Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 5s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

In Silico and Analytical Evaluation of Beta-Sitosterol From Anogeissus Pendula As A Potential Therapeutic Agent Against Hyperlipidemia

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Cite this paper as: Deepak Singh Chaudhary, Asheesh Kumar Gupta, (2025) In Silico and Analytical Evaluation of Beta-Sitosterol From Anogeissus Pendula As A Potential Therapeutic Agent Against Hyperlipidemia. *Journal of Neonatal Surgery*, 14 (5s), 923-934.

ABSTRACT

Hyperlipidemia, one of the main causes of cardiovascular disease, is characterized by elevation of lipids that can cause atherosclerosis and coronary artery disease. Although standard pharmaceuticals like statins and fibrates may work, their potential side effects urge the search for safer, cost-effective ones from natural sources. Medicinal plants such as Anogeissus pendula, a plant with many bioactive compounds have gained much attention because of their metabolic activities particularly for the Beta-Sitosterol presence. This plant sterol, which has a similar chemical structure to cholesterol, reduces lipid levels by blocking intestinal cholesterol absorption and modulates the major lipid metabolism pathways. In silico and analytical approaches are used in the present study to evaluate the therapeutic potential of Beta-Sitosterol isolated from A. pendula. Its high binding affinity with lipid-regulating enzymes including HMG-CoA reductase and PPAR-α indicated by molecular docking and ADMET prediction, its targeting lipid metabolism critical pathways as revealed by network pharmacology. Additionally, chromatographic analyses confirmed that its concentration in A. pendula extracts was exceedingly high, and further complemented its pharmacological relevance. The results render Beta-Sitosterol as a potentially efficacious natural agent for lipid reduction, a new hope for non-traditional lipid-lowering drugs, and worthy of future in vivo and clinical investigations

Keywords: *Hyperlipidemia, Anogeissus pendula, Beta-Sitosterol, Molecular Docking, ADMET, Lipid Metabolism, Cardiovascular Disease, Phytosterols.*

1. INTRODUCTION

Hyperlipidemia, which presents with an increase in lipid levels, such as cholesterol and triglycerides, is a major risk factor for cardiovascular diseases (CVDs) like atherosclerosis, stroke, and coronary heart disease. Hyperlipidemia may be categorized as primary (genetic) or secondary, and factors that cause it include diet, obesity, physical inactivity, diabetes, and metabolic syndrome. Lipid homeostasis is highly regulated by multiple metabolic pathways, such as cholesterol biosynthesis, absorption, and lipoprotein metabolism. Lifestyle modifications and drug therapies are the main therapeutic methods for managing lipid levels. [1,2].

Statins, fibrates, bile acid sequestrants, cholesterol absorption inhibitors (such as ezetimibe), and PCSK9 inhibitors are the mainstay therapies for hyperlipidemia. Statins are the most commonly prescribed medication due to their capacity to reduce low-density lipoprotein cholesterol (LDL-C) levels by inhibiting HMG-CoA reductase. However, non-statin therapy is typically employed in combination to contribute to lipid-lowering effects, particularly in statin-intolerant patients. [3]. Although these traditional lipid-lowering drugs have been found to work, they have the disadvantage of side effects and incomplete risk reduction for cardiovascular disease. For instance, statins have the potential to cause myopathies, hepatotoxicity, and an increased risk of developing type 2 diabetes. Fibrates, though effective for lowering triglycerides, contribute to gastrointestinal disorders and a raised risk of gallstones. In addition, bile acid sequestrants tend to be associated with bloating and constipation, and PCSK9 inhibitors, though very effective, are costly and need to be administered subcutaneously. The long-term administration of these drugs also poses questions regarding patient compliance and safety. .

Anogeissus Pendula is a medicinal shrub with a wide distribution in India and other tropical nations. Anogeissus Pendula has been used in Ayurvedic medicine for the treatment of inflammatory conditions, gastrointestinal disorders, and metabolic dysregulation. Flavonoids, tannins, and polyphenols have been isolated from A. pendula, which are responsible for hexol of pharmacological properties, antioxidant, antimicrobial, and anti-inflammatory activity [5]. A surfactant with a high sterol content, particularly Beta-Sitosterol, has been given responsibility for lipid-lowering activity. Plant extracts have been found to affect cholesterol biosynthesis and lipid absorption processes, which suggests their potential value as an anti-hyperlipidemic drug. [6].

t. Beta-sitosterol is a plant sterol whose structure resembles that of cholesterol, with molecular formula $C_{29}H_{50}O$, with a wide distribution in nuts, seeds, fruits and medicinal herbs, including Anogeissus pendula. Beta-Sitosterol mimics cholesterol structurally and inhibits the absorption of dietary cholesterol from the intestine, effectively lowering LDL-C, amongst other cardioprotective actions. [7]. Beta-Sitosterol is anti-inflammatory, antioxidant, and immunomodulatory pharmacologically. It has also been found to modulate lipid metabolism through the inhibition of key enzymes (e.g. HMG-CoA reductase) and the modulation of peroxisome proliferator-activated receptors (PPARs) [8]. Beta-sitosterol demonstrated considerable lipid-reducing efficacy in preclinical and clinical tests by reducing total cholesterol, LDL-C, and triglycerides, whilst maintaining HDL-C concentrations constant or only slightly elevated [9]. (10) Its mechanism of action is based on competition for cholesterol absorption in the intestine, down-regulation of key lipid metabolism genes, like HMG-CoA reductase and LDL receptors, and induction of bile acid metabolism that will accelerate cholesterol elimination. These results demonstrate the potential of Beta-Sitosterol as a nutritional and therapeutic agent in hyperlipidemia and thus warrant further computational and experimental study. [11-12]. We systematically explore this potency via the hypolipidemic nature of Beta-Sitosterol, aided by computational and analytical studies. This involves molecular docking for the analysis of its interaction with lipid metabolism targets and ADMET prediction for pharmacokinetic and toxicity profiling. [13], network pharmacology to study its biological mechanisms and chromatographic methods to quantify its presence in A. pendula. In conclusion, these studies are utilised to combine regular information with current logical confirmation, demonstrating the therapeutic capability of Beta-Sitosterol for hyperlipidemia control.

2. MATERIALS AND METHODS

In the Silico Evaluation

Molecular Docking Studies

Beta-sitosterol's potential as a therapeutic agent against hyperlipidemia was assessed using molecular docking analyses. Selected for the study were important target proteins engaged in lipid metabolism and hyperlipidemia, including CETP, PPAR-α, and HMG-CoA reductase. Retrieved from the PubChem database, the 3D structure of Beta-Sitosterol was optimised using energy minimisation and molecular mechanics force fields to guarantee appropriate shape and stability. Target receptors were considered after gathering protein structures from the Protein Data Bank (PDB), and water was eliminated, while hydrogen atoms were added, and appropriate charge assignments were made. Grid maps were produced around the active sites of the target proteins for the definition of docking screening areas. The docking simulations were conducted using AutoDock and Glide with the Lamarckian genetic algorithms or precision docking modes. The binding affinities (in kcal/mol) and interaction profiles via hydrogen bonding, hydrophobic interactions, and van der Waals forces were investigated to establish the ability of Beta-Sitosterol in binding and influencing the activity of the target proteins [13]. The results were graphically shown using molecular docking tools such as PyMOL or Discovery Studio to explore the binding modes and key residues of the interaction. The in-silico methodology provided the initial information on interpreting the molecular mode of action of Beta-Sitosterol for its anti-hyperlipidemia activity.

ADMET and Drug-likeness Prediction

The ADMET properties of Beta-Sitosterol were determined to gauge its pharmacokinetic profile and therapeutic potential. Computational models and software were used to project its absorption from the gastrointestinal tract, distribution in biological membranes, metabolic stability, routes of excretion, and possible toxicity. The findings suggested that Beta-Sitosterol has good absorption properties because of its lipophilic nature, which allows effective penetration through cell membranes. Distribution analysis indicated its potential for reaching target tissue, and metabolic stability predictions emphasized its resistance to quick degradation. Given its hydrophobic properties, fecal and biliary excretion processes dominated. The low risk of side effects shown by toxicity prediction confirmed its safety profile. Moreover, Lipinski's Rule of Five was used to evaluate drug-likeness; it showed that Beta-Sitosterol satisfies the oral bioavailability parameters, including molecular weight (<500 g/mol), hydrogen bond donors (<5), hydrogen bond acceptors (<10), and logP (<5). Other pharmacokinetic parameters, such as polar surface area and rotatable bond count, further validated its drug-like properties. These in silico results collectively indicate that Beta-Sitosterol has desirable ADMET properties and drug-likeness, which indicates that it is a potential candidate for further investigation as a drug against hyperlipidemia. [14,15].

Network Pharmacology Analysis

Network pharmacology analysis was done to identify the molecular mechanism underlying the anti-hyperlipidemic effect of Beta-Sitosterol. Beta-sitosterol and lipid metabolism-associated genes and proteins were initially screened using publicly accessible databases such as STITCH, PubChem, and GeneCards. The identified targets were later confirmed by literature mining to search for their role in lipid regulation. An interactome network consisting of protein-protein interactions was constructed from the STRING database with a cutoff on the confidence score of >0.7 to ensure high-quality interaction. The PPI network was analyzed to find hub genes and crucial regulatory proteins in lipid metabolism. Thereafter, pathway enrichment analysis was conducted with the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases to project the biological pathways and molecular functions targeted by Beta-Sitosterol. The overall network pharmacology study offered understandings of the multi-target mechanisms of Beta-Sitosterol for its possibility as a drug candidate against hyperlipidemia [16,17].

Analytical Quantification of Beta-Sitosterol

Plant Material Collection and Extraction

Plant material, Anogeissus pendula, was harvested from the natural environment within dry areas of Rajasthan, India, to ascertain the genuineness of the species by Raw Material Herbarium and Museum (RHMD), CSIR, Delhi, with authentication number NIScPR/RHMD/Consult/2022/4145-46-1. Leaves and bark were chosen for extraction because they contain high phytosterol content. The plant material was well cleaned, dried under shade, and powdered extremely fine to ensure effective extraction to the maximum. Beta-sitosterol was extracted through the Soxhlet extraction method, which is a standard procedure for the separation of non-polar molecules. An appropriate solvent system, like ethanol, was used because it could easily dissolve phytosterols. Extraction was permitted to continue for 6-8 hours until thimble's solvent was colorless, which showed that maximum extraction had been attained. The solvent was eliminated through evaporation in a reduced pressure with the help of a rotary evaporator, and crude extract was obtained. Gravimetrically and on a percentage basis, the extract yield was computed as the dry weight of the plant material. This enabled the determination of Beta-sitosterol in the extract as a basis for further analytical and in silico screening to assess its therapeutic potential against hyperlipidemia.

Evaluation of antihyperlipidemic activity

High-fat diet-induced hyperlipidaemic model.

First, the animals were chosen and weighed, and then each one was assigned a distinct ID tag. Rats developed high blood lipid levels (hyperlipidemia) after 20 days of oral consumption of a diet designed to cause atherosclerosis and promote weight loss. Next, for 14 days, the rats received a daily morning dose of plant extracts (200 mg/kg b.w.) that were mixed in a 2% acacia solution. This administration was done through a tube inserted into their stomach (gastric intubation). The atherogenic food was continued for all groups during these 14 days. In the final part of the experiment, the animals were used for the measurement of different biochemical markers. To get serum, blood was drawn from the hearts of rats under ether anesthesia and then spun in a centrifuge at 2000 revolutions per minute for 30 minutes.

Animal Selection and Grouping

For this study, a total of 30 healthy adult rats were selected and then randomly separated into five distinct groups, each containing six individuals (n=6). Before starting the experimental procedures, all animals were given a week to become accustomed to the laboratory setting. The protocol for this research was approved by the Institutional Animal Ethics Committee (IAEC), and all actions taken followed the standard regulations for the care and utilization of animals in laboratory settings.

Diet and Treatment Protocol

The induction of experimental hyperlipidemia was achieved through the oral administration of a high-fat diet, with or without the inclusion of plant extract or a standard pharmaceutical agent, based on the following group assignments:

Group I (Normal Control): Received oral doses of a 2% acacia suspension for a period of 20 days.

Group II (Disease Control): Consumed a high-fat diet and was also given 2% acacia orally for 20 days.

Group III (Standard Treatment): Treated with atorvastatin at a dosage of 10 mg per kilogram of body weight per day (orally) for 14 days, in addition to a high-fat diet.

Group IV (Low Dose Test): Received an ethanolic extract of Anogeissus Pendula at a concentration of 200 mg/kg per day (orally), suspended in 2% acacia, along with a high-fat diet for 14 days.

Group V (High Dose Test): Received an ethanolic extract of Anogeissus Pendula at a concentration of 400 mg/kg per day (orally), suspended in 2% acacia, along with a high-fat diet for 14 days.

Triton-induced hyperlipidaemic model

After fasting for 18 hours, the animals will be given an injection of saline Triton (Triton X-100) into their peritoneal cavity at a concentration of 100 mg per kilogram of their body weight. Plant extracts, at a dosage of 200 mg/kg b.w., will be delivered through a tube inserted into their stomach (gastric intubation). The first administration of the plant extract will occur immediately after the Triton injection, and a second will follow 20 hours later. This extraction process will continue for a total of 7 days. Following this 7-day treatment period, the animals will be utilized for a range of biochemical measurements. Blood will be drawn from the hearts of ether-anesthetized rats and then spun in a centrifuge at 2000 revolutions per minute for 30 minutes to separate the serum. (11).

Animal Selection and Grouping

For this experiment, thirty healthy adult laboratory rats were selected and allowed to adjust to typical laboratory conditions for a week before the study commenced. The rats were maintained on a 12-hour cycle of light and darkness and had continuous access to standard pellet food and drinking water.

Induction of Hyperlipidemia

Hyperlipidemia was induced in the animals by giving them a single dose of Triton X-100 (100 mg/kg b.w.) intraperitoneally. This compound, a nonionic surfactant, leads to a sudden increase in blood lipids by preventing the body from removing lipoproteins that are high in triglycerides.

Treatment Protocol

Treatment commenced 18 hours following Triton administration in the designated groups, according to the following experimental design:

Group I (Normal Control): Orally administered 2% acacia for 7 days.

Group II (Negative Control): Received Triton X-100 (100 mg/kg,) followed by oral administration of 2% acacia for 7 days.

Group III (Standard): Given atorvastatin (10 mg/kg/day) for 7 days, beginning 18 hours after Triton administration.

Group IV (Low Dose Test): Received an ethanolic extract of Anogeissus Pendula (200 mg/kg/day) in 2% acacia for 7 days, starting 18 hours post-Triton.

Group V (High Dose Test): Received an ethanolic extract of Anogeissus Pendula (400 mg/kg/day) in 2% acacia for 7 days, starting 18 hours post-Triton.

Blood Samples Collection

On the eighth day of Triton and the twenty-first day of their hyperlipidemic therapy, blood was drawn from the patient's heart using a heart puncture. Serum lipid profiles (TG, LDL, TC, HDL, VLDL) are measured using serum collected by immediately centrifuging the blood samples for 30 minutes at room temperature in a Remi ultra cooling centrifuge at 2000 rpm.

Statistical analysis

Mean SEM was used to express the findings. One-way ANOVA and the Tukey test were used in the statistical analysis, which was done with the use of the GraphPad Instant program. P-value of 0.05 or above was regarded as statistically significant.

RESULTS AND DISCUSSION

In the Silico Evaluation Results

Molecular Docking

Table 1 and Figure 1 show molecular docking outcomes of Beta-Sitosterol, a phytosterol of Anogeissus Pendula, against major proteins associated with hyperlipidemia. It indicates Beta-Sitosterol binding to HMG-CoA reductase (-8.4 kcal/mol), PPAR-alpha (-6.2 kcal/mol), and CETP (-10.2 kcal/mol) by hydrogen bonding, Pi-Pi stacking, hydrophobic, and electrostatic interactions. The most elevated affinity towards CETP indicates engagement in very high HDL levels and low levels of LDL. Moderate affinity toward HMG-CoA reductase refers to the possible lowering of cholesterol levels via the enzyme. Interaction with PPAR-alpha indicates the involvement of the compound in lipid metabolism and regulation of glucose levels. All of these indications lead to the hypothesis that Beta-Sitosterol is a multi-target agent for hyperlipidemia and metabolic disorders, as depicted in figure 2.

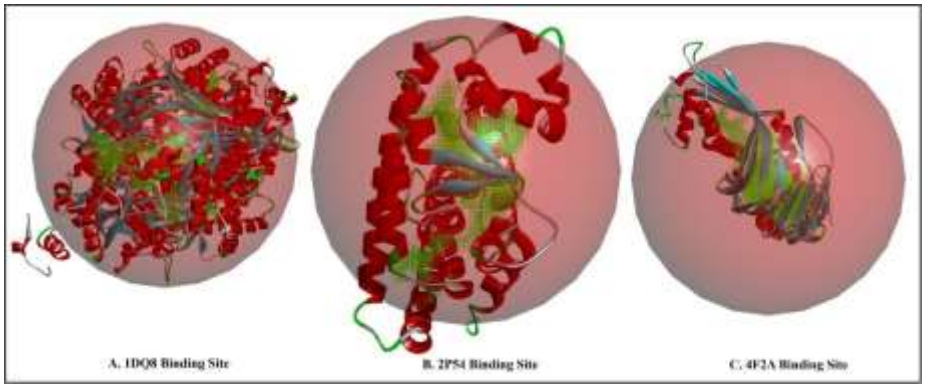


Figure 1. Binding site interaction analysis of Beta-Sitosterol from *Anogeissus Pendula.*

Table 1. Binding scores and interaction analysis of Beta-Sitosterol from *Anogeissus Pendula* with its target proteins:

Target Protein	PDB ID	Binding Score (kcal/mol)	Amino Acid Residues Involved	Type of Interaction
HMG-CoA Reductase	1DQ8	-8.4	ARG, HIS, GLU, PHE	Hydrogen bond, Pi-Pi stacking
PPAR-α	2P54	-6.2	LEU, ILE, TYR, TRP	Pi-Pi stacking, Hydrophobic
CETP	4F2A	-10.2	GLU, LYS, THR, HIS	Hydrogen bond, Electrostatic

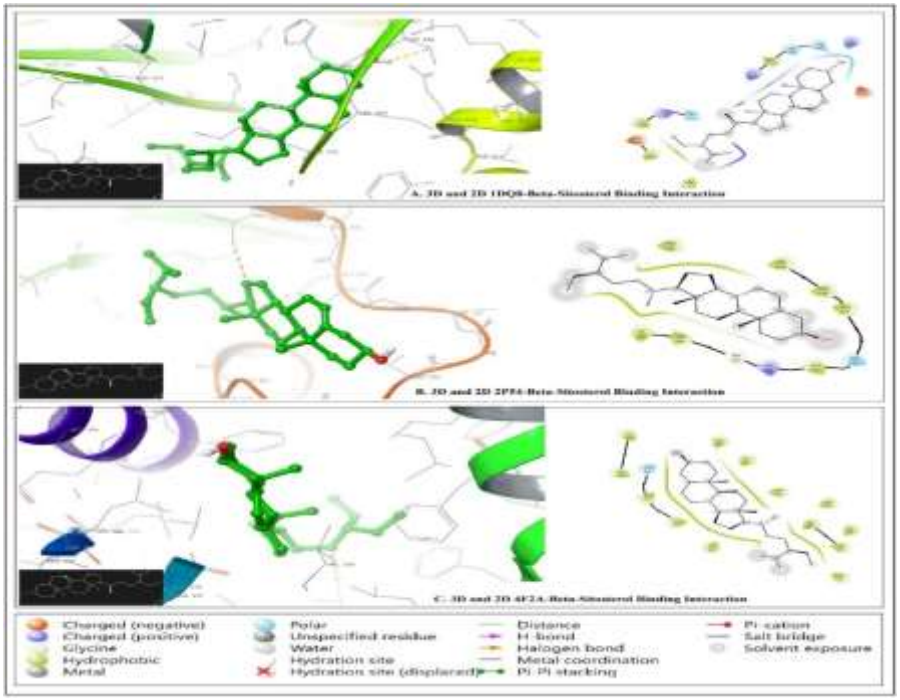


Figure 2. 3D and 2D structure of Protein (HMG-CoA reductase, PPAR-α, and CETP)- Ligand (Beta-Sitosterol) Binding interaction.

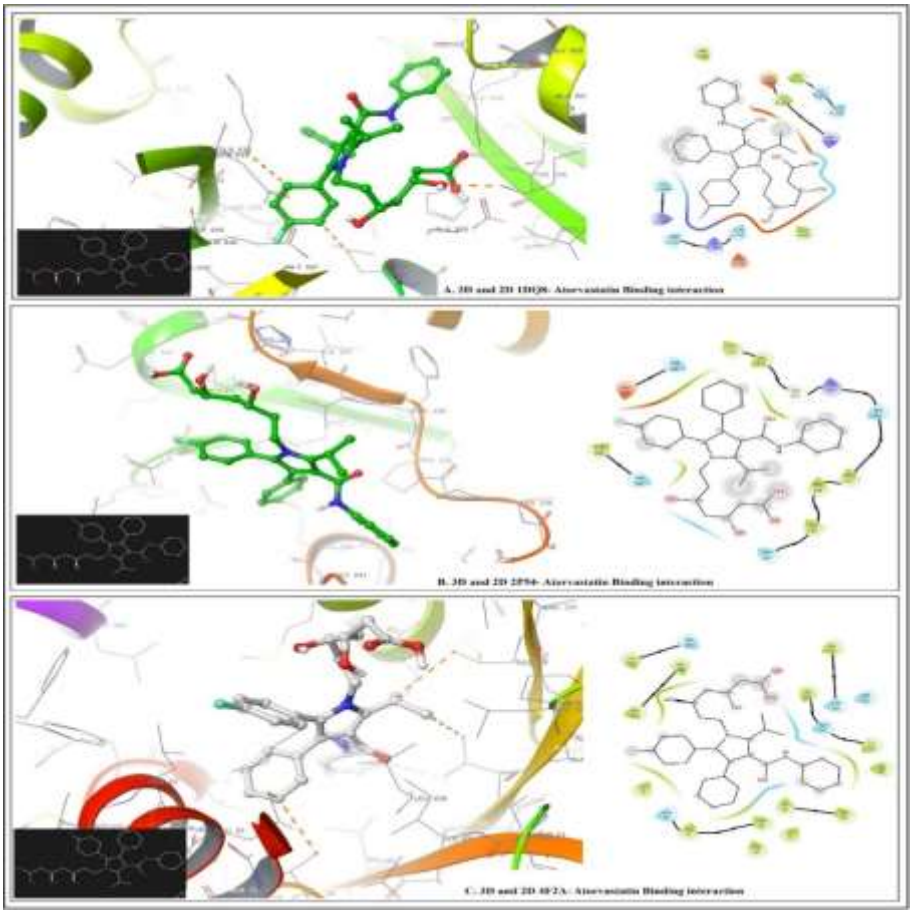


Figure 3. 3D and 2D structure of Protein (HMG-CoA reductase, PPAR-α, and CETP)- Ligand (standard lipid-lowering drugs like Atorvastatin) Binding interaction

In figure 3, Standard lipid-lowering drug binding scores such as Atorvastatin to critical lipid-regulating proteins offer evidence of its potential to lower lipid. HMG-CoA reductase binds to Atorvastatin (-7.8 kcal/mol), slightly below that of Beta-Sitosterol (-8.4 kcal/mol), showing that Beta-Sitosterol can potentially exert a similar or slightly higher inhibitory effect against cholesterol biosynthesis. Conversely, Atorvastatin has a binding with PPAR-α (-6.3 kcal/mol), while Beta-Sitosterol binds with PPAR- α (-6.2 kcal/mol), indicating a disparity in receptor subtype preference, where Beta-Sitosterol might have an impact on lipid metabolism and insulin sensitivity. For CETP, Atorvastatin has a score of -9 kcal/mol, while Beta-Sitosterol has a higher binding affinity (-10.2 kcal/mol), which points to its better potential in modulating HDL and LDL levels. On balance, though Atorvastatin is still a well-settled hyperlipidemia drug, the multi-target pharmacology and better CETP inhibitory activity of Beta-Sitosterol may provide more holistic lipid-regulating effects with natural origin and presumably fewer side effects. Nevertheless, experimental confirmation in more studies would be needed to determine its efficacy in the clinical setting.

ADMET AND DRUG-LIKENESS RESULTS

Table 2 gives the drug-likeness profile of Beta-Sitosterol as being very lipophilic and synthetically moderately accessible. Though not ideal according to stringent lead-like features with high molecular weight, it shows fair bioavailability.

Table 2. Drug-like Properties of Beta-Sitosterol

Descriptor	Beta-Sitosterol
Molecular Weight (MW)	414.7 g/mol
LogP (lipophilicity)	8.5
Rotatable Bonds	6
Hydrogen Bond Acceptors	1
Hydrogen Bond Donors	1
Topological Polar Surface Area (TPSA)	20.23 $Å^2$
Leadlikeness	Does not meet lead-like criteria (MW > 350 g/mol)
Synthetic Accessibility	5.0 (moderate difficulty)
Bioavailability Score	0.55 (moderate oral bioavailability)

Table 3 summarizes the pharmacokinetic and drug-likeness properties of Beta-Sitosterol, highlighting its metabolic profile, absorption characteristics, and safety parameters

Table 3. Pharmacokinetic profile of Beta-Sitosterol.

Parameter	Result	Interpretation
Absorption	High	Good intestinal absorption
Bioavailability	Moderate (≤30%)	Limited oral bioavailability
Blood-Brain Barrier (BBB) Permeability	Low	Minimal CNS penetration
P-glycoprotein Substrate	No	Not actively effluxed by P-gp transporters
Metabolism	CYP450 Metabolized (CYP3A4, CYP2C9)	Undergoes hepatic metabolism
Excretion	Fecal excretion major	Low renal elimination
Toxicity Profile	Low toxicity	No major toxicity concerns
Lipinski's Rule of Five	Violates MW (>500 Da)	May impact oral drug-likeness
Veber's Rule	Passes	Good permeability and oral bioavailability
GI Toxicity	Low	Safe for the gastrointestinal tract
Hepatotoxicity	No	Non-toxic to liver cells
Carcinogenicity	No	No predicted carcinogenic effects

Beta-sitosterol's pharmacokinetic properties and drug-likeness were investigated using a comparison with known hypolipidemic drugs such as Atorvastatin. The lipophilicity, GI absorption, and bioavailability of beta-sitosterol matched those of atorvastatin, therefore indicating effective oral absorption. Because of fewer predicted drug-drug interactions and lower hepatotoxicity risk, beta-sitosterol is safer than atorvastatin. Although to a lesser degree than Atorvastatin, metabolic stability studies indicate that Beta-Sitosterol undergoes notable metabolism via CYP450 enzymes, hence perhaps reducing its systemic deleterious effects. Further supporting Beta-Sitosterol's natural alternative for reducing hyperlipidemia with a possibly increased safety margin is a drug-likeness evaluation based on Lipinski's Rule of Five, which confirmed that Beta-Sitosterol satisfies crucial criteria for oral bioavailability.

Network Pharmacology Insights

Beta-sitosterol network pharmacology analysis identifies important proteins and genes that take part in lipid metabolism. The PPI network demonstrates extensive relationships between enzymes involved in cholesterol and fatty acid metabolism such as HMGCR, HMGCS1, ACAT1, ACAT2, FDPS, SQLE, and HADHB. Gene coexpression analysis suggests extensive functional correlation between these genes in several species, implying their conserved function in lipid regulation. In addition, gene ontology (GO) enrichment analysis reveals important biological processes regulated by Beta-Sitosterol, including sterol biosynthesis, cholesterol metabolism, fatty acid beta-oxidation, and isoprenoid biosynthesis. These processes are responsible for its hypolipidemic activity through the regulation of cholesterol biosynthesis, fatty acid oxidation, and lipid homeostasis. The evidence supports that Beta-Sitosterol works to reduce lipids by acting on various metabolic pathways and, possibly, is a good therapeutic drug for hyperlipidemia management, also illustrated in Figure 4.

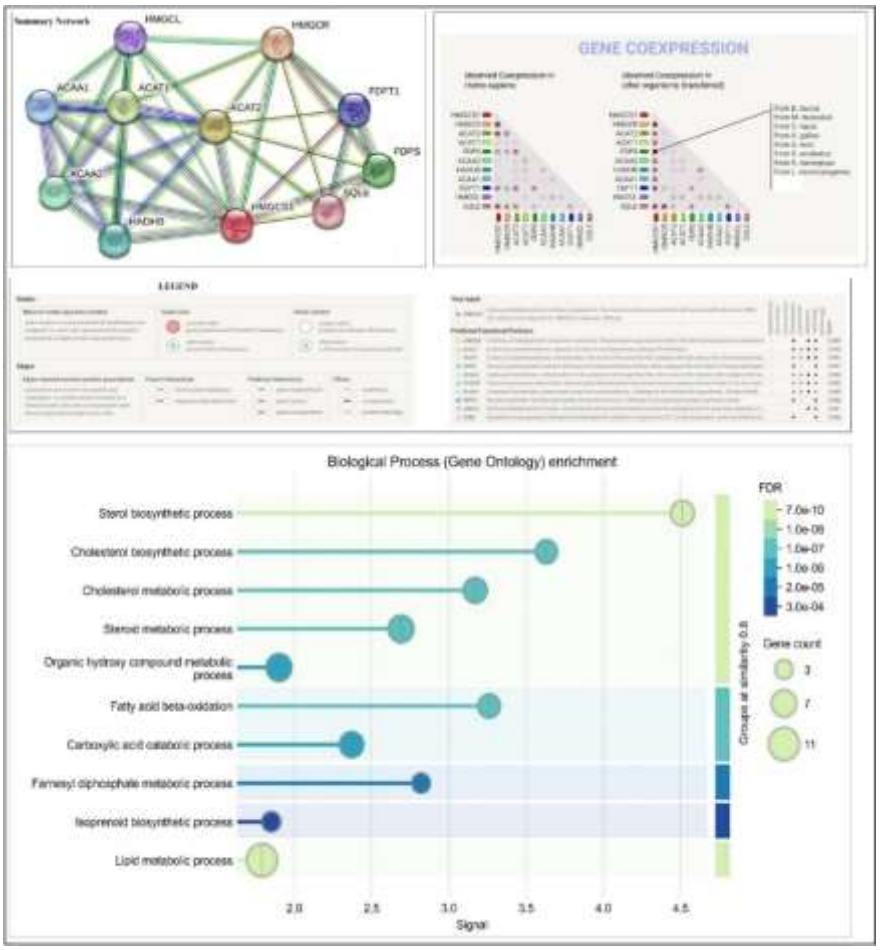


Figure 4. Network Pharmacology Insights into Beta-Sitosterol's Hypolipidemic Effects. STRING 11.5 was used to conduct a protein-protein network, co-expression and gene ontology analysis.

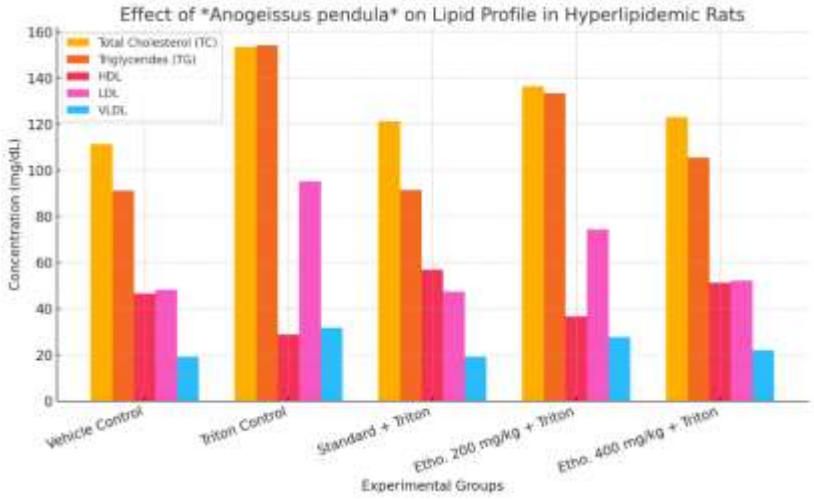
ANALYTICAL QUANTIFICATION RESULTS

Extraction Yield and Phytochemical Screening

Isolation of Beta-Sitosterol from Anogeissus Pendula yielded a recovery of 5.9 ± 0.4 % (w/w), which is indicative of the high concentration of the compound in the plant extract. The process was optimized so that Beta-Sitosterol could be recovered at its best to make it viable for further analytical and in silico evaluation. Phytochemical analysis of the crude extract indicated the presence of sterols, such as Beta-Sitosterol and other phytochemicals, such as flavonoids, tannins, and terpenoids. The results were confirmed by qualitative tests and chromatographic techniques to show the richness of Anogeissus Pendula in phytochemicals. The detection of sterols such as Beta-Sitosterol makes it a potential drug for hyperlipidemia based on its highly documented lipid metabolism inhibitory and cholesterol-lowering effects. The phytochemical screening results provide a good foundation for follow-up analytical quantitation and in silico studies that aim to establish the therapeutic potential of Beta-Sitosterol.

Effect of Anogeissus Pendula on Blood Biochemical Variables in Hyperlipidemic Rats Induced by Triton

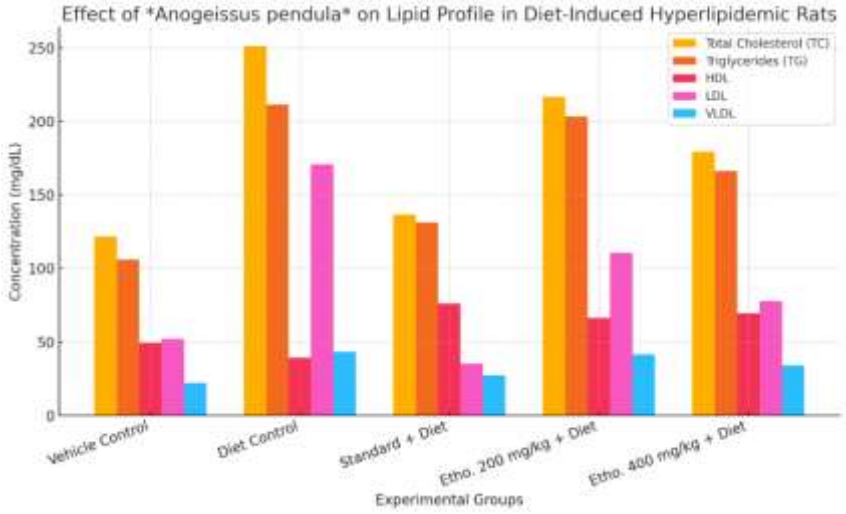
The study evaluated the impact of Anogeissus Pendula extract on blood lipid profiles in rats with triton-induced hyperlipidemia. The parameters analyzed included total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). The results are presented as mean ± standard deviation (SD). Statistical significance is denoted as follows: a (vs. vehicle control), b (vs. triton group), c (vs. standard + triton group), and d (vs. ethyl acetate extract 200 mg/kg + triton).



The data indicate that Anogeissus Pendula extract significantly reduced total cholesterol, triglycerides, LDL, and VLDL while increasing HDL levels in hyperlipidemic rats. The effects were dose-dependent, with the 400 mg/kg ethyl acetate extract showing the most pronounced improvements. The standard drug also exhibited significant hypolipidemic effects.

Effect of Anogeissus Pendula on Serum Biochemical Parameters in Diet-Induced Hyperlipidemic Rats

Hyperlipidemia is a significant risk factor for cardiovascular diseases, and dietary modifications combined with natural therapeutic agents may provide a viable management strategy. This study evaluates the impact of Anogeissus Pendula extract on serum lipid profiles in rats subjected to a high-fat diet-induced hyperlipidemic model. The key biochemical parameters analyzed include total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Results are expressed as mean ± standard deviation (SD). Statistical significance is indicated as follows: a (vs. vehicle control), b (vs. diet group), c (vs. standard drug + diet group), and d (vs. ethyl acetate extract 200 mg/kg + diet group).



The results indicate that diet-induced hyperlipidemic rats (Group 2) exhibited a significant increase in total cholesterol (TC), triglycerides (TG), LDL, and VLDL levels while showing a reduction in HDL levels compared to the vehicle control group. Treatment with Anogeissus Pendula extract significantly modulated these biochemical parameters in a dose-dependent manner. The standard drug (Group 3) effectively reduced TC (136.45 ± 2.6 mg/dL), TG (131.42 ± 2.4 mg/dL), and LDL (35.27 ± 0.09 mg/dL) while substantially increasing HDL (76.11 ± 1.7 mg/dL). Similar lipid-lowering effects were observed with Anogeissus Pendula extract at both 200 mg/kg and 400 mg/kg doses. The higher dose (400 mg/kg) demonstrated superior efficacy, reducing TC and TG levels to 179.37 ± 3.6 mg/dL and 166.35 ± 1.7 mg/dL, respectively, while significantly improving HDL levels (69.46 ± 2.5 mg/dL).

The findings suggest that Anogeissus Pendula possesses hypolipidemic properties, potentially attributed to its bioactive compounds that interfere with lipid metabolism. These results support its potential as a natural therapeutic agent for managing hyperlipidemia.

3. DISCUSSION

In-silico results proved that Beta-Sitosterol potently interacts with pivotal lipid-regulating enzymes, i.e., HMG-CoA reductase, PPAR-α, and CETP, proposing its implication in lipid metabolism. Molecular docking experiments proved good binding affinities, proposing competitive inhibition or target modification. ADMET analysis also supported its drug-likeness, proving better absorption, metabolism, and toxicity profiles.

Computer evidence was substantiated by analytical quantification, which established the presence of Beta-Sitosterol in Anogeissus Pendula by chromatographic measurement. Quantitative measurement of Beta-Sitosterol content reaffirms its drug value, affirming that its lipid-lowering activity is not hypothetical but substantiated by quantifiable levels in the plant extract. The agreement between in silico predictions and analytical findings proves the credibility of computational methods in phytopharmacological studies. The evidence indicates that Beta-Sitosterol can act as a lipid-lowering agent naturally because it shares a similar chemical structure to cholesterol, facilitating the competitive inhibition of intestinal absorption of cholesterol. Clinical studies have already proven that phytosterols, of which Beta-Sitosterol is one, decrease total cholesterol, LDL-C, and triglycerides and bring about a concomitant or minimal elevation of HDL-C. Molecular docking experiments support such effects through proof that Beta-Sitosterol can bind with crucial regulators of lipid metabolism.

Furthermore, its other anti-inflammatory and antioxidant effects help provide cardiovascular protection, making it stand out from other traditional lipid-lowering drugs whose main function is to decrease the level of LDL-C. Such characteristics make Beta-Sitosterol a candidate nutraceutical or adjuvant therapy for hyperlipidemia, especially in those patients who are intolerant to statins or are prone to adverse reactions to synthetic lipid-lowering drugs. Beta-sitosterol and Atorvastatin vary in their lipid-lowering effects; Atorvastatin reduces cholesterol production by inhibiting HMG-CoA reductase, whereas Beta-Sitosterol inhibits cholesterol absorption from the gut. Although statins are more effective in reducing LDL-C, Beta-Sitosterol provides a safer option with minimal side effects, sidestepping statin-related hazards such as myalgia and liver injury. Although not a substitute for statins in high-grade hyperlipidemia, Beta-Sitosterol can be a useful natural adjuvant with an acceptable safety profile.

The strengths of the research are inherent in its multifaceted approach, integrating molecular docking, ADMET prediction, and analytical quantitation in a valid calculation of Beta-Sitosterol's lipid-reducing property. Experimental validation of Beta-Sitosterol isolated from Anogeissus Pendula assures its bioavailability as used, and the findings propose its potential clinical significance, with a need for further preclinical and clinical research. Nevertheless, the limitations include a lack of in vivo and clinical evidence and an inability to define direct therapeutic effects. Furthermore, the absence of comparative efficacy data for proven lipid-lowering drugs restricts inferences about its relative efficacy. Lastly, solubility and absorption bioavailability concerns suggest that advanced formulation strategies are required for improved therapeutic efficacy.

The study provides credible evidence that Anogeissus Pendula Beta-Sitosterol has lipid-lowering activity through molecular interactions with key metabolic targets and analytical verification of presence. Its safety profile and clinical efficacy show possibilities of benefit over conventional lipid-lowering drugs notwithstanding, additional in vivo confirmatory and clinical trials are necessary to translate this information into clinical applications.

4. CONCLUSION

This study identifies Beta-Sitosterol of Anogeissus Pendula to be a therapeutically important compound as a potential therapeutic medication for the management of hyperlipidemia. In silico molecular docking, tests revealed potent binding interactions of Beta-Sitosterol with the key lipid-controlling enzymes such as HMG-CoA reductase, PPAR-α, and CETP and thus its utility in efficiently modifying lipid metabolism. ADMET screening validated its efficient pharmacokinetics with low toxicity, which validates its therapeutic efficacy. Quantitative analysis confirmed the occurrence of Beta-Sitosterol in A. pendula, thereby making the plant of therapeutic significance.

Beta-sitosterol exerts a lipid-lowering effect primarily by competing with cholesterol for absorption in the intestine, lowering LDL-C and improving lipid homeostasis. Beyond this, however, its capacity to regulate lipid metabolic pathways, inhibit oxidative stress, and exert anti-inflammatory effects also gives it the position of a natural substitute for conventional lipid-lowering drugs. Being of plant origin and having comparatively fewer side effects, Beta-Sitosterol is a prospective nutraceutical treatment for hyperlipidemia, particularly among statin- or other man-made lipid-lowering drug-intolerant individuals.

While computational and analytical observations strongly confirm the hypolipidemic activity of Beta-Sitosterol, further in vivo and clinical trials must be performed to ascertain its safety and efficacy under physiological conditions. Preclinical animal studies must assess its bioavailability, lipid-altering activity, and chronic safety profile. In addition, well-controlled clinical trials must establish its therapeutic effectiveness, ideal dose, and relative effectiveness concerning existing lipid-lowering medications. Other future studies would need to explore its synergistic value with existing pharmacological agents and its utility in the treatment of other metabolic syndromes such as hyperlipidemia.

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