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GC-MS Fatty Acid Profile and In silico Studies of *Telfairia occidentalis* Hook f. (Fluted Pumpkin) and *Sphenostylis stenocarpa* (African Yam bean) Seeds Oil on Human 5 Alpha Reductase II

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Abstract

Increased testosterone levels in older men are linked to a higher risk of benign prostate hyperplasia (BPH). This risk comes from the conversion of testosterone to the stronger hormone dihydrotestosterone (DHT) by the 5-alpha reductase enzyme. Managing BPH focuses on blocking this enzyme. This study examines how fatty acids from *Telfairia occidentalis* (Fluted pumpkin) and *Sphenostylis stenocarpa* (African Yam bean) seed oil affect human 5α-reductase II, using computer modeling. Gas mass spectrophotometry identified the fatty acids in the extracted oils. Computer analyses (molecular docking and MM-GBSA) were performed using the Maestro interface. GC-MS found six compounds with the most notable peaks in African yam bean seeds, and nine in fluted pumpkin seeds. Molecular docking identified 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (from African yam bean seeds, 6.24 kcal/mol) and Cyclopentadecanone, 2-hydroxy- (from fluted pumpkin seeds, -6.40 kcal/mol**)** as the most promising inhibitors of human steroid 5α-reductase II. MM-GBSA validation showed values of -87.06 kcal/mol and -60.49 kcal/mol for these compounds, respectively. 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester formed bonds with GLU57, GLU197, and TYR91 residues in the enzyme, while Cyclopentadecanone, 2-hydroxy- formed a bond with ARG114. In summary, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester may be a promising inhibitor of human 5α-reductase II for managing BPH. Further animal and laboratory studies are needed to confirm these results.

Keywords: Androgen; Urology; Phytotherapy; Benign prostate hyperplasia; Fatty acids; 5α-reductase II

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Introduction

Benign prostatic hyperplasia (BPH) is the most prevalent age-related disease of the prostate gland for men (Yusoff, 2023). Its symptoms include urinary tract obstruction, frequent urination, urinary retention, decreased diameter of the urinary tube and pressure of urine flow, and dribbling at the end of urination (Yaqoob and McCafferty, 2020). The disease is characterized by prostate gland enlargement due to hyperproliferation of cellular components such as mesenchymal cells. In Nigeria, Ezeanyika *et al*. (2006) reported that one-in-four men older than 40 years, who reside in Nsukka, Enugu State Nigeria have symptoms suggestive of BPH. The condition often affects individual's quality of life and may progress into bladder dysfunction and eventually lead to acute urinary retention if not corrected (Zhang *et al*., 2022).

Benign prostatic hyperplasia (BPH), a non-cancerous proliferation of epithelial and stromal cells of the prostate gland, is one of the most common age-related diseases in aging men (Asare *et al*., 2024; Song *et al*., 2021). 5α-Reductase inhibitors and alpha-1-adrenergic antagonists are two main agents commonly used to treat BPH. 5α-Reductase is an essential enzyme that converts testosterone to dihydrotestosterone (DHT) (Abaci and Orhan, 2024). Finasteride is a classical 5α-reductase inhibitor that decreases the DHT level, resulting in a decrease in the prostate volume (PV) and symptoms of patients with BPH (Stroomberg *et al*., 2022). It has been well established that a1-adrenoreceptor blockers relax prostatic smooth muscles thereby increasing urine flow while decreasing the prostate size and Prostatic SpecificAntigen (PSA) (Lanman *et al*., 2025).

Alpha-adrenergic receptor blockers and 5-α reductase inhibitors cause side effects, and the high prices of some of these drugs have led to an increasing tendency to use natural compounds as a source of lead compounds for drug design for the treatment of BPH or as a supplementary drug. 5-α reductase inhibitors are reported to exhibit numerous adverse side effects, for instance erectile dysfunction, ejaculatory dysfunction and loss of libido. This has led to a surge of interest on plant-derived alternatives that might offer favourable side effects and less toxic profiles. Phytochemicals from plants are shown to exhibit numerous

medicinal properties in various studies targeting many major illnesses including benign prostatic hyperplasia (Azizi *et al*., 2021).

Studies have shown that alternative and complementary treatment are remarkable options for the management of mild BPH patients including Serona repen, *Pygeum africanum*, and *Secale cereal* (Sadeghimanesh *et al*., 2021). There are several reasons for significant attitude for this global approach including their availability, low cost as well as showing better safety profile than the current pharmaceutical medications.

Pumpkin (Cucurbita spp.) has received considerable attention because of the nutritional and health-protective value of its seed oil (Kaur and Dhatt, 2024). It contains a wide variety of bioactive compounds, including fatty acids, phytosterols, vitamins, among many others (Stevenson *et al*., 2007; Montesano *et al*., 2018; Morakul *et al*., 2019). Vitamins A, C, D and E are associated with decreased BPH symptoms. Vitamin D has the greatest impact on BPH. Vitamin D deficiency has been indicated in prostatic inflammation (Das and Buchholz, 2019; Cho *et al*., 2021; La Vignera and Basile, 2022)

Pumpkin seeds, in addition contains protein and fatty acids, vitamins (folates, niacin (B3), vitamins A, C, and especially E), minerals (zinc, phosphorus, manganese, potassium, magnesium, copper and iron) plus phytonutrients such as β-carotene, β-cryptoxanthin, lutein, and zeaxanthin) (Devi *et al*., 2018; Devi *et al*., 2019; Ramak and Mahboubi, 2019). In 2021, Kang *et al*. reported using a rat model that pumpkin seed oil ameliorated BPH parameters by lowering prostate 5α-reductase enzyme expression, which confirmed previous results demonstrating this benefit (Kang *et al*., 2021).

African Yam bean (AYB) (*Sphenostylis stenocarpa*) is an herbaceous (tuberous) leguminous plant occurring throughout tropical Africa and grows during the wet season (Onwughalu *et al*., 2025).

African yam bean, *Sphenostylis stenocarpa* (Hochst. ex A. Rich.), is an underutilized annual tuberous legume that serves as a food and nutritional security crop in sub-Saharan Africa and is the most economically important species in the genus *Sphenostylis* (National Research Council, 1975). It produces beans in pods with varying seed patterns and colors (Asoiro and Ani, 2011). The seed is rich in protein (19.5%), carbohydrates (62.6%), fat (2.5%), vitamins and minerals.

It is cultivated both for the seeds and tubers because of its valuable and prominent source as plant protein (Amoatey *et al*., 2000). Nigeria is very significant for AYB production where extensive cultivation had been reported in the eastern, western and northern areas of the country, where it is known as Geri-Geri in Hausa (Northern, Nigeria) and Ahuma in Tiv (North Central, Nigeria).
Based on these previous studies, the present work is aimed at assessing the activities of fatty acids from *Telfairia occidentalis* Hook f. (Fluted pumpkin) and *Sphenostylis stenocarpa* seeds oil on human 5 alpha reductase II using in silico approach.

Materials and Methods

Plant material

Mature fluted pumpkin seeds (3 kg to 5 kg) was purchased from Railway market in Makurdi, Benue State, Nigeria. The fruits were sliced open and the seeds harvested, cleaned and decocted manually using a sharp steel knife. The seeds were washed and allowed to dry at room temperature without exposing to sunlight. The dried seeds were identified and authenticated in the Department of Botany, Joseph Sarwuan Tarka University, Makurdi and a voucher number of (JoSTUM-FPS-134) was assigned. The seeds was dried in an oven at 30°C to a constant weight, and then milled into a fine powder using an electric blender (Akang *et al*., 2010).

The seeds of *Sphenostylis stenocarpa* (African yam bean) was purchased from Saturday Market, Kwande LGA, of Benue State, Nigeria. The African yam bean seeds was sorted to remove stones and dirt by sieving. The seeds was soaked in water for 24 hours and then the water was removed and the seeds air dried for 3 days. The seeds was identified and authenticated in the Department of Botany, Joseph Sarwuan Tarka University, Makurdi and a voucher number of (JoSTUM-AYB-139) assigned. The seeds was toasted for 35 minutes in a frying pan, milled using hammer mill to obtain African yam bean powder (AYBP) and stored in bags for use.

Extraction of Pumpkin Seed Oil (PSO）

Extraction of oil from pumpkin seed was done by the method described by Uba and Muhammad (2019). The Soxhlet extraction method was utilized to extract the crude oil from pumpkin seeds with a few changes. The pulverized pumpkin seeds (50 g) was appropriately packed into the thimble of the Soxhlet extractor, and petroleum ether (300 ml) was put into the Soxhlet extractor's 500 ml round bottom flask. The oil from pumpkin seeds will be extracted using the Soxhlet extraction system with petroleum ether (boiling point 60-80 °C). The extraction process took about 6 hours. The extract was filtered using filter paper before being dried in a rotary evaporator at 40 °C. The recovered oil was stored in a vial at 4 °C until further examination.

Roasting and Extraction of African Yam Bean Seed Oil

Roasting: Samples of African yam bean seeds (2 kg) was placed in a pot and mixed with clean fine sand. The pot was heated with naked fire from firewood. Frequent stirring or turning was carried out at intervals to prevent burning of the seeds coat and to ensure uniform distribution of heat. The heating was done for thirty (30) minutes before the sand was separated from the seed using a sieve and then allowed to cool (Christopher *et al*., 2002). For the oil extraction, the Method of Nwankwo and Onah (2021) was adopted with slight modifications .The extraction of oil from the AYB was further carried out using soxhlet extraction with petroleum ether at 60-80^{0}C as the extracting solvent. In this, 50 g of the ground seeds was put in the extractor and allowed to run for 6 hours. Finally, the oil-solvent mixture collected in the flask was dried in a rotary evaporator at 40^{0}C.

Determination of fatty acid using gas chromatography-mass spectrometry (GC-MS) of Pumpkin Seed and African Yam Bean Seed Oils

A. Preparation of fatty acid methyl esters (FAME).

Conversion of fatty acids into their derivatives, such as fatty acid methyl ester, is required so that they can be measured by gas chromatography-mass spectrometric (GC-MS) analysis. According to Uba and Muhammad (2019), 1 g of extracted oil and 2% methanolic KOH (6 ml) will be added to a 50 ml round-bottom flask. The condenser was connected to the round-bottom flask, and the mixture was heated in a water bath for 1 hour at 60-70°C with constant stirring. The reaction mixture was allowed to cool

to room temperature. After adding 40 ml of n-hexane to the solution, it was transferred to a separating funnel. The organic layer (upper layer) was separated and filtered through Whatman filter paper (110 mm) after being dried over anhydrous sodium sulfate.

B. Gas chromatography-mass spectrometry.

The fatty acid compositions was determined using an Agilent Technology 5977E MSD with an auto-sampler and an Agilent 7820A GC system (USA). The chromatographic separation was performed on a DB-1701 micro-column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) at an 8 psi pressure and a 1 ml/min flow rate (Hagos, 2023). The fatty acids were identified by comparing the retention times of a standard mixture to the retention times of the fatty acids, and by comparing with NIST spectral library.

In silico ADMET Analysis

To evaluate the druggability and therapeutic potential of the identified compounds, an integrated computational workflow involving molecular modelling, molecular docking, and pharmacokinetic prediction was applied using several software and web-based tools. PubChem (NCBI) provided compound IDs, canonical SMILES, and 3D structures. SwissADME facilitated drug-likeness screening, including Lipinski's Rule of Five, bioavailability, and pharmacokinetic profiling. ADMET profiles were further evaluated using ProTox-II servers. All compounds were screened for drug-likeness using SwissADME, and only those meeting Lipinski's Rule of Five (MW ≤ 500 Da, LogP ≤ 5, hydrogen bond donors ≤ 5, hydrogen bond acceptors ≤ 10) and Veber's criteria (TPSA ≤ 140 Å², rotatable bonds ≤ 10) were retained for docking analysis.

Preparation of Protein Structures and Grid Generation

Human steroid 5α-reductase 2 (SRD5α2) (7BW1) was obtained from the RCSB (https://www.rcsb.org/structure) database and prepared using the protein preparation wizard in the Maestro panel. During the preparation of protein bond orders were assigned and hydrogen atoms were added as well. Water molecules were removed within 3 Å of het groups (Sinha *et al*., 2019). Finally, the OPLS-2005 force field was applied to minimize the structure of the protein (Schrodinger, LLC, NY, USA, 2009) (Halgren *et al*., 2004). Further receptor grid boxes were generated using "Glide's Receptor Grid Generation" module at the active site (with the radius of 20 Å around the crystal structure) of the co-crystallized ligand with the computing cubic box of 10 Å × 10 Å × 10 Å (Jasuja *et al*., 2014).

Ligand Preparation

The 3D chemical structures of the selected ligands were geometry optimized and minimized using algorithms monitored in Schrödinger Maestro v 11.4 (Subramaniyan *et al*., 2018). LigPrep module (Schrodinger, LLC, NY, USA, 2009) was used from the Maestro builder panel to prepare ligand and generate 3D structure of the ligands by adding hydrogen atoms and removing salt and ionizing at pH (7 ± 2) (Modi *et al.,* 2018). Energy minimization was performed using OPLS_2005 force field by using the standard energy function of molecular mechanics and RMSD cut off 0.01 Å to generate the low-energy ligand isomer (Pradeep and Rajanikant, 2012).

Molecular Docking Studies

Molecular docking is a structure-based drug design approach to identify the essential amino acid interactions between the selected protein and generated ligands with low energy conformation (Carlesso *et al*., 2019). Minimum interaction of the ligands characterized by the scoring function which used to foretell the binding affinity with the receptor. Glide Standard precision (SP), docking protocol was applied without smearing any constrain. Flexible docking with Glide Standard precision (SP) protocol was performed to predict the binding affinity and ligand efficiency as inhibitor of human steroid 5α-reductase 2 (SRD5α2) (7BW1) target. Concluding energy assessment was done with the dock score. Visualization of docked ligands was also done by Maestro interface (Schrödinger Suite, LLC, NY).

Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) Calculations

Binding free energies were calculated using MM-GBSA, VSGB 2.0 implicit solvation model, and OPLS-2005 via Prime (Lyne *et al*., 2006). The binding free energy was calculated using the equation:

ΔGbind = Gcomplex - Gprotein - Gligand

Where, G complex, G protein, and G ligand represent the free binding energy of the protein-ligand complex; protein; and ligand, respectively.

Results

GC-MS and characterization of the fatty acids present in the oil extracts

The results from the GC-MS spectrum and identified compounds for fluted pumpkin seed oil are shown in Figure 1 and Table 1. The spectrum identified the presence of twelve (12) compounds with different retention times and percentage abundance (area, %). The spectrum and identified compounds for African yam bean oil, shown in Figure 2 and Table 2, identified the presence of sixteen (16) bioactive compounds (sixteen peaks) with distinct retention times and percentage abundance (area, %)

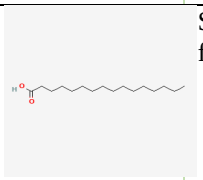
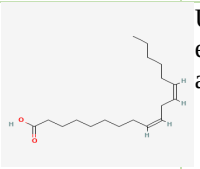
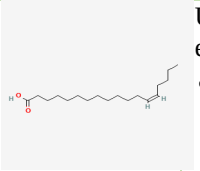
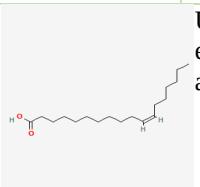
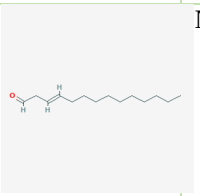
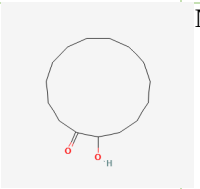
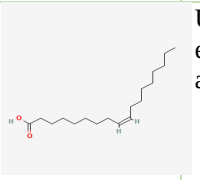
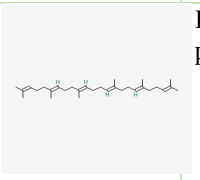
ADME Properties of Bioactive Compounds present in the fluted pumpkin seeds oil

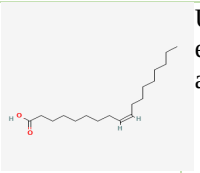
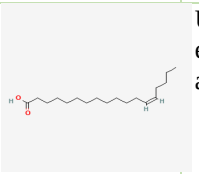
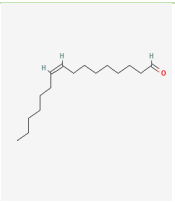
The ADME profile of bioactive compounds in fluted pumpkin seed oil, as shown in Table 3, revealed that eight compounds violated one Lipinski rule of five, while two violated none. Only 13-Tetradecenal and 2-hydroxy-Cyclopentadecanone showed consensus LogPO/W values below 5.0; the rest had values above 5.0. Nonyl tetradecyl ether alone exhibited low gastrointestinal absorbance. None of the analysed compounds inhibited CYP 3A4. In contrast, n-Hexadecanoic acid, 13-Tetradecenal, 2-hydroxy-Cyclopentadecanone, and cis-9-Hexadecenal permeated the blood-brain barrier.

ADME Properties of Bioactive Compounds present in the African yam bean seeds oil

The ADME profile of the bioactive compounds present in the African yam bean seed oil, as shown in Table 4, revealed nine (9) bioactive compounds that violated one Lipinski rule of five; they all had consensus LogPO/W greater than 4.5, and six bioactive compounds did not violate any rule. Most constituents of the African yam bean oil were absorbed in the gastrointestinal tract. Only six bioactive compounds were reported to be able to pass through the blood-brain barrier (BBB). Only 9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester can be transported by the P-glycoprotein efflux pump. 4-methyl-4-oxide Morpholine was the only bioactive compound in the African yam bean oil to be able to inhibit the enzyme CYP 3A4

Table 1: Characterized Identified Compounds in Fluted Pumpkin Seed Oil

S/N	Retention Time	Percent of Peak Area (%)	Name of Compound	IUPAC Name	Molecular Formular	Structure	Type of Compound
1	14.446	2.11	n-hexadecanoic acid (Palmitic acid)	hexadecanoic acid	$C_{16}H_{32}O_2$		Saturated fatty acid
2	18.134	8.53	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	(9Z,12Z)-octadeca-9,12-dienoic acid	$C_{18}H_{32}O_2$		Unsaturated fatty acid
3	18.325	13.58	cis-13-Octadecenoic acid	(*Z*)-octadec-13-enoic acid	$C_{18}H_{34}O_2$		Unsaturated fatty acid
4	18.758	13.01	cis-Vaccenic acid (Asclepic acid)	(*Z*)-octadec-11-enoic acid	$C_{18}H_{34}O_2$		Unsaturated fatty acid
5	23.263	6.53	13-Tetradecenal	tetradec-13-enal	$C_{14}H_{26}O$		Non-lipid
6	28.536	7.95	Cyclopentadecanone, 2-hydroxy-	2-hydroxycyclopentadecan-1-one	$C_{15}H_{28}O_2$		Non-lipid
7	31.782	7.89	Oleic Acid	(Z)-octadec-9-enoic	$C_8H_{17}CH{=}CH(CH_2)7COOH$		Unsaturated fatty acid
8	35.512	0.82	Squalene	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	$C_{30}H_{50}$		Iso-prenol

9	37.090	12.60	Oleic Acid	(Z)-octadec-9-enoic	$C_8H_{17}CH=CH(CH_2)7COOH$		Unsaturated fatty acid
10	37.921	1.19	Nonyl tetradecyl ether	1-nonoxytetradecane	$C_{23}H_{48}O$		Non-lipid
11	38.833	8.79	cis-13-Octadecenoic acid	(Z)-octadec-13-enoic acid	$C_{18}H_{34}O_2$		Unsaturated fatty acid
12	42.377	16.99	cis-9-Hexadecenal	(Z)-hexadec-9-enal	$C_{16}H_{30}O$		Fatty aldehyde

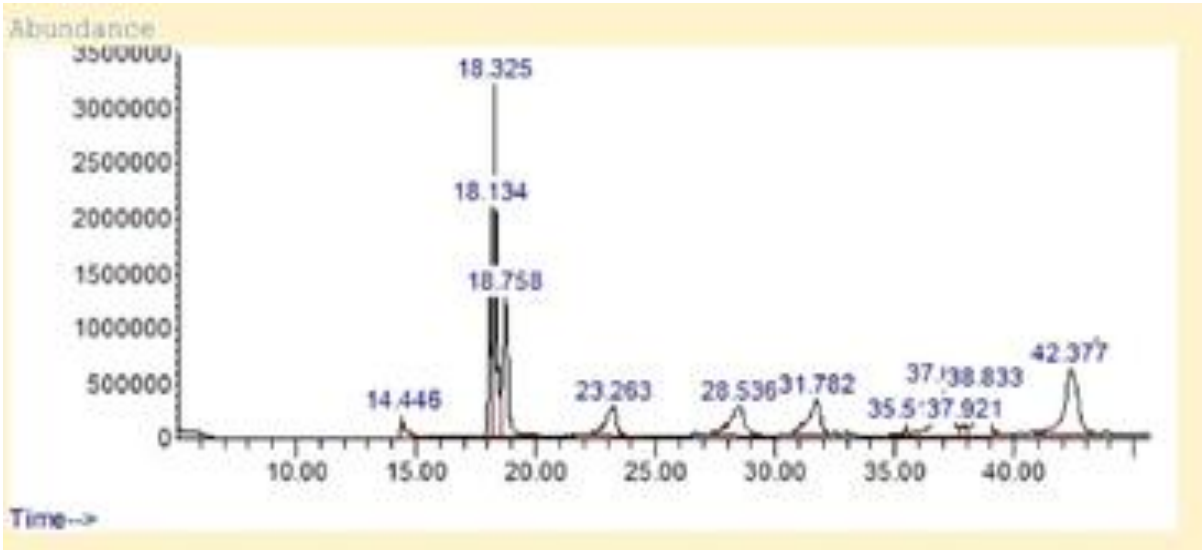
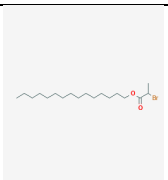
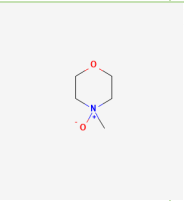
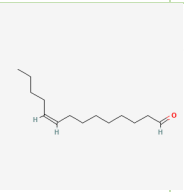
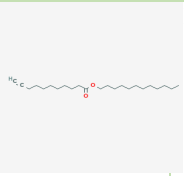
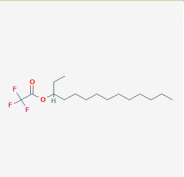
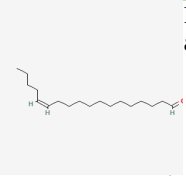
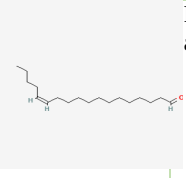
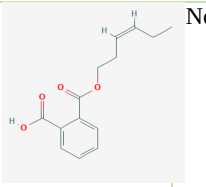
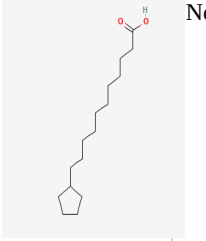
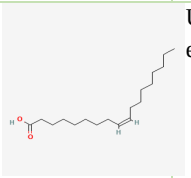
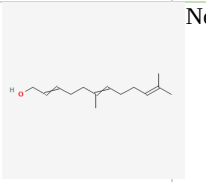
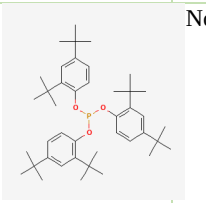
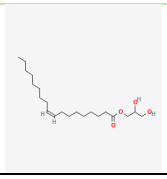


Figure 1: GC-MS Spectrum for Fluted Pumpkin Seed Oil

Table 2: Characterized Identified Compounds in African Yam Bean Seeds Oil

S/N	Retention Time	Percent of Peak Area (%)	Name of Compound	IUPAC name	Mol. Formular	Structure	Type of compound
1	7.287	0.99	2- Bromopropionic acid, pentadecyl ester	(pentadecyl 2-bromopropanoate)	$C_{18}H_{35}BrO_2$		Non-lipid

2	9.189	18.66	1-Tridecene	(tridec-1-ene)	$C_{13}H_{26}$		Hydrocarbon
3	14.411	1.90	n-Hexadecanoic acid (Palmitic Acid)	(hexadecanoic acid)	$C_{16}H_{32}O_2$		Saturated fatty acid
4	14.975	0.92	Morpholine, 4-methyl-, 4-oxide	(4-methyl-4-oxidomorpholin-4-ium)	$C_5H_{11}NO_2$		Non-lipid
5	16.461	12.06	n-Hexadecanoic acid (Palmitic acid)	(hexadecanoic acid)	$C_{16}H_{32}O_2$		Saturated fatty acid
6	18.223	1.40	9-Tetradecenal, (Z)-	((Z)-tetradec-9-enal)	$C_{14}H_{26}O$		Fatty aldehyde
7	18.975	0.34	Undec-10-ynoic acid, dodecyl ester	(dodecyl undec-10-ynoate)	$C_{23}H_{42}O_2$		Non-lipid
8	19.381	0.52	3-Trifluoroacetoxytetradecane	(tetradecan-3-yl 2,2,2-trifluoroacetate)	$C_{16}H_{29}F_3O_2$		Non-lipid
9	19.613	0.25	13-Octadecenal, (Z)-	((Z)-octadec-13-enal)	$C_{18}H_{34}O$		Fatty aldehyde
10	22.478	24.20	13-Octadecenal, (Z)-	((Z)-octadec-13-enal)	$C_{18}H_{34}O$		Fatty aldehyde

11	27.807	0.06	(Z)-2-((Hex-3-enyloxy)carbonyl)benzoic acid	(2-[(Z)-hex-3-enoxy]carbonylbenzoic acid)	$C_{14}H_{16}O_4$		Non-lipid
12	31.109	0.35	Cyclopentaneundecanoic acid	(11-cyclopentylundecanoic acid)	$C_{16}H_{30}O_2$		Non-lipid
13	34.473	15.74	Oleic Acid	((Z)-octadec-9-enoic)	$C_8H_{17}CH=CH(CH_2)7COOH$		Unsaturated fatty acid
14	35.505	0.36	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	(6,11-dimethyldodeca-2,6,10-trien-1-ol)	$C_{14}H_{24}O$		Non-lipid
15	37.515	0.75	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	(tris(2,4-ditert-butylphenyl) phosphite)	$C_{42}H_{63}O_3P$		Non-lipid
16	40.857	21.51	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	(2,3-dihydroxypropyl (Z)-octadec-9-enoate)	$C_{21}H_{40}O_4$		Non-lipid

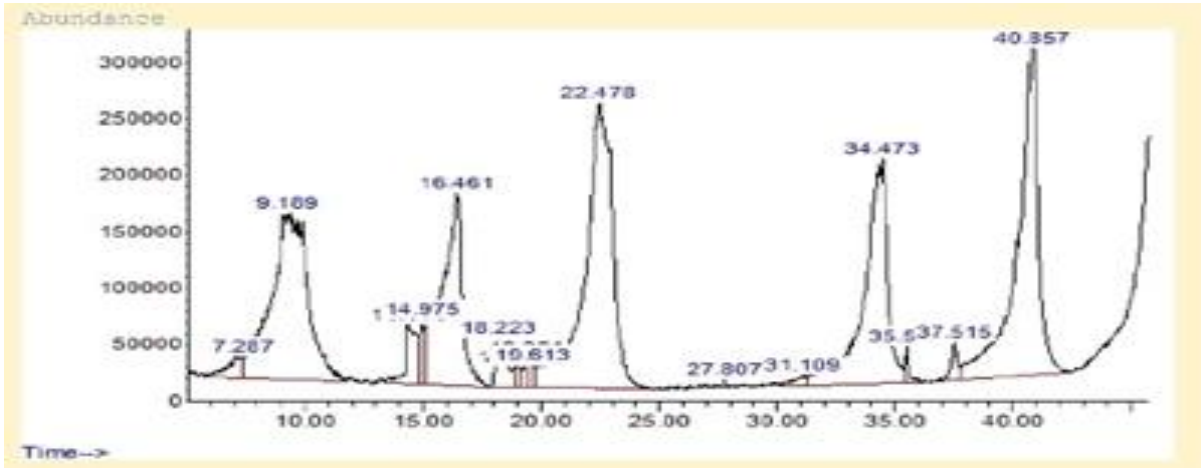


Figure 2: GC-MS Spectrum for African Yam bean Seed Oil

Table 3: ADME Properties of Compounds Found in Fluted Pumpkin Seed Oil

Peak number	Name of Compound	Mol. Wt (g/mol)	No. Of rotatable bonds	No. Of h-bond acceptors	No. Of h-bond donors	Consensus log $p_{o/w}$	Gi absorption	Bbb permeant	P-gp substrate	Cyp 3a4 inhibitor	Lipinski
1	n-Hexadecanoic acid	256.42	14	2	1	5.20	High	Yes	No	No	Yes; 1 violation: MLOGP>4.15
2	9,12-Octadecadienoic acid (Z,Z)	280.4	14	2	1	5.88	NI	NI	NI	NI	NI
3	cis-13-Octadecenoic acid	282.5	15	2	1	5.62	High	No	No	No	Yes; 1 violation: MLOGP>4.15
4	cis-Vaccenic acid	282.5	15	2	1	5.70	High	No	No	No	Yes; 1 violation: MLOGP>4.15
5	13-Tetradecenal	210.36	12	1	0	4.48	High	Yes	No	No	Yes; 0 violation
6	Cyclopentadecanone, 2-hydroxy-	240.38	0	2	1	3.58	High	Yes	No	No	Yes; 0 violation
7	Oleic Acid	282.5	15	2	1	5.65	High	No	No	No	Yes; 1 violation: MLOGP>4.15
8	Squalene	410.7	15	0	0	NI	NI	NI	NI	NI	NI
9	Oleic Acid	282.5	15	2	1	5.65	High	No	No	No	Yes; 1 violation: MLOGP>4.15
10	Nonyl tetradecyl ether	340.6	21	1	0	8.09	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
11	cis-13-Octadecenoic acid	282.5	15	2	1	5.62	High	No	No	No	Yes; 1 violation: MLOGP>4.15
12	cis-9-Hexadecenal	238.41	13	1	0	5.19	High	Yes	No	No	Yes; 1 violation: MLOGP>4.15

Table 4: ADME Properties of Compounds found in African Yam Bean Seed Oil

S/N	Name of compound	Mol. Wt (g/mol)	No. Of rotatable bonds	No. Of h-bond acceptors	No. Of h-bond donors	Consensus log $p_{o/w}$	Gi absorption	Bbb permeant	P-gp substrate	Cyp 3a4 inhibitor	Lipinski
1	2- Bromopropionic acid, pentadecyl ester	363.4	16	2	0	6.30	High	No	No	No	Yes; 1 violation: MLOGP>4.15
2	1-Tridecene	182.35	10	0	0	5.29	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
3	n-Hexadecanoic acid	256.42	14	2	1	5.20	High	Yes	No	No	Yes; 1 violation: MLOGP>4.15
4	Morpholine, 4-methyl-, 4-oxide	117.15	0	2	0	-1.01	High	No	No	Yes	Yes; 0 violation

5	n-Hexadecanoic acid	256.42	14	2	1	5.20	High	Yes	No	No	Yes; 1 violation: MLOGP>4.15
6	9-Tetradecenal, (Z)-	210.36	11	1	0	4.37	High	Yes	No	No	Yes; 0 violation
7	Undec-10-ynoic acid, dodecyl ester	350.6	20	2	0	7.22	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
8	3-Trifluoroacetoxytetradecane	310.39	14	5	0	5.90	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
9	13-Octadecenal, (Z)-	266.5	15	1	0	5.84	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
10	13-Octadecenal, (Z)-	266.5	15	1	0	5.84	Low	No	No	No	Yes; 1 violation: MLOGP>4.15
11	(Z)-2-((Hex-3-enyloxy)carbonyl)benzoic acid	248.27	7	4	1	2.70	High	Yes	No	No	Yes; 0 violation
12	Cyclopentaneundecanoic acid	254.41	11	2	1	4.79	High	Yes	No	No	Yes; 0 violation
13	Oleic Acid	282.5	15	2	1	5.65	High	No	No	No	Yes; 1 violation: MLOGP>4.15
14	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	208.34	7	1	1	3.86	High	Yes	No	No	Yes; 0 violation
15	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	646.9	NI	NI	NI	NI	NI	NI	NI	NI	NI
16	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	356.5	19	4	2	5.09	High	Yes	Yes	No	Yes; 0 violation

Docking and MM-GBSA score of GC-MS Identified compounds in African yam bean seed oil

The GC-MS analysis revealed several compounds, with six (6) having the highest retention time and area peak. These compounds were further subjected to molecular docking and post-docking analysis using the MM-GBSA method. 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester emerged as the hit compound against human steroid 5α-reductase 2 with a docking score of -6.24 (kcal/mol) and MM-BGSA of -87.06 (kcal/mol), respectively. The 2-D interaction also revealed hydrogen bond interactions with GLU57, GLU197, and TYR91 residues (Table 5 and Figure 3).

Docking and MM-GBSA score of GC-MS Identified compounds in fluted pumpkin seed oil

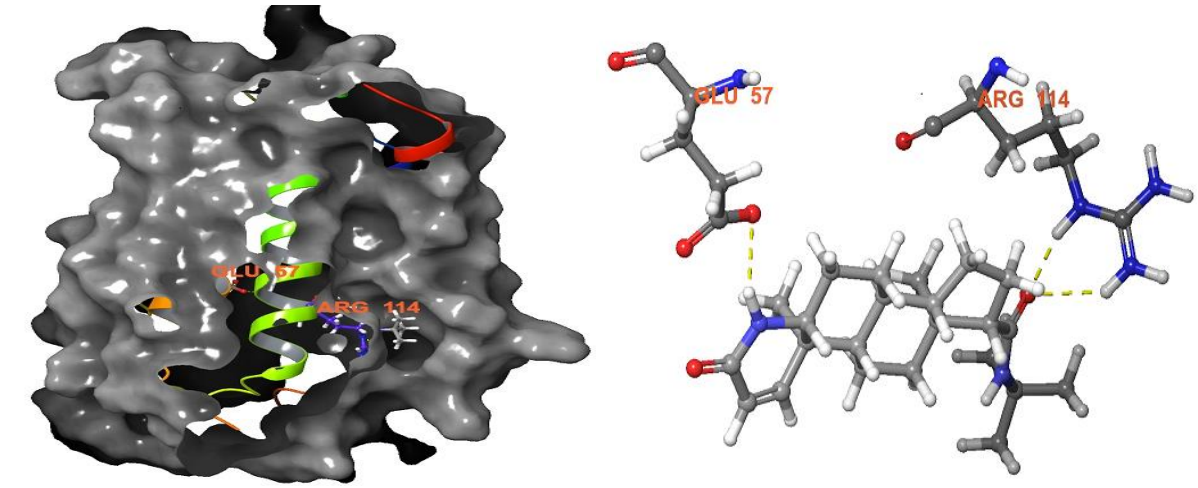
Similarly, the GC-MS analysis revealed several compounds with nine (9) having the highest retention time and area peak. These compounds were further subjected to molecular docking and post-docking analysis using the MM-GBSA method. Cyclopentadecanone, 2-hydroxy- emerged as the hit compound against human steroid 5α-reductase 2 with a docking score of -6.40 (kcal/mol) and MM-BGSA of -60.49 (kcal/mol), respectively. The 2-D interaction also revealed hydrogen bond interactions with the ARG114 residue (Table 6 and Figure 4).

Table 5: Docking and MM-GBSA score of GC-MS Identified compounds with notable peaks in African yam bean seed oil

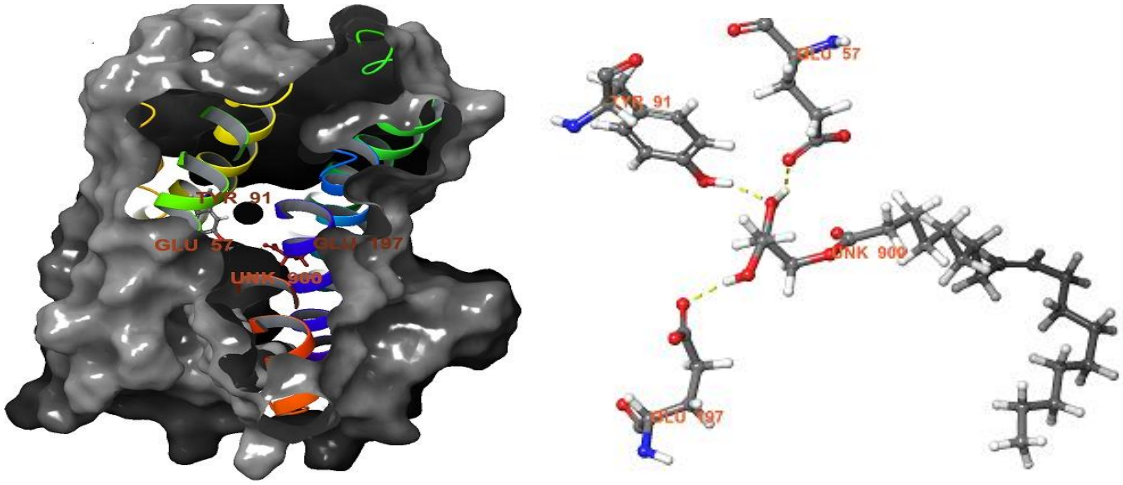
S/N	Name of Compound	Docking score (kcal/mol)	Hydrogen Bond Interactions	MM-GBSA (kcal/mol)
1	Finasteride (Control)	-7.23	GLU57, ARG114	-92.25
2	1-Tridecene	2.61	-	-57.11
3	n-Hexadecanoic acid (Palmitic acid)	-0.26	TYR178,ARG171,ARG105	-72.07
4	9-Tetradecenal, (Z)-	-0.01	ARG94	-62.62
5	13-Octadecenal, (Z)-	0.28	TYR33	-71.95
6	Oleic Acid	-1.49	ARG171,TYR178,ARG105	-75.95
7	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	-6.24	GLU57,GLU197,TYR91	-87.06

Table 6: Docking and MM-GBSA score of GC-MS Identified compounds with notable peaks in fluted pumpkin seeds oil

S/N	Name of Compound	Docking score (kcal/mol)	Hydrogen Bond Interaction	MM-GBSA
1	Finasteride (Control)	-7.231	GLU57,ARG114	-92.25
2	n-hexadecanoic acid (Palmitic acid)	-0.26	ARG171,TYR178,ARG105	-72.07
3	9,12-Octadecadienoic acid (Z,Z) (Linoleic acid)	-2.08	ARG171,TYR178,ARG105	-77.98
4	cis-13-Octadecenoic acid	-3.38	ARG171,TYR178,ARG105	-67.01
5	cis-Vaccenic acid (Asclepic acid)	-1.54	TYR178,ARG105,ARG171	-76.84
6	13-Tetradecenal	1.63	ARG94	-60.71
7	Cyclopentadecanone, 2-hydroxy-	-6.40	ARG114	-60.49
8	Oleic Acid	-1.49	TYR178,ARG171,ARG105	-75.95
9	cis-13-Octadecenoic acid	0.28	TYR33	-71.95
10	cis-9-Hexadecenal	0.04	ARG114	-61.75



A



B

Figure 3: Surface and 2-d Interaction of compounds with amino acids in the active site of human 5 alpha reductase II. A: standard drug (finstaride) and B: hit compound (9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester) for African yam bean seed oil.

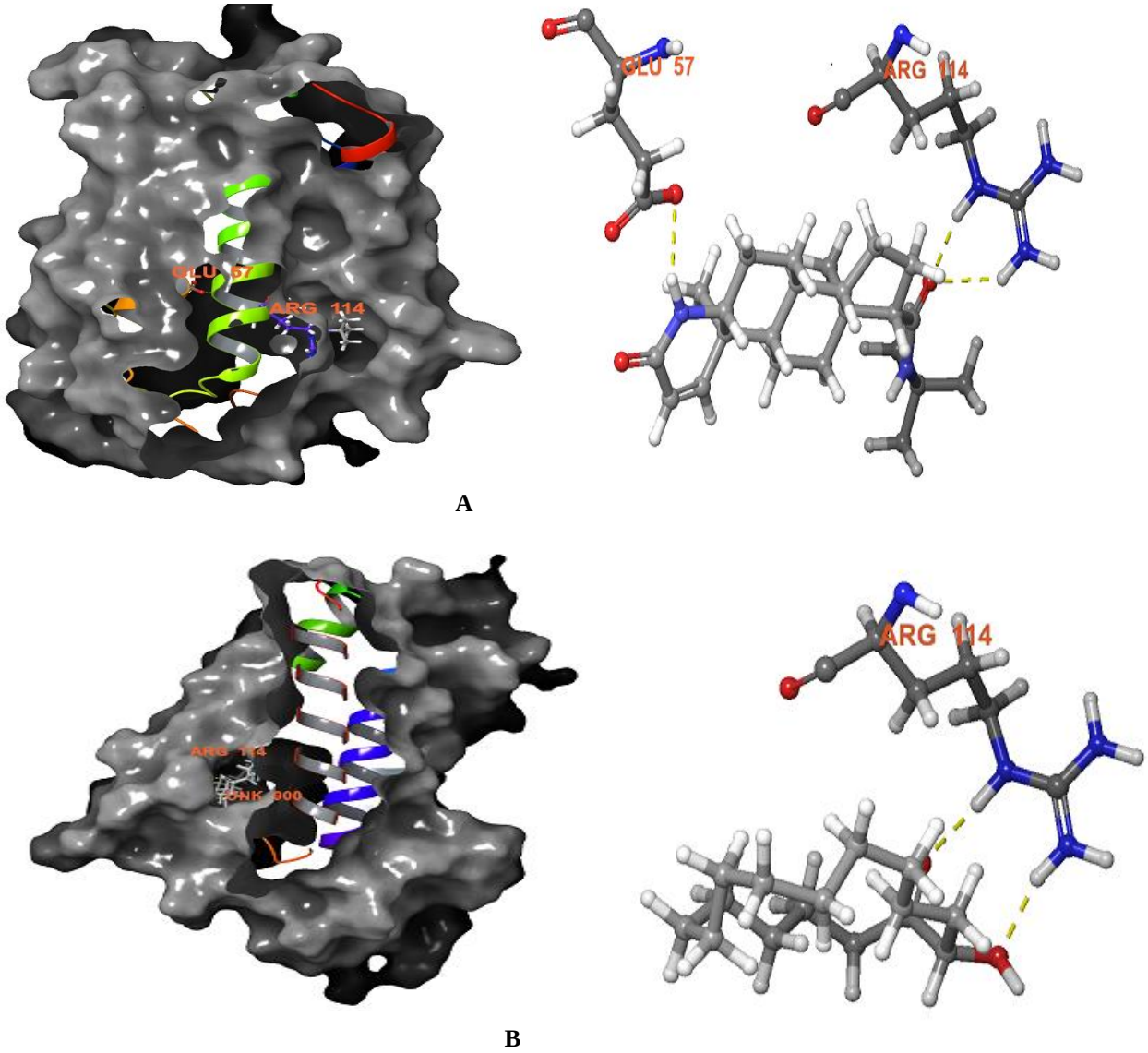


Figure 4: Surface and 2-d Interaction of compounds with amino acids in the active site of human 5 alpha reductase II, A: standard drug (finastaride) and B: hit compound Cyclopentadecanone, 2-hydroxy- for fluted pumpkin seed oil

Discussion

The enhanced endogenous testosterone levels, especially in aging males, have been linked to the risk of BPH development (Kusuma *et al*., 2020). This risk is attributed to the metabolism of testosterone to dihydrotestosterone (DHT), a more potent form of the hormone, by the 5-alpha reductase enzyme (Andriole *et al*., 2004). DHT is predominantly generated by prostatic 5-alpha-reductase (Björnebo *et al*., 2022). Higher DHT activity in prostate tissues is a permissive mediator of the development of BPH69 and prostate cancer (Björnebo *et al*., 2022). The enzyme 5-alpha reductase exists in two forms, type 1 and type 2. Type 1 is produced primarily in the liver and skin and is carried to the prostate, whereas Type 2 is the major form in the prostate (Marcelli and Mediwala, 2020). Therefore, 5-alpha-reductase type 2 inhibitors are typical therapeutic agents that are used against BPH to reduce DHT production and prostate size (Devlin *et al*., 2020).

The GC-MS analysis of fluted pumpkin seed oil revealed about 2.11% saturated fatty acids, 64.40% unsaturated fatty acid, 17.81% non-fatty acid lipids and 15.67% non-lipids, with n-hexanoic (palmitic) acid (2.11%), and Cis-13-octadecenoic (linoleic) acid (22.37%), being the most predominant saturated and unsaturated fatty acids respectively. This doesn't correlate with the result gotten by Samuel et al.(2017) where the concentration of saturated and unsaturated fatty acids were 18.4% and 80.2% respectively in fluted pumpkin seed oil. These drastic differences may have arisen due to regional differences. Similarly, the GC-MS results of African yam bean seed oil revealed the African yam bean seeds oil contained 13.96% saturated fatty acid, 15.74% unsaturated fatty acids, 18.66% hydrocarbon, 25.85% fatty Aldehydes and 25.80% non-lipid bioactive compounds. It was also discovered that palmitic and oleic acid were the only saturated and unsaturated fatty acids constituted in the seed oil. This also does not align with the findings of Adeyeye et al.(1999) who reported the

presence of six saturated fatty acid and five unsaturated fatty acids. In his findings, palmitic acid was 19.78% and Oleic acid was trace (<2.00%). Also the extraction and treatment methods used on the African yam beans may be different, this nevertheless is not a certain reason since the extraction method used by Adeyeye et al. was not stated.

The ADME properties for fluted pumpkin seeds oil revealed eight (8) compounds violated one Lipinski rule of 5, while 2 violated none. Only 13-Tetradecenal and 2-hydroxy-Cyclopentadecanone showed consensus LogPO/W below 5.0, while the rest had values above 5.0. Having a consensus LogPO/W above 5.0 results to bio-availability issues; their relative absorption into the bloodstream may be significantly hindered due to their tendency to remain sequestered in lipid environments rather than dissolving in the aqueous environment of the gastrointestinal tract (Bhal *et al*.,2007). Also, they may accumulate in fatty tissues due to their lipophilic nature which is toxic. Nonyl tetradecyl ether had a low gastro-intestinal absorbance.

The bioactive compounds in the fluted pumpkin seed oil are not transported by the P-glycoprotein efflux pump and does not inhibit the action of the enzyme CYP 3A4; this would result in a consistent diminishing of the compound in the body systems as the enzyme would convert the lipophilic chemical compounds into more hydrophilic metabolites, which can then be excreted from the body. Nevertheless, n-Hexadecanoic acid, 13-Tetradecenal, 2-hydroxy-Cyclopentadecanone and cis-9-Hexadecenal can pass through the blood brain barrier (BBB), suggesting that these bioactive compounds have relatively small molecular sizes and are psychotropic in nature.

The ADME properties for African yam bean seeds oil revealed Nine (9) bioactive compounds violated one Lipinski rule of five, they all had consensus LogPO/W greater than 4.5 and six bioactive compounds did not violate any rule. This suggests that the oil bioactive components may likely accumulate in fatty tissues due to its high lipophilicity. Most constituents of the African yam bean seeds oil can be absorbed in the gastrointestinal tract. Only six bioactive compounds were reported to be able to pass through the blood brain barrier (BBB) this also revealed their psychotropic nature . Only 9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester can be transported by the P-glycoprotein efflux pump. 4-methyl-4-oxide Morpholine was the only bioactive compound in the African yam bean oil to be able to inhibit the enzyme CYP 3A4, which suggests that it would be better retained in the blood stream without being easily converted to lipophobic metabolites to be excreted.

Molecular docking is a computer-based predictive method used in drug discovery to evaluate and model how a small molecule (ligand) fits into the binding site of a biological target, typically a protein, and to estimate the strength and stability of that interaction based on scoring functions and structural complementarity. It enables in silico prediction of binding modes and affinities, facilitating virtual screening and lead optimization in structure-based drug design (Anusha and Govinda 2025).

The molecular docking activities of GC-MS identified compounds from fluted pumpkin seed oil against 5α-reductase type 2 revealed that 2-hydroxycyclopentadecanone was the hit compound with a docking score of when compared with finasteride (-7.23 kcal/mol). 2-hydroxycyclopentadecanone is a flexible, C15 aliphatic ketone with a single hydroxyl group, structurally dissimilar to typical steroid substrates and finasteride. It may make hydrophobic contacts or weak, reversible hydrogen bonds, or act via indirect (membrane/allosteric) effects. This implies that the mechanism of this compound interacting with 5α-reductase type 2 is quite different from that of finasteride, which fits the 5α-reductase type II (SRD5A2) steroid tunnel, undergoes hydride transfer from NADPH and is trapped as an NADP dihydrofinasteride adduct; binding is tight and essentially irreversible on physiological timescales (crystallographically observed; key residues include E57 and Y91) (Xiao *et al*., 2020).

The biological activity of 2-hydroxycyclopentadecanone against 5α-reductase type II (SRD5A2) can be attributed mainly to its β-hydroxy ketone functional group**,** rather than to the hydrocarbon ring itself. The adjacent hydroxyl (–OH) and carbonyl (C=O) groups provide dual hydrogen-bonding capability that is critical for molecular recognition within the SRD5A2 catalytic cavity. The hydroxyl group can act as a hydrogen-bond donor**,** while the carbonyl oxygen serves as a hydrogen-bond acceptor, enabling stabilising interactions with key polar residues. Structural studies of SRD5A2 demonstrate that such polar interactions are essential for aligning ligands relative to the NADPH cofactor and for modulating hydride transfer during testosterone reduction. Consequently, the β-hydroxy ketone moiety functions as a pharmacophoric element**,** allowing non-steroidal compounds like 2-hydroxycyclopentadecanone to interfere with enzyme activity by mimicking critical substrate enzyme interactions exploited by established inhibitors (Xiong *et al*., 2020; Di Costanzo *et al*., 2021).

Molecular docking and post-docking MM-GBSA free energy calculations revealed marked differences in the interaction profiles and binding strengths of finasteride and 2-hydroxycyclopentadecanone with the active site of 5α-reductase type II (SRD5A2). Finasteride formed stable hydrogen bond interactions with GLU57 and ARG114, two residues that are critically involved in substrate recognition and catalysis within the enzyme active site (Yin *et al*., 2020), and exhibited a highly favorable MM-GBSA binding free energy of −92.25 kcal/mol. In contrast, 2-hydroxycyclopentadecanone formed a hydrogen bond interaction only with ARG114, with a comparatively less favorable MM-GBSA binding free energy of −60.49 kcal/mol. The involvement of GLU57 in finasteride binding is particularly significant, as crystallographic and mechanistic studies have identified this residue as a key catalytic amino acid responsible for proton transfer during the NADPH-dependent reduction of testosterone. Interaction with GLU57 facilitates optimal positioning of finasteride within the catalytic pocket, enabling hydride transfer and subsequent formation of the stable NADP dihydrofinasteride adduct that underlies finasteride's mechanism-based, irreversible inhibition of SRD5A2 (Khantham *et al*., 2021). The additional hydrogen bond with ARG114 further stabilizes finasteride within the predominantly hydrophobic transmembrane cavity,

contributing to its strong bonding. This finding aligns with prior reports that many natural, non-steroidal compounds such as cyclopentadecanone, 2-hydroxy- can bind SRD5A2 moderately in silico but rarely replicate finasteride's mechanism without further chemical features or experimental confirmation.

Molecular docking analysis of compounds in African yam bean seed oil identified from GC-MS revealed that 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester as the hit compound. 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester exhibited a moderate docking score of −6.24 kcal/mol and formed stable hydrogen-bond interactions with Glu57, Glu197 and Tyr91 within the active site of human steroid 5α-reductase type II (SRD5A2). These residues are located within or proximal to the catalytic pocket and are known to play important roles in substrate recognition and inhibitor binding (Yin *et al*., 2020). Glu57 is implicated in the catalytic mechanism, occupation of its vicinity raises the possibility of competitive inhibition. In addition, interaction with Tyr91, a residue lining the hydrophobic tunnel of the enzyme, further stabilizes ligand positioning within the active site, enhancing binding affinity (Yin *et al*., 2020). The additional hydrogen bond observed with Glu197 indicates extended polar interactions that may contribute to improved binding stability. Tight binding alone does not guarantee the same pharmacological outcome: finasteride's mechanism-based covalent/NADP adduct and long residence time are a function of both its chemical reactivity and its precise steroid geometry. The mono-acylglyceride, although potentially strongly bound, lacks the structural features for hydride transfer and adduct formation; therefore the functional consequence is likely to be reversible competitive inhibition.

Furthermore, the biological activities of 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester can be explained by the combined action of its polar glycerol-derived diol–ester head group and hydrophobic unsaturated fatty acid chain**.** The 2,3-dihydroxypropyl moiety provides multiple hydrogen-bond donors and acceptors, enabling stabilising interactions with key polar residues in the 5α-reductase type II (SRD5A2) catalytic cavity that are involved in ligand orientation and NADPH-dependent catalysis. The ester carbonyl further contributes to binding by acting as a hydrogen-bond acceptor, helping to anchor the ligand within the active site. In parallel, the cis-oleyl chain promotes hydrophobic interactions within the membrane-embedded binding pocket, enhancing affinity and allowing conformational adaptation. (Xiong *et al*., 2020; Di Costanzo *et al*., 2021; Vanlaeys *et al*., 2023).

Although the docking score suggests moderate affinity, MM-GBSA analysis predicted a highly favorable binding free energy (−87.06 kcal/·mol), comparable to values reported for known SRD5A2 inhibitors. This discrepancy reflects methodological differences, as docking scores are heuristic, whereas MM-GBSA incorporates molecular mechanics and solvation energy terms, often yielding stronger estimates of binding for ligands with extensive hydrophobic and polar contacts (Genheden and Ryde, 2015). The long hydrophobic aliphatic chain of the ligand likely enhances van der Waals interactions within the lipophilic enzyme tunnel, while the glycerol moiety contributes stabilizing hydrogen bonds.

Conclusion

In conclusion, the hit compounds; 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (from African yam bean seeds) revealed a docking score of 6.24 kcal/mol) and Cyclopentadecanone, 2-hydroxy- (from fluted pumpkin seeds, -6.40 kcal/mol**)**. MM-GBSA validation further revealed -87.06 kcal/mol and -60.49 kcal/mol for these compounds, respectively against human steroid 5α-reductase type II (SRD5A2). Hence these compounds may be suitable in managing benign prostate hyperplasia disorder.

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