitates electron transfer and oxygen activation[13]. Also, tetradecanoic acid binds within the kinase domain of INSR with a moderate binding affinity (ΔGbind = −4.10 kcal/mol; Ki = 986.66 μM). The ligand formed hydrogen bonds with Asp1083 and Leu1002, contributing to stabilization within the ATP-binding region. The three-dimensional docked complexes of tetradecanoic acid with CYP1A2 and INSR are shown in Figures 2 and 3, respectively. In figure 2b and 3b, target-ligand illustrating key non-covalent interactions. Interaction types are color-coded as follows: van der Waals (vdW) interactions in green, π-alkyl interactions in pink, and π-sigma interactions in purple. These interactions collectively contribute to the stabilization of tetradecanoic acid within the active site of CYP1A2 and potential modulation of INSR kinase activity.

Figure 2. (A) Hydrogen bond interactions between tetradecanoic acid and Cytochrome P450 1A2 (CYP1A2). (B) Three-dimensional representation of the tetradecanoic acid–CYP1A2 docked complex.

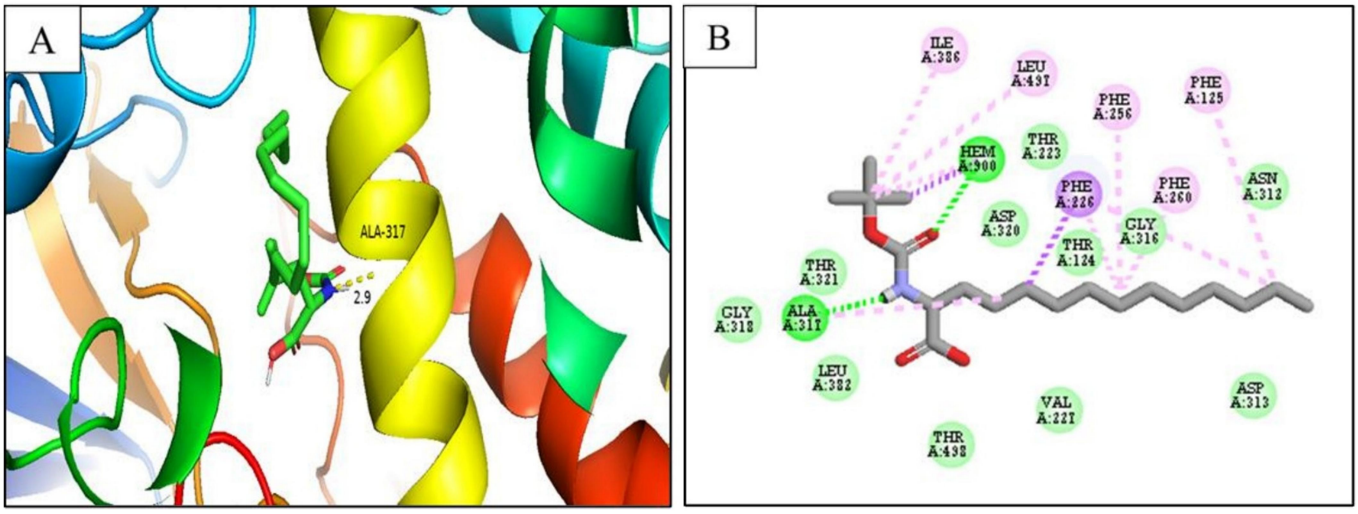
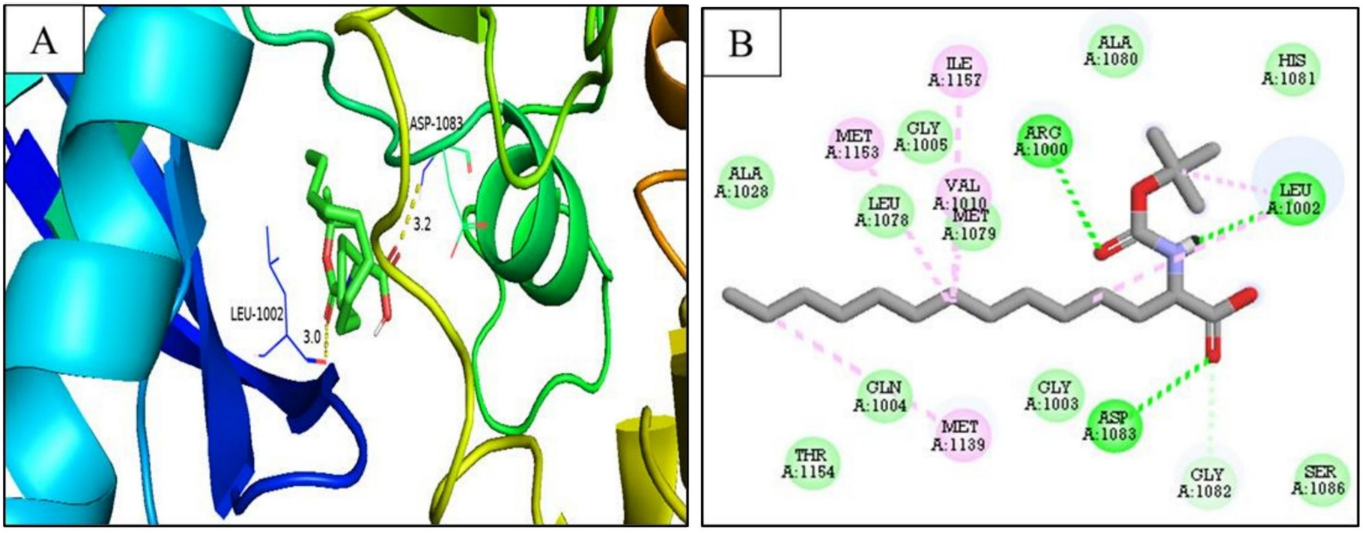


Figure 3. (A) Hydrogen bond interactions between tetradecanoic acid and the insulin receptor tyrosine kinase domain (INSR). (B) Three-dimensional docked complex of tetradecanoic acid with INSR.



DISCUSSION

Cytochrome P450 1A2 (CYP1A2) is essential in oestrogen metabolism, their inhibition can modulate oestrogen clearance, thereby maintaining physiologically optimal oestrogen levels. This regulation may support ovarian function[14], follicular maturation[15], endometrial stability[16], and overall reproductive cyclicity[17]. The carboxylate moiety of tetradecanoic acid anchors near the catalytic region through hydrogen bonding and metal coordination, while the long aliphatic chain extends along the hydrophobic access channel of CYP1A2. This binding orientation suggests a competitive or mixed-type inhibitory mechanism, wherein tetradecanoic acid may hinder substrate access to the heme iron, thereby reducing catalytic turnover. Furthermore, extensive hydrophobic and π–alkyl interactions with aromatic residues lining the active site, including Phe125, Phe226, Phe256, and Phe260, enhance ligand retention within the cavity. Such interactions are commonly observed for fatty acid–like ligands in CYP enzymes[18], supporting the hypothesis that tetradecanoic acid may act as a substrate mimic, interfering with normal metabolic processes. The insulin receptor (INSR) is a receptor tyrosine kinase that regulates glucose homeostasis through ATP-dependent autophosphorylation of tyrosine residues[19]. However, the relatively high Ki value and the predominance of hydrophobic interactions indicate that tetradecanoic acid is unlikely to function as a strong ATP-competitive inhibitor. Instead, the long aliphatic chain occupies a hydrophobic region adjacent to the catalytic site, suggesting a weak or indirect modulatory mechanism rather than potent kinase inhibition[20]. The docking-based mechanistic analysis highlights target-dependent modes of action for tetradecanoic acid. The stronger binding affinity, favorable energetics, and direct interaction with the heme iron indicate that CYP1A2 is the primary molecular target, and supporting a secondary or modulatory role in insulin receptor kinase.

In addition, Octanoic acid present in Shatavari has been shown to regulate the INSR gene and enhance insulin sensitivity via the Akt–mTOR signalling pathway. This mechanism is particularly significant in PCOS, where insulin resistance contributes to hyperandrogenism and anovulation[21]. The metabolic effects collectively underscore Shatavari's potential as a supportive therapy for reproductive–metabolic interface disorders, including PCOS. The findings suggest Shatavari's potential as a multi-modal therapeutic agent, relevant for both fertility enhancement and the management of complex endocrine-metabolic disorders.

CONCLUSION

The study shows *Asparagus racemosus* (Shatavari) as a potent natural reproductive health enhancer, as evidenced by GC-MS identification of bioactive compounds that alter oestrogen homeostasis by inhibiting important metabolic enzymes such as CYP1A2. Docking with the insulin receptor (INSR) revealed weaker but notable hydrophobic interactions, indicating a modulatory effect on kinase activity that could influence insulin signaling and glucose homeostasis. These computational insights validate and extend the GC–MS findings, linking specific phytochemicals to their potential molecular mechanisms of action. Such evidence supports Shatavari's efficacy in the treatment and prevention of hormonal imbalance-related illnesses such as PCOS, menopausal symptoms, and estrogen-regulated reproductive dysfunction. This work establishes a mechanistic framework for further pharmacological studies and underscores the potential of *A. racemosus* in evidence-based complementary therapies.

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