

Inhibitory Actions of Ethanolic Fraction of *S. linifolia* L. Leaves on Erectile Dysfunction-Relevant Enzymes in Male Rat Penile Tissue Homogenates: An *ex-vivo* and *in-silico* Approach

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Received: 29.10.2024; Accepted: 11.04.2025; Published: 20.12.2025

Abstract: This study explored the propensity of the ethanolic fraction of *Sida linifolia* leaves (EtOHFSL) to inhibit enzymes linked to erectile dysfunction in rat penile tissue homogenate. The *ex vivo* study was conducted using conventional methods. Molecular docking studies (MDS) were performed using AutoDock Vina. From the result, EtOHFSL at different concentrations (0.2–1.0 mg/ml) demonstrated appreciable inhibitory effects on enzymes linked to ED, such as AChE, arginase, PDE-5, and angiotensin-I converting enzyme (ACE). The inhibition increased with concentration and was at par with respective standard inhibitors (galantamine, L-NOHA, sildenafil, and captopril). Perhaps the observed enzyme-inhibitory actions of EtOHFSL could be attributed to its high polyphenolic content and flavonoid composition. The IC₅₀ value range (0.66–0.84 mg/ml) of EtOHFSL, compared with that of the reference inhibitors (0.53–0.67 mg/ml), indicates moderate enzyme inhibition. MDS revealed that ellagic acid, lunamarin, and rutin, among other compounds identified in EtOHFSL, showed potent inhibitory activity against these enzymes and were comparable to reference inhibitors. The ADMET and toxicity profiles of the studied compounds showed excellent drug candidacy and no potential toxicity, respectively. This preliminary study suggests that *S. linifolia* leaves may possess male sexual-boosting potential and represent a viable source of phytochemicals for therapeutic research.

Keywords: erectile dysfunction; PDE5; arginase; ACE; AChE; polyphenolics.

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1. Introduction

Sexual erection occurs via the participation and interplay of endocrine, vascular, neuronal, and local secretory actions. Both autonomic and somatic nerves are involved in erectile function, and the limbic and hypothalamic pathways are important in this process [1]. Penile erection comprises the dilation of nearby blood vessels, trabecular smooth muscle relaxation, and initiation of the veno-occlusive mechanism of spongiosum and corpus cavernosum tissues [2]. Conversely, erectile dysfunction (ED) refers to the inability of a sexually mature male to attain and sustain a penile tumescence sufficient for desired sexual activity [3]. Basically, men with ED experience difficulty in achieving a proper penile erection to satisfy their sexual needs [4]. Several risk factors have been linked to ED, including neurological, hormonal, and neurovascular disorders, as well as oxidative stress and metabolic syndromes [5]. By implication, ED continues to represent a global human health concern due to its widespread occurrence in both young and old males; about 300 million cases are predicted by 2025 [6].

Clinically, key enzymes, such as angiotensin-converting enzyme (ACE), acetylcholinesterase (AChE), arginase, and phosphodiesterase type 5 (PDE5), are elevated in penile corpus cavernosum tissues of patients with ED [7]. Thus, these enzymes represent therapeutic targets in the management of ED [8]. Basically, ED could result from a decrease in nitric oxide (NO) bioavailability, caused by impairment of endothelial nitric oxide synthase (eNOS) activity and/or elevated NO clearance [9]. Essentially, NOS converts L-arginine to NO, which activates guanylyl cyclase in the smooth muscles, resulting in the generation of 3'-5'-cyclic guanosine monophosphate (cGMP) required for the relaxation of penile smooth muscle [10, 11]. However, rapid breakdown of second messengers, such as cAMP and cGMP, has been linked to ED [12]. Cytosolic concentrations of these metabolites are downregulated by cyclic nucleotide phosphodiesterase [13]. PDE5 occurs primarily in the smooth muscle of the corpora cavernosum. Therefore, modulating PDE5 activity has been used as a therapeutic target in the management of ED [14]. Also, the urea cycle produces ornithine and urea by converting L-arginine, a process catalyzed by ACE. Hence, in cells, NOS and ACE compete for the substrate L-arginine [15].

Furthermore, the renin-angiotensin system (RAS) plays a significant role in the progression of erectile dysfunction. Elevated activity of ACE simultaneously results in the liberation of angiotensin-II, which stimulates tonic contraction of smooth muscles of the corpus cavernosum during penile erection, resulting in ED [16, 17]. Hence, a decrease in ACE activity correlates with improved NOS function and reduced ED. In relation to this, recent studies have shown that inhibiting ACE activity could improve erectile function and simultaneously reduce angiotensin-II levels in patients with ED [7, 18].

Penile erection is a neurovascular event characterized by the dilation of arteries that supply blood to the corpora spongiosum and corpora cavernosa of the penis; at the same time, the smooth muscles of the bulbospongiosus and ischiocavernosus compress the corpora cavernosa veins, preventing blood from exiting the penis and confer extra rigidity of the erected penis [19]. Another target for ED therapy is the inhibition of acetylcholinesterase (AChE), which controls ACh levels and causes NO-dependent relaxation of smooth muscle during the erection process [20]. Penile tissue is a reservoir of the ACh molecule and of cholinergic neurons [21]. However, elevated AChE activity reduces ACh levels by catalyzing its

conversion to choline and acetate [8]. Therefore, it has been proposed that suppressing AChE activity increases ACh levels, thereby improving penile erection and stiffness [22].

The use of natural products and plant-based medicines to manage disease conditions has been strongly advocated in the scientific community. This is due to the growing evidence demonstrating their excellent pharmacological potential and reduced toxicity compared to synthetic remedies [7, 23]. In the same vein, the search for medicinal herbs with sexual-boosting abilities may represent an inexpensive, reliable, and alternative remedy to male sexual dysfunctions since synthetic drugs used to manage sexual disorders are not without several adverse effects [18]. Inhibitors of enzymes linked to ED are reported to cause a variety of adverse effects, including nasal congestion, headache, dyspepsia, and visual impairment. In a typical African civilization, herbs with established traditional pharmacopeias and folklore claims are abundant and used for nourishment and disease management.

Furthermore, the use of plants as food or medicine is an important element of most African beliefs and cultures [24]. Several species of the genus *Sida* have been shown to contain a wealth of pharmacologically active phytochemicals with several health benefits. Some representative species include *S. cordifolia*, *S. rhombifolia*, *S. acuta*, *S. tiagii*, *S. corymbosa*, and *S. linifolia* [25].

Sida linifolia L., also referred to as flax leaf fanpetals, is a plant of the Malvaceae family and Sidagenus that thrives abundantly in dry woodland areas of Sub-Saharan Africa (particularly in Nigeria and Sierra Leone), North America, as well as in South America. The plant is called Yaya in Hausa (Nigeria), kpawulo (Sierra Leone), Trebol sabanero (Venezuela), flor de Sabana (Dominican Republic), and Guanxuma (Brazil) [26]. Preliminary studies on the plant leaf extract have been documented, including its ethnopharmacological relevance and biological activities, such as antioxidant, anti-inflammatory, antimalarial, neuromodulatory, and aphrodisiac properties, as well as its rich phytochemistry [27-30]. Some botanicals of the *Sida* genus have also been reported to exhibit potent therapeutic potential in managing male sexual dysfunction [31, 32]. However, information on the plant species' possible sexual-boosting potential is limited. Due to several ethical considerations associated with the use of laboratory animals at the early stages of drug discovery, investigators are encouraged to use *in vitro* or *ex vivo* studies that limit animal use while still affording rich insights into cellular responses [7, 8, 18]. Moreover, in the case of ED, it is difficult to directly assay the inhibition of these culprit enzymes (arginase, PDE5, ACHE, and ACE) in the biological system due to their complex nature [5, 7]. Perhaps synchronous and uncontrollable events, such as hormonal and immune responses, as well as the absorption, distribution, metabolism, and excretion of enzyme inhibitors, may influence the experimental outcome, making it difficult to accurately interpret the effects of this enzyme inhibition on ED pathology *in vivo* [8]. Conversely, *ex vivo* studies are conducted on isolated tissues or organs and allow better control over experimental conditions while retaining the natural architecture, drug-tissue interactions, and tissue functions [18]. This could afford a closer approximation of drug concentration, enzyme activity, and tissue responsiveness, and of how an enzyme inhibitor might work *in vivo*, without the complexities of a whole organism. In addition, *ex vivo* ED-related enzyme-inhibition studies have recently been reported [5, 7, 8, 18]. Hence, it may be more rational to use *in vitro/ex vivo* assays in the initial screening of medicinal herbs for their potential pharmacological properties. Herein, the propensity of the hydroalcoholic fraction of *S. linifolia* leaves to inhibit enzymes relevant to ED was investigated in rat penile tissue homogenate.

2. Materials and Methods

2.1. Chemicals and reagents.

The chemicals employed in the present study were procured from reputable merchants and dealers of scientific equipment and chemicals, and were of analytical grade.

2.2. Collection and identification of the plant.

The leaves of the *S. linifolia* plant were harvested fresh from grazing land in Enugu. The collected leaves were air-dried in a room that was out of the sun's reach. Plant identification and authentication (Vouch. No. BDCP20220704) was carried out by a botanist stationed at the Bio-Resources Development and Conservation Program (BDCP) research facility. A sample of the plant leaves was kept in the herbarium for easy future retrieval.

2.3. Extraction and fractionation procedures.

Powdered *S. linifolia* leaves were subjected to extraction and fractionation procedures following conventional methods [33, 34], respectively. Dried *S. linifolia* leaves were pulverized with an industrial blender, and 3.5 kg of powdered plant sample was macerated in 4 Liters of ethanol (98 %) for 72 hours, then filtered with Whatman Number 1 filter paper. Afterward, a rotary evaporator operating at low pressure and 45°C was utilized to concentrate the resultant crude extract. Thereafter, a silica gel-equipped fractionating column was used to fractionate the resulting concentrated leaf extract with varying solvents of increasing polarity (n-hexane, ethyl acetate, and ethanol). The resulting fractions were separately concentrated by evaporation (under similar conditions) and then preserved in sterilized, appropriately labeled screw-capped jars at 3 - 5°C until further studies. The study adopted the ethanol leaf fraction for further analysis. The choice of plant fraction is consistent with the previous method reported [30].

2.4. Gas chromatography-flame ionization detection (GC-FID) analysis.

2.4.1. Sample preparation.

The procedure documented by Kelly and Nelson [35] was deployed in this study. One gram of the test sample was dispensed into a test tube, and measured volumes of KOH (10 mL, 50 % (m/v)) and ethanol (15 mL) were introduced into the test tube. The reaction mixture was left to incubate at 60°C in a water bath for 60 min. Then, the test tube content was transferred into a separatory funnel. Washing of the test tube was done in successive rounds using 20 mL of ethanol, cold water (10 mL), 10 mL of hot water, and hexane (3 mL), and collectively dispensed into the funnel. Thereafter, a volume of dilute ethanol solution (10 mL 10 % (v/v), was used to wash thrice. Following this, anhydrous sodium sulfate was introduced to dry the resultant mixture, and then solvent evaporation ensued. A volume of pyridine (1000 mL) was used to dissolve the resultant sample, after which a portion (200 mL) was dispensed into the vial for study.

2.4.2. GC-FID quantification.

A flame ionization detector-equipped BUCK M910 Gas chromatography system was utilized in screening and quantifying the various phytochemicals present in the sample. The column used in the study was a RESTEK 15-meter MXT-1 1 (15 m × 250 mm × 0.15 mm)

column. The study also deployed 5.0 psi of Helium, with a 40 mL/min flow rate, to serve as the carrier gas. The injector temperature was 280°C with a splitless injection of 2 mL of sample and a linear velocity of 30 cm/s. The initial operating temperature of the oven was 200°C, and it was heated to 330°C at a rate of 3°C min⁻¹, which was maintained for 5 min. The detector was operated at a temperature of 320°C. Analysis of the phytochemical composition of plant material was performed by evaluating the area and mass ratio of internal standards compared to those of the identified phytochemicals [35]. The various concentrations of the phytochemicals were presented in mg/g (which equals ppm and mg/mL).

2.5. Animal handling.

Four (4) male adult Wistar albino rats with a weight range of 225 ± 15 g, aged 8–12 weeks old, were procured from the University Animal housing unit, Department of Zoology and Environmental Biology. Animals were handled in ways consistent with the guidelines enshrined in the Manual for the Care and Use of Laboratory Animals, issued by the National Institutes of Health and the National Academy of Sciences (USA). Ethical procedures were strictly adhered to, in accordance with the National and Institutional ethical guidelines, to ensure the safety and welfare of experimental animals during the experiments. Animals were left to acclimatize for 2 weeks in well-ventilated cages kept at room temperature, under proper laboratory conditions (12 h light/dark cycle), and allowed free access to Vital animal chow and water *ad libitum*. Thereafter, they were anesthetized with Diethyl ether and euthanized via cervical dislocation, and the penile tissues were harvested, washed, dried, and weighed. The preparation of tissue homogenate to obtain crude enzymes for enzyme inhibition studies was performed following the method of Ojo *et al.* [18].

2.6. Phosphodiesterase-5 (PDE5) inhibition assay.

The inhibitory activity of EtOHFSL on PDE5 was assayed using the methods outlined by Oboh [7]. Briefly, the penile tissue was mixed with cold saline (1/10 w/v) and homogenized at 1200 rpm. Then, it was centrifuged for 10 minutes at 3000 rpm. The resultant supernatant served as a crude enzyme source and was used in the enzyme inhibition assay. The reaction solution, which comprised 5 mM p-nitrophenyl phenyl phosphonate (substrate), 100 µl of penile tissue supernatant (crude extract), Tris-HCl (20 mM, pH 8.0), and EtOHFSL (0.2-1.0 mg/ml) or sildenafil, was incubated for 10 minutes at 37°C. The quantity of p-nitrophenol generated was measured as a change in absorbance at 5 min of reaction using a spectrophotometer set at 400 nm. The control solution (Contr) was prepared similarly, but it was void of EtOHFSL and sildenafil. The percentage inhibition of PDE5 activity exerted by EtOHFSL was evaluated using the formula below:

$$\% \text{ Enzyme Inhibition} = 1 - \frac{\text{Absorbance of Test}}{\text{Absorbance of Contr}} \times 100 \quad (1)$$

2.7. Arginase inhibition assay.

The inhibitory potential of EtOHFSL on arginase activity in penile tissue homogenate was ascertained according to the procedures outlined by Adefegha [36]. Briefly, to prepare the penis and testicular homogenates, a known weight (10 g (w/v)) of penile tissue was homogenized in a cold phosphate buffer of pH 7.2. The resultant homogenate was centrifuged for 20 minutes at 4000 rpm. Thereafter, the resultant supernatant was adopted as the source of

enzymes. The amount of urea produced upon the addition of Ehrlich's reagent was used to measure arginase activity. The reaction medium comprised 1.0 mM MnCl₂, 0.1 mM arginine, Tris-HCl buffer (1.0mM, pH 9.5), and 1.0 mL of EtOHFSL (0.2-1.0 mg/ml) or LNOHA (L-2-amino-[4-(20-hydroxyguanidino)] butyric acid). After incubating for 10 minutes at a temperature of 37°C, the solution was quenched by introducing 2.5 mL of Ehrlich reagent (p-dimethyl amino benzaldehyde (2.0 g) in a volume (20 mL) of strong hydrochloric acid and distilled water (100 mL). Spectrophotometric reading was recorded at 450 nm after a reaction time of 20 min. The control experiment was done without EtOHFSL or LNOHA. The % inhibitory action of EtOHFSL on arginase activity was evaluated using the formula in Eq. 1;

2.8. *Acetylcholinesterase inhibition assay.*

The propensity of EtOHFSL in inhibiting acetylcholinesterase (AChE) activity in penile tissue homogenate was assayed following the method described by Akomolafe [8]. Briefly, to obtain crude AChE enzyme preparation, penile tissue samples were homogenized in cold phosphate buffer (0.1 M, pH 7.2). The inhibitory effect of EtOHFSL, or standard compound galanthamine, on AChE activity was determined at different concentrations (0.2 - 1.0 mg/ml). The reaction medium comprised 200 μ L penile tissue homogenate, 100 μ L of 3.3 mM 5,5-dithiol-bis (2-nitrobenzoic) acid (DTNB) in phosphate-buffered solution (0.1 M, pH 7.0), comprising NaHCO₃ (6 mM), and EtOHFSL or galanthamine. The solution thus formed was incubated for 20 min at 25°C before the addition of acetylthiocholine iodide. Thereafter, spectrophotometric readings at 412 nm were recorded. The % AChE inhibition exerted by EtOHFSL at various concentrations was evaluated using the formula in Eq. 1;

2.9. *Angiotensin-I-converting enzyme (ACE) inhibition assay.*

The ability of EtOHFSL to inhibit ACE activity was assayed using the procedure outlined by Akomolafe [37]. The plant material or captopril at varying concentrations (0.2 - 1.0 mg/ml) was mixed with 50 μ L of penile tissue homogenate (4 mU/mL) and pre-incubated for 15 minutes at 37°C. Briefly, to initiate the reaction, a volume (200 μ L) of 8.33 mM ACE substrate [hippuryl-L-histidyl-L-leucine (HHL)] dissolved in Tris-HCl buffer (125 mM, pH 8.3) was introduced into the reaction medium and allowed to incubate at 37°C for 30 min. Thereafter, a volume (300 μ L) of HCl (1 M) was added to the resultant solution to quench the reaction. The reaction produced hippuric acid (Bz-Gly), which was extracted with a known volume (2 mL) of ethyl acetate and centrifuged to remove the ethyl acetate layer. The mixture was then transferred to a volumetric flask and evaporated to dryness. Thereafter, a volume of distilled water was used to reconstitute the residue, and the solution was monitored at 228 nm. Essentially, the control mixture was prepared similarly, except that the solution was void of EtOHFSL or captopril. The % inhibition of ACE activity was evaluated using Eq. (1).

2.10. *In silico studies.*

2.10.1. Protein retrieval.

The crystal structures of Human Arginase (PDB ID: 3GMZ), Human Acetylcholinesterase (PDB ID: 4EY7), Human Phosphodiesterase 5A (PDB ID: 1XOZ), and Human Angiotensin Converting Enzyme (PDB ID: 1O8A) were retrieved from the Protein

Data Bank (PDB) (Additional file 1: Figure S1). Cleaning and refinement of the retrieved protein structures were performed using Discovery Studio.

2.10.2. Ligand retrieval.

The 3D structures of some selected phytochemicals identified in EtOHFSL (epicatechin, flavone, rutin, kaempferol, lunamarin, naringenin, ellagic acid, quercetin, and chlorogenic acid) and four FDA-approved drugs/compounds (Sildenafil, L-NOHA, Galantamine, Captopril) were downloaded from the PubChem database as SDF files. The Pymol software [38] was used to convert these SDF files to PDB format for molecular docking.

2.10.3. Protein and ligand preparation.

The AutoDock tools were used to prepare the ligands and target proteins, which entailed removing bound complexes and water molecules, adding charges and polar hydrogens, and setting up the grid. The Molecular Operating Environment was used to perform the active site investigation [39].

2.10.4. Molecular docking.

A molecular docking study was performed using AutoDock Vina [40]. The Pymol software [38] was used to generate 2D (Figure 6) and 3D (surface) views of the protein-ligand interactions (see Additional file 1: Figure S3a-d). In addition, the physicochemical attributes of the studied compounds, including solubility, pharmacokinetics, lipophilicity, and Lipinski drug-likeness, were determined using Protox and SwissADME Server [41].

2.11. Statistical analysis.

One-way and two-way ANOVA were used to analyze the study data using Statistical Product and Service Solutions (version 23.0, Chicago, Illinois, USA). The results from descriptive tables were represented as mean \pm standard deviation. Significant differences in values across the various groups were described using the Duncan *post hoc* table. The level of Significance was established at $p < 0.05$. GraphPad Prism version 6.5 (GraphPad Software, Inc., California, USA) was used in the results presentation.

3. Results and Discussion

3.1. Results.

3.1.1. GC-FID profile of EtOHFSL.

The phytochemical composition of EtOHFSL, as determined by GC-FID analysis, is presented in Table 1. From the result, different concentrations of pharmacologically relevant phytochemicals were present, such as epicatechin (290.27 ppm), flavonoids (222.239 ppm), steroids (358.4 ppm), tannins (5.6680 ppm), catechins (10.1884 ppm), cyanogenic glycosides (10.8555 ppm), rutin (13.5812 ppm), saponins (3.4794 ppm), kaempferol (9.0841 ppm), cardiac glycosides (7.8854 ppm), oxalate (85.9552 ppm), lunamarine (14.0509 ppm), naringenin (9.3649 ppm), flavonones (7.5546 ppm), phytate (7.1886 ppm), proanthocyanins (52.0734 ppm), flavan-3-ols (3.8774 ppm), spartein (8.2814 ppm), and resveratrol (1.9882 ppm) were present in EtOHFSL. The chromatogram of the GC-FID depicting the

phytochemical profile of EtOHFSL is shown in Figure 1. (see Additional file 1: Figure S2 for HPLC data)

Table 1. Gas chromatography-flame ionization detector (GC-FID) phytochemical profile of ETOHFSL.

| Compounds | Retention time (min) | Peak area | Peak height | Conc. (ppm)* | Formula | Class of compounds | MW (g mol ⁻¹) |
|-----------------------|----------------------|------------|-------------|--------------|---|-------------------------|---------------------------|
| Epicatechin | 34.600 | 5872.83 | 109.164 | 290.27 | C ₁₅ H ₁₄ O ₆ | Flavonoids | 32.8801 |
| Flavone | 10.366 | 19532.12 | 355.819 | 222.239 | C ₆ H ₄ OC ₃ HO | Flavonoids | 14.5446 |
| Steroids | 22.730 | 9444.27 | 173.278 | 358.4 | C ₁₉ H ₂₈ O ₂ | Steroids | 12.8502 |
| Tannins | 7.470 | 8487.2413 | 661.982 | 5.6680 | C ₇₆ H ₅₂ O ₄₆ | Tannins | 11.1121 |
| Catechin | 2.390 | 12421.8376 | 963.612 | 10.1884 | C ₁₅ H ₁₄ O ₆ | Flavonoid | 10.8069 |
| Cyanogenic glycosides | 27.536 | 11537.7968 | 889.916 | 10.8555 | C ₁₀ H ₁₇ NO ₆ | Glycosides | 10.6995 |
| Rutin | 6.016 | 18238.4729 | 1390.783 | 13.5812 | C ₂₇ H ₃₀ O ₁₆ | Flavonoids | 10.6547 |
| Sapogernin | 15.460 | 4978.4178 | 390.100 | 3.4794 | C ₅₁ H ₈₂ O ₂₁ | Saponins | 10.5154 |
| Kaempferol | 25.650 | 10090.3523 | 784.201 | 9.0841 | C ₁₅ H ₁₀ O ₆ | Flavonoids | 9.0087 |
| Cardiac glycosides | 12.970 | 6253.5744 | 489.557 | 7.8854 | C ₁₄ H ₆₄ O ₁₃ | Steroidal glycosides | 8.7167 |
| Oxalate | 36.876 | 6997.3518 | 539.543 | 5.9552 | C ₂ O ₄ ⁽²⁻⁾ | Oxalate | 8.5897 |
| Lunamarin | 0.206 | 6102.8514 | 493.551 | 14.0509 | C ₁₈ H ₁₅ NO ₁₄ | Glycoside | 8.2778 |
| Naringenin | 17.963 | 11350.8426 | 881.534 | 9.3649 | C ₁₅ H ₁₂ O ₅ | Flavonoids | 8.2466 |
| Flavonones | 20.316 | 12766.214 | 888.829 | 7.5546 | C ₁₅ H ₁₀ O ₂ | Flavonoids | 7.3861 |
| Phytate | 29.860 | 5485.4772 | 430.055 | 7.1886 | C ₆ H ₁₈ O ₂₄ P ₆ | Steroids | 5.8161 |
| Proanthocyanin s | 42.276 | 3509.9406 | 275.546 | 2.0734 | C ₃₁ H ₂₈ O ₁₂ | Phenols | 5.0795 |
| Flavo-3-ol | 4.120 | 6544.0460 | 511.833 | 3.8774 | C ₁₅ H ₁₄ O ₂ | Flavonoids | 4.6501 |
| Sparte in | 10366 | 19619.6586 | 1493.612 | 8.2814 | C ₁₅ H ₂₆ N ₂ | Alkaloids | 2.0607 |
| Resveratrol | 39.200 | 10236.5203 | 788.825 | 5.8222 | C ₁₄ H ₁₂ O ₃ | Phenols | 1.9882 |
| Epihedrine | 44.170 | 10548.9686 | 819.790 | 32.4385 | C ₁₀ H ₁₅ NO | Phenethylamine alkaloid | 165.24 |

* 1 ppm = 10⁻¹ mg/100g = 1 µg/g = 1 µg/ml = 1 mg/L

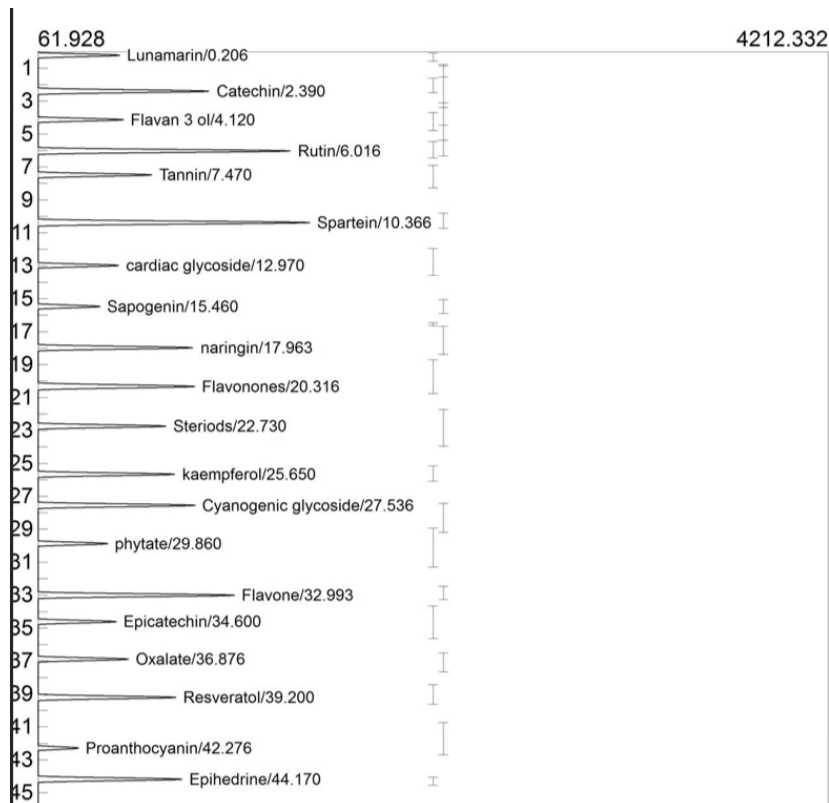


Figure 1. Chromatogram of the GC-FID depicting the phytochemicals in EtOHFSL.

3.1.2. Inhibitory effect of EtOHFSL on PDE5 activity.

The inhibition action of EtOHFSL on PDE5 activity is shown in Figure 2. The results showed that EtOHFSL demonstrated moderate inhibitory activity on PDE5, with effects dependent on concentration and comparable to those of the standard drug (sildenafil). At low doses (0.2, 0.4, and 0.6 mg/ml), EtOHFSL displayed moderately (< 50 %) effective inhibition of PDE5 activity (30.11 ± 0.51 , 34.83 ± 0.52 , and 45.57 ± 0.51 %), respectively, which was inferior ($p < 0.05$) to that produced with sildenafil (34.10 ± 0.14 , 43.93 ± 0.43 , and 54.53 ± 0.65), at the respective concentrations. Nevertheless, EtOHFSL displayed relatively higher inhibition of PDE5 activity (59.20 ± 0.55 and 64.69 ± 0.51 %) at high concentrations (0.8 and 1.0 mg/ml), respectively; yet, at these concentrations, sildenafil showed superior ($p < 0.05$) inhibitory potential in PDE5 activity (61.75 ± 1.67 and 72.25 ± 0.58 %), when compared to EtOHFSL. The peak % PDE5 inhibition was produced at the highest assayed concentration (1.0 mg/ml) of the leaf fraction. Additionally, a higher IC_{50} value of 0.66 mg/ml was recorded for EtOHFSL compared to sildenafil, with a relatively lower IC_{50} (0.53 mg/ml).

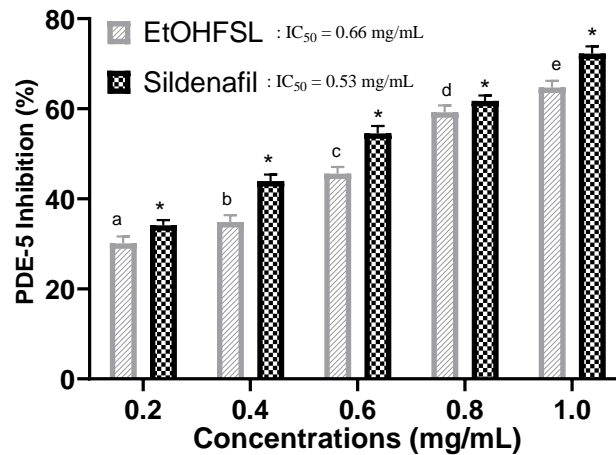


Figure 2. Inhibitory effect (%) of EtOHFSL on phosphodiesterase-5 (PDE5) activity. Results (n=3) are expressed as mean \pm standard deviation. Columns with different small case alphabets are significantly ($p < 0.05$) different, whereas subsets of each twin column having an asterisk (*) are different ($p < 0.05$).

3.1.3. Inhibitory effect of EtOHFSL on ACE activity.

The suppressive action of EtOHFSL on ACE relative to a reference anti-ACE drug (captopril) is presented in Figure 3. From the results, EtOHFSL effectively suppressed ACE activity in a manner that was positively proportional to the concentrations of the test samples and was on par with captopril. At lower concentrations (0.2 and 0.4 mg/ml), EtOHFSL exerted moderate (< 50 %) inhibition (28.28 ± 1.24 and 41.97 ± 5.61 %) on ACE activity, respectively. However, at similar concentrations, the % inhibitory action of captopril on ACE was significantly ($p < 0.05$) lower (25.38 ± 0.71 %) at 0.2 mg/ml but significantly ($p < 0.05$) higher (48.14 ± 1.20 %) at 0.4 mg/ml, compared to EtOHFSL. Furthermore, the degree of inhibition exerted by EtOHFSL on ACE activity (51.24 ± 1.48 , 72.36 ± 1.07 , and 76.57 ± 1.17 %) improved markedly ($p < 0.05$) at higher concentrations (0.6, 0.8, and 1.0 mg/ml), respectively; however, at these concentrations, captopril demonstrated superior ($p < 0.05$) ACE inhibition (59.95 ± 1.67 , 74.91 ± 1.69 , and 85.85 ± 2.51 %). From the results, the maximum anti-ACE activity of EtOHFSL occurred at 1.0 mg/mL. Notably, the IC_{50} value of EtOHFSL (0.76 mg/ml) was higher than that of captopril (0.60 mg/ml).

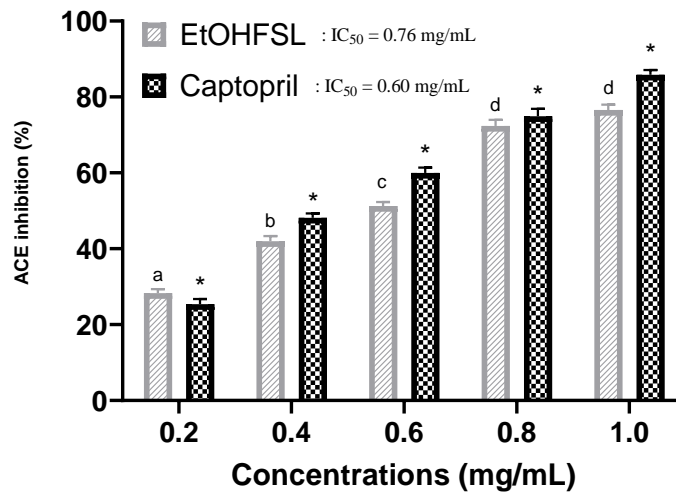


Figure 3. Inhibitory effect (%) of EtOHFSL on angiotensin-I-converting enzyme (ACE) activity. Results (n=3) are expressed as mean \pm standard deviation. Columns with different small case alphabets are significantly ($p < 0.05$) different, whereas subsets of each twin column having an asterisk (*) are different ($p < 0.05$).

3.1.4. Inhibitory effect of EtOHFSL on arginase activity.

The inhibitory potential of EtOHFSL on arginase activity is presented in Figure 4. From the results, EtOHFSL effectively inhibited arginase activity in a concentration-dependent manner and was comparable to LNOHA, a standard arginase inhibitor. At low concentrations (0.2 and 0.4 mg/ml), EtOHFSL demonstrated moderate ($< 50\%$) arginase inhibition (33.52 ± 0.30 and $38.24 \pm 0.36\%$), respectively; however, LNOHA showed superior ($p < 0.05$) % arginase inhibition (36.00 ± 0.43 and $40.67 \pm 1.92\%$) at similar concentrations, respectively. Furthermore, at high concentrations (0.6, 0.8, and 1.0 mg/ml), the inhibitory effect of EtOHFSL on arginase activity (45.38 ± 0.73 , 53.71 ± 0.43 , and $59.24 \pm 0.36\%$) improved significantly ($p < 0.05$), respectively; yet, the standard arginase inhibitor LNOHA demonstrated superior ($p < 0.05$) arginase inhibition (45.43 ± 0.57 , 52.29 ± 0.43 and $64.48 \pm 0.36\%$), at similar concentrations. According to the result, the peak % arginase inhibition exerted by EtOHFSL was recorded at 1.0 mg/ml (the highest concentration); however, even at this concentration, LNOHA exhibited superior ($p < 0.05$) arginase inhibition. Additionally, a higher IC_{50} value (0.72 mg/ml) was observed for EtOHFSL compared to LNOHA ($IC_{50}=0.67$ mg/ml).

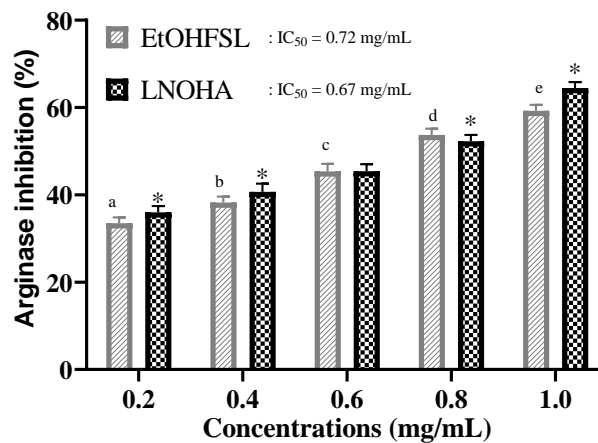


Figure 4. Inhibitory effect (%) of EtOHFSL on arginase activity. Results (n=3) are expressed as mean \pm standard deviation. Columns with different small case alphabets are significantly ($p < 0.05$) different, whereas subsets of each twin column having an asterisk (*) are different ($p < 0.05$).

3.1.5. Inhibitory effect of EtOHFSL on AChE activity.

The inhibitory potential of EtOHFSL on AChE activity is presented in Figure 5. From the results, EtOHFSL effectively inhibited AChE activity in a concentration-dependent manner, similar to galanthamine, a reference AChE inhibitor. Moderate (< 50 %) AChE % inhibition (35.14 ± 1.42 , 39.99 ± 1.28 , 42.99 ± 1.46 and 49.94 ± 1.41 %) was produced with EtOHFSL at low and mid concentrations (0.2, 0.4, 0.6, and 0.8 mg/ml), respectively; however, galanthamine showed superior ($p < 0.05$) % AChE inhibition (34.01 ± 1.34 , 41.23 ± 1.17 , 50.06 ± 1.41 and 65.09 ± 1.99 %) at similar concentrations, respectively. Furthermore, at the maximum concentration (1.0 mg/ml), EtOHFSL exerted a more potent (>50%) inhibitory action on AChE activity (53.74 ± 1.64 %); the standard AChE inhibitor, galanthamine, demonstrated superior ($p < 0.05$) AChE inhibition (68.85 ± 1.23 %) at similar concentrations. According to the result, the peak % AChE inhibition exerted by EtOHFSL was recorded at 1.0 mg/ml (the highest concentration); however, even at this concentration (1.0 mg/ml), galanthamine showed stronger ($p < 0.05$) AChE inhibition. Additionally, a higher IC_{50} value (0.84 mg/mL) was observed for EtOHFSL compared to LNOHA ($IC_{50} = 0.56$ mg/mL).

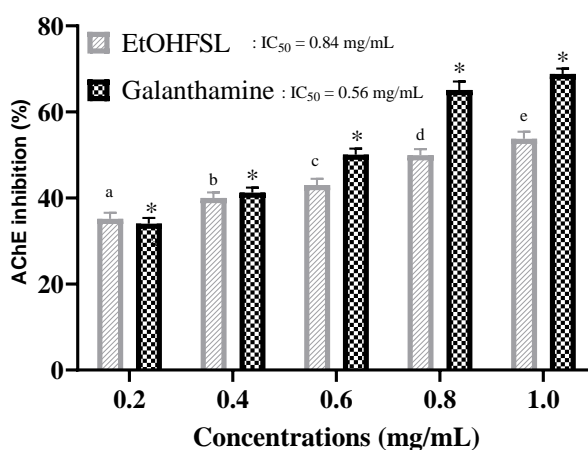


Figure 5. Inhibitory effect (%) of EtOHFSL on acetylcholinesterase (AChE) activity. Results (n=3) are expressed as mean \pm standard deviation. Columns with different small case alphabets are significantly ($p < 0.05$) different, whereas subsets of each twin column having an asterisk (*) are different ($p < 0.05$).

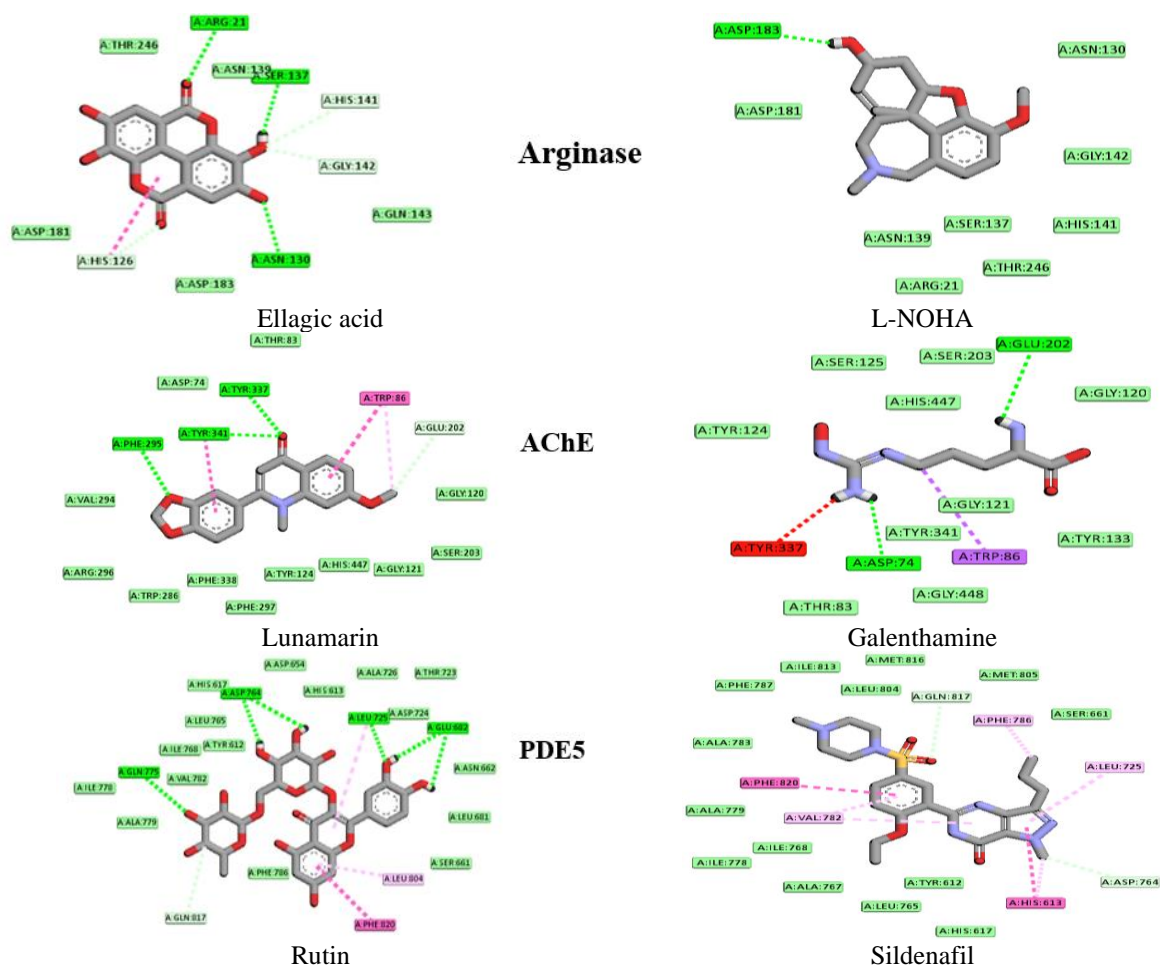
3.1.6. Molecular docking of identified compounds of EtOHFSL against selected erectile dysfunction-relevant proteins.

According to molecular docking studies, the selected compounds identified in EtOHFSL with GCFID and HPLC (see Additional file 1: Figure S2) demonstrated strong inhibitory activity against selected erectile dysfunction-relevant proteins, owing to their high binding affinities (low binding energies), as shown in Table 2. Ellagic acid, lunamarin, kaempferol, rutin, naringenin, chlorogenic acid, epicatechin, flavone, and quercetin exhibited considerably high inhibitory effects across all the studied proteins (targets) (acetylcholinesterase, arginase, ACE, and PDE5) when compared to their respective standard drugs (galantamine, L-NOHA, captopril, and sildenafil). Their active-site interactions via several types of bonding are highlighted in Figure 6. Ellagic acid, lunamarin, and rutin competed favorably in inhibiting arginase, AChE, and PDE5, respectively, when compared to their corresponding reference compound (L-NOHA, galantamine, and sildenafil). Also, rutin showed a superior inhibitory effect on ACE compared with captopril (the reference drug). In addition, the 2D diagrams and active-site view interactions between the identified compounds

in EtOHFSL and the target proteins are shown in Figure 6. In the diagram, varying forms of interaction through different bonding were observed between the functional groups of the studied compounds and the active-site amino acids of the target proteins. For instance, the hydroxyl groups of ellagic acid were found to interact with Arg 21, ASN 130, and SER 137 of Arginase via a conventional hydrogen bond, while the benzene ring of ellagic acid exhibited van der Waals interactions with HIS 126. The rest of the interactions are indicated in the attached legend.

Table 2. Binding affinities of protein-ligand interactions (Kcal/mol).

| Ligands | Arginase | Acetylcholinesterase | Phosphodiesterase-5 | ACE |
|------------------|----------|----------------------|---------------------|-------|
| Epicatechin | -6.9 | -10.0 | -8.7 | -8.3 |
| Flavone | -7.0 | -10.4 | -9.2 | -8.0 |
| Rutin | -7.0 | -9.9 | -10.1 | -10.4 |
| Kaempferol | -7.3 | -9.3 | -8.9 | -8.4 |
| Lunamarin | -7.8 | -10.7 | -9.3 | -9.0 |
| Naringenin | -6.9 | -10.3 | -9.0 | -8.3 |
| Ellagic Acid | -8.1 | -10.5 | -9.2 | -8.2 |
| Quercetin | -7.9 | -10.2 | -8.7 | -8.6 |
| Chlorogenic Acid | -7.2 | -9.8 | -8.9 | -8.5 |
| Sildenafil | --- | --- | -9.4 | --- |
| Captopril | --- | --- | --- | -5.4 |
| Galantamine | --- | -6.5 | --- | --- |
| L-NOHA | -6.4 | --- | --- | --- |



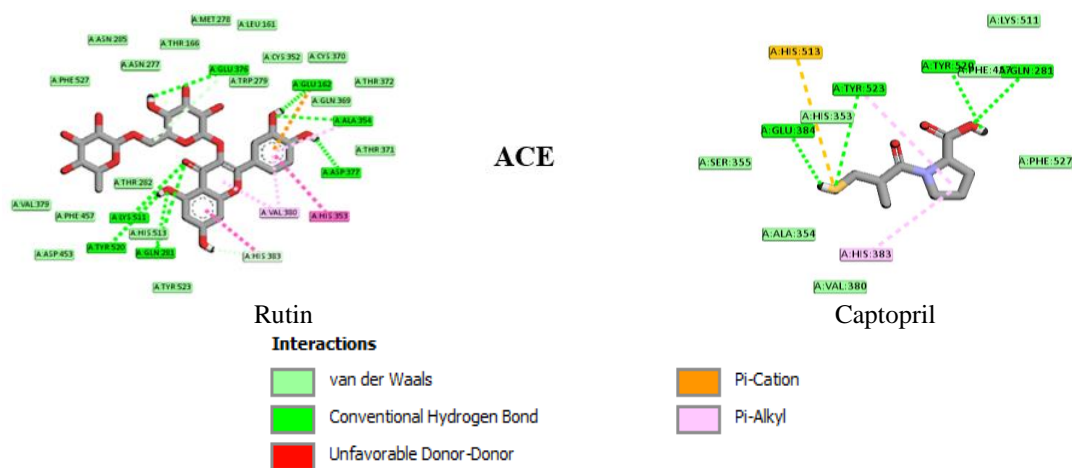


Figure 6. Active site views of Protein-Ligand interaction of top-scoring selected compounds in EtOHFSL and standard drugs showing various types of interactions.

3.1.7. Drug likeness and pharmacokinetics of selected EtOHFSL phytochemicals.

The drug-likeness and pharmacokinetic properties (ADMET analysis) of some selected phytochemicals identified in EtOHFSL and standard drugs were analyzed. The Physicochemical, lipophilicity, solubility, pharmacokinetics, and Lipinski drug-likeness properties predicted by SwissADME and PROTOX are shown in Table 3. All top-scoring compounds of EtOHFSL had good properties as drug candidates and also complied with the Lipinski Rule of Five, except rutin (MW > 500, NorO > 10, NHorOH > 5). Results of the PROTOX-predicted toxicity profile study also showed no toxicity for most of the studied compounds.

Table 3. Drug likeness and pharmacokinetic properties of selected EtOHFSL phytochemicals.

| Parameters | Epicatechin | Flavone | Rutin | Kaempferol | Lunamarin | Naringenin | Ellagic Acid | Quercetin | Chlorogenic Acid |
|-------------------------------------|------------------|--------------------|---|------------------|--------------------|------------------|------------------|------------------|----------------------------|
| Molecular weight(g/mol) | 290.27 | 222.24 | 610.52 | 286.24 | 309.32 | 272.25 | 302.19 | 302.24 | 354.31 |
| Num. H-Bond acceptor | 6 | 2 | 15 | 6 | 4 | 5 | 8 | 7 | 9 |
| Num. H-Bond donor | 5 | 0 | 10 | 4 | 0 | 3 | 4 | 5 | 6 |
| Predicted LD50 value (mg/kg) | 10000 | 2500 | 5000 | 3919 | 400 | 2000 | 2991 | 159 | 5000 |
| Lipophilicity CLogPo/w value | 0.85 | 3.18 | -1.29 | 1.58 | 2.87 | 1.84 | 1.0 | 1.23 | -0.38 |
| Water solubility | Soluble | Moderately soluble | Soluble | Soluble | Moderately soluble | Soluble | Soluble | Soluble | Very soluble |
| GI absorption | High | High | Low | High | High | High | High | High | Low |
| Druglikeness: Obeyes Lipinski rule? | Yes; 0 violation | Yes; 0 violation | No; 3 violations: MW>500, NorO>10, NHorOH>5 | Yes; 0 violation | Yes; 0 violation | Yes; 0 violation | Yes; 0 violation | Yes; 0 violation | Yes; 1 violation: NHorOH>5 |
| Toxicity class | 6 | 5 | 5 | 5 | 4 | 4 | 4 | 3 | 5 |
| Hepato-toxicity | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| Carcinogenicity | Inactive | Active | Inactive | Inactive | Active | Inactive | Active | Active | Inactive |
| Immunotoxicity | Inactive | Inactive | Active | Inactive | Active | Inactive | Inactive | Inactive | Active |
| Mutagenicity | Inactive | Inactive | Inactive | Inactive | Active | Inactive | Inactive | Active | Inactive |
| Cytotoxicity | Inactive | Active | Inactive | Inactive | Active | Active | Inactive | Inactive | Inactive |

3.2. Discussion.

The prevalence of erectile dysfunction globally is rising steadily every year and continues to pose serious threats to world health. Over the years, FDA-approved drugs for managing this disease, such as sildenafil, among others, have been effective. However, these drugs have their own set of negative effects. Scientists are now focusing on screening medicinal herbs with folkloric relevance to make alternative therapeutic options available. Africa is home to an abundance of medicinal plants used to treat and manage a wide range of diseases, including erectile dysfunction. Many Africans still rely on traditional medicine to treat serious health conditions because of limited access to orthodox medications and quality health facilities. The ability of African herbs to promote erectile functions is one aspect of phytomedicine that has been grossly explored, dating back to antiquity. Several native African plants have been proven to have a significant capacity to reduce erectile dysfunction via years of extensive study on medicinal herbs [42, 43].

The diverse composition of phytoactive compounds inherent in medicinal herbs makes them a rich repository of health-promoting biologically active principles and templates for developing novel therapeutic modalities [44]. These validate the growing interest in identifying plant-based therapies as alternatives to synthetic modalities, which are nearly never free of side effects when used in disease management [45]. *Sida linifolia* L. remains an understudied medicinal plant with various folklore uses in African traditional medicine. However, recent studies have reported rich phytochemistry in the plant leaf extracts. In addition, the folkloric claims and preliminary studies on the plant extract as an aphrodisiac, anti-hypertensive, and a genital stimulant/depressant [26-28] warranted the present study. Moreover, using GC-FID techniques, our study showed that *S. linifolia* ethanol leaf fraction is composed of biologically active phytochemicals such as phenols (resveratrol, proanthocyanin), tannins, flavonoids (flavo-3-ol, epicatechin, rutin, catechin, flavone, naringenin, kaempferol, and flavanones, and ephedrine), saponin, alkaloids, steroids, lunamarin, phytate and oxalate at varying concentrations. In another recent study, HPLC profiling of the bioactive composition of the ethanolic fraction of *S. linifolia* leaves revealed the preponderance of phenolics such as ferulic acid, quercetin, and ellagic acid, and flavonoids such as chlorogenic, vanillic, 3,4-dimethoxybenzoic, gallic, sinapic, and 4-methoxy cinnamic acid at varying concentrations (Additional file 2) [30]. The presence of these phytochemicals in the plant material suggests its possible pharmacological properties. Herein, the present study assessed the propensity of an ethanolic fraction of *S. linifolia* leaves (EtOH/FSL) to modulate enzymes linked to erectile dysfunction (ED).

Sexual arousal causes NO, a retrograde neurotransmitter that works in synapses, to be released from the endothelium of the cavernous spaces and nerve endings by nitric oxide synthase (NOS) (non-cholinergic and non-adrenergic), resulting in penile erection [46]. NOS utilizes molecular oxygen and L-arginine as substrates to liberate NO. The synthesized NO further diffuses into smooth muscles. In the smooth muscles, NO activates the enzyme guanylyl cyclase, which, in turn, converts guanosine-5'-triphosphate (GTP) into 3'-5'-cyclic guanosine monophosphate (cGMP) [11]. Furthermore, cGMP activates the cGMP-dependent protein kinase (PKG), which phosphorylates proteins necessary for muscle relaxation [10]. Additionally, cGMP reduces intracellular concentrations of calcium ions (Ca^{2+}). Resultantly, the trabecular and arterial smooth muscles relax, causing arterial dilation, increased circulation of blood into the penile tissue, and the penis becomes more erect and stiffer [2]. PDE5 exerts

inhibitory feedback on cyclic GMP, resulting in arteriolar vasoconstriction and penile detumescence [47]. However, increased PDE5 activity causes lower cGMP levels, resulting in ED. Therefore, inhibiting PDE5 activity increases cGMP levels and enhances penile erections [48]. The use of inhibitors of PDE5, such as tadalafil, vardenafil, and sildenafil, not only increases cGMP levels [49] but also induces cGMP activation and improves NO bioavailability for penile tissue relaxation, resulting in penile erection [50]. However, these synthetic inhibitors cause a variety of adverse effects, including nasal congestion, headache, visual anomalies, dyspepsia, among others [49].

Furthermore, recent studies have demonstrated that medicinal plant extracts rich in flavonoids can effectively decrease PDE5 activity [50, 51]. Our results showed that EtOHFSL exerted moderate inhibitory effects on PDE5 in a dose-dependent manner and was comparable to the standard drug sildenafil. The higher IC₅₀ value (0.66 mg/ml) for EtOHFSL compared to sildenafil (0.53 mg/ml) suggests moderate inhibition of PDE5 activity without adverse effects. Studies have previously reported a rich phenolic composition, including tannins, flavonoids, and other phenols, in EtOHFSL [29, 30]. Perhaps the enzyme-inhibitory potential of the EtOHFSL could be attributed to its polyphenolic composition. Moreover, previous *in silico* studies reported strong interactions between natural polyphenols and PDE5 [52-56]. The *Sida* genus consists of several ethnopharmacologically relevant herbs with sexual-boosting properties and rich phytochemistry. For instance, in Western Uganda, the leaves of *Sida tenuicarpa* (Malvaceae) are pounded, boiled, and administered orally to manage sexual impotence and ED [57]. In a systematic review of medicinal herbs used to manage erectile dysfunction in Ethiopia, Asmerom *et al.* [58] listed several members of the *Sida* genus, including *Sida tenuicarpa* leaves, *Sida schimperiana* root, and *Sida rhombifolia* root, as representative herbal remedies used in African folklore for treating ED. Besides, preliminary studies highlighted the traditional use of *Sida* plant decoctions, including *S. linifolia*, as an aphrodisiac and an anti-hypertensive [26-28]. In line with this, the ability of EtOHFSL to modulate PDE5 activity validates the folkloric uses of the plant leaf decoction in enhancing sexual functions [27].

The renin-angiotensin system (RAS) regulates body fluid, blood pressure, and electrolyte balance. Renin, an enzyme secreted by the kidney, cleaves liver-derived angiotensinogen into angiotensin (Ang)-I. Angiotensin-converting enzyme (ACE) released by the lungs converts Ang-I to active Ang-II [59]. Resultantly, Ang-II, the main component of RAS, causes tonic contraction of corpus cavernosum smooth muscles during penile erection [1]. However, higher levels of Ang II have been linked to ED and cardiovascular diseases (CVDs) [24]. Chronically elevated levels of Ang-II activate the angiotensin type 1 receptor (AT1R), promoting renal sodium and water retention and vasoconstriction, accompanied by decreased endothelial NO concentration and increased blood pressure, ultimately leading to erectile dysfunction [60, 61]. It also stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to excess superoxide production [62]. Therefore, treatment with ACE inhibitors, renin inhibitors, and Ang-II receptor blockers has been associated with modulation of Ang-II and other approaches for the management of ED [63]. From the result of the study, EtOHFSL effectively inhibited ACE activity in a dose-dependent fashion and was at par in activity with the conventional anti-ACE drug (captopril). The inhibitory potential of the plant leaf fraction on ACE activity could be attributed to its rich composition in flavonoids and other phenolic compounds. Our result is consistent with the report of Ahmad *et al.* [64], which posited that closely related plants such as *Sida acuta*, *Sida cordifolia*, and *Sida retusa* exhibited

appreciable ACE inhibition at different potencies and linked these activities to their phenolic composition. Plant-derived phenolics are excellent inhibitors of ACE activity. Several reports have shown that plant-derived phenolic compounds and some pure flavonoids reduce blood pressure by suppressing ACE activity [65, 66]. In line with these studies, investigators have reported that certain flavonoids, such as resveratrol (a stilbene), quercetin, rutin, and other phenolics (tannic acid and gallic acid), inhibit ACE [67-69]. Phenolic compounds have been shown to inhibit the catalytic activity of ACE via their excellent interaction with the zinc ion positioned in the active site of the enzyme, which is further stabilized by other interactions involving hydrogen bonding between their hydroxyl groups and amino acids in the enzyme active site [70].

The cholinergic nerve within the corpus cavernosum smooth muscles of the penis produces the parasympathetic neurotransmitter Acetylcholine (ACh). ACh relaxes the corpus cavernosum smooth muscle and increases penile tumescence by increasing endothelial NO generation [1, 71]. However, acetylcholinesterase (AChE) reduces ACh level by cleaving it to choline and acetate, resulting in ED [37]. Suppressing AChE activity causes an increase in ACh levels, which further enhances penile erections [7, 25]. Our results showed that EtOHFSL effectively inhibited AChE activity in a concentration-dependent manner and was comparable to galanthamine, a potent conventional AChE inhibitor. The inhibitory action of the plant leaf fraction on AChE activity is consistent with recent reports demonstrating that plant-derived AChE inhibitors are effective in managing ED as well as avert oxidative stress in penile neuronal cells [7, 18]. The presence of phenolics with notable activities in EtOHFSL could explain its suppressive effect on AChE activity. Copious evidence has shown that phenolic-rich plants can suppress AChE [72, 73]. Our result also agrees with recent studies [74, 75] that have demonstrated the anti-cholinergic potentials of extracts from closely related species, such as *S. rhombifolia*. These activities are anchored in the plant extract's rich polyphenolic content. It has been established that polyphenolics interact with amino acid residues positioned at the active site of AChE via the formation of hydrogen bonds (H-bonds), Pi-Pi (π - π) interactions, and hydrophobic interactions [76, 77]. The multiple reactive hydroxyl groups in the phenolic molecule are thought to improve its inhibitory activity on AChE by increasing molecular interactions [78]. These molecular interactions explain the enzyme-inhibitory potential of most phenolic compounds; however, not all exhibit the same mechanism of action [79]. In addition, Kundo *et al.* [80] found a high association between proanthocyanidin, flavonoids, and phenolic concentration and cholinesterase inhibitory activity of *Loranthus globosus*. Naringenin identified in EtOHFSL is known to exhibit excellent anti-AChE activity [81]. Also, quercetin, gallic, vanillic, and ferulic acids found in EtOHFSL have been reported to exhibit potent anti-cholinergic effects at varying potencies [81-84].

Arginase and Nitric oxide synthase (NOS) in the endothelium and smooth muscles vie for L-arginine as a substrate for their different catalytic activities. The use of L-arginine as substrate by arginase to exert its activity in vascular endothelial and smooth muscles of penile tissue decreases the activity of NOS and, as a result, reduces NO concentration, a key factor for proper penile erection [85]. Therefore, inhibiting arginase activity offers an additional therapeutic approach for managing ED, as it may improve penile erection by increasing L-arginine bioavailability and promoting NO biosynthesis via NOS catalysis. Our results showed that EtOHFSL suppressed arginase activity in a dose-dependent manner and was comparable with LNOHA, a standard arginase inhibitor. The plant leaf fraction's ability to inhibit arginase

aligns with previous reports that plant materials rich in phenolics, such as quercetin, epicatechin, and rutin, exhibit potent arginase-inhibitory activity [37, 86].

From the molecular docking studies (Tables 2 and 3), ellagic acid, lunamarin, rutin, quercetin, naringenin, kaempferol, and chlorogenic acid identified in EtOHFSL demonstrated excellent inhibitory activity against arginase, AChE, PDE5, and ACE. It was comparable to respective standard inhibitors (L-NOHA, galantamine, sildenafil, and captopril). The toxicity profile studies, as predicted by PROTOX, showed no potential toxicity for most of the studied compounds, as all top-scoring compounds of EtOHFSL had good properties as drug candidates and also complied with the Lipinski Rule of Five, except rutin (MW>500, NorO>10, NHorOH>5). Also, the 2D (surface) views of the protein-ligand interactions of top-scoring selected compounds (Figure 6) showed the participation of several bonding types and molecular interactions resulting in the observed inhibitory effect of the phytochemicals on the target proteins, similar to standards (see Additional file 1: Figures S3a-d for detailed 3D interactions). These were evident in the formation of conventional hydrogen bonds between the studied compounds and the active residues of the target proteins via their various functional groups. Further formation of other attractions, such as van der Waals, π -cation, π -Alkyl, and π - π attractions, contributed to the stabilization of the Protein-Ligand interaction. Our data are consistent with previous studies demonstrating the potential of plant-derived phenolic compounds as potent inhibitors of enzymes linked to erectile dysfunction [8, 68, 87, 88]. Similarly, Oboh *et al.* [7] reported excellent inhibitory actions of extracts of ripe and unripe plantain fruit peels on enzymes implicated in ED penile tissue homogenate and linked these properties to their rich composition in phenolic compounds, such as ellagic, chlorogenic, and gallic acids, rutin, kaempferol, catechin, and quercetin. In addition, Ojo *et al.* [18], Akomolafe *et al.* [37], and Oboh *et al.* [86] in their study, attributed the considerably high inhibitory effect of extracts from *Ocimum gratissimum*, *Ficus capensis*, and *Moringa oleifera* leaves, respectively, on erectile dysfunction-relevant enzymes to their rich polyphenolic profile.

4. Conclusions

This study revealed that the ethanolic fraction from *S. linifolia* leaves exhibited considerable inhibitory effects on key enzymes implicated in ED, which could be attributable to its rich composition of various phenolics and flavonoids. Perhaps the plant leaves could represent a potential reservoir of pharmacologically potent phytochemicals that could be explored in the medical and pharmaceutical industries. In addition, the rich phytochemical composition and potent enzyme inhibition properties of the plant leaf fraction make it a potential candidate for cancer research. Hence, further research on the plant leaves is warranted.

Author Contributions

Conceptualization, E.C.E.; methodology, A.Y.S. and E.C.E.; software, A.Y.S.; validation, G.O.O., K.V.E., and G.O.O.; formal analysis, N.O.A. and J.O.G.; investigation, A.Y.S., E.C.E. and E.C.A.; resources, E.C.E.; data curation, S.A.I., N.S.V., C.D.A., C.P.O., and Y.B.I.; writing—original draft preparation, E.C.E.; writing—review and editing, E.C.E.; visualization, S.C.O. and C.C.U.; supervision, E.C.A. and A.C.M.; project administration, E.C.A., E.C.E. and N.M.I.; funding acquisition, All authors. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The animal study protocol was approved by the Ethics Committee with Approval No: UNN/FBS/EC/1029 on 21/02/2023.

Informed Consent Statement

Not applicable

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

The authors funded the project.

Acknowledgments

The authors wholeheartedly acknowledge the University of Nigeria, Nsukka, staff for their solidarity and assistance during the experiment. The contributions of members of other institutions visited during the study are very much appreciated. The authors also acknowledge Mr. Alfred Ozioko of the International Center for Ethnomedicine and Drug Development (Inter CED) for his initial insights into the plant's folklore relevance.

Conflicts of Interest

The authors declare that they have no competing interests.

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Supplementary materials

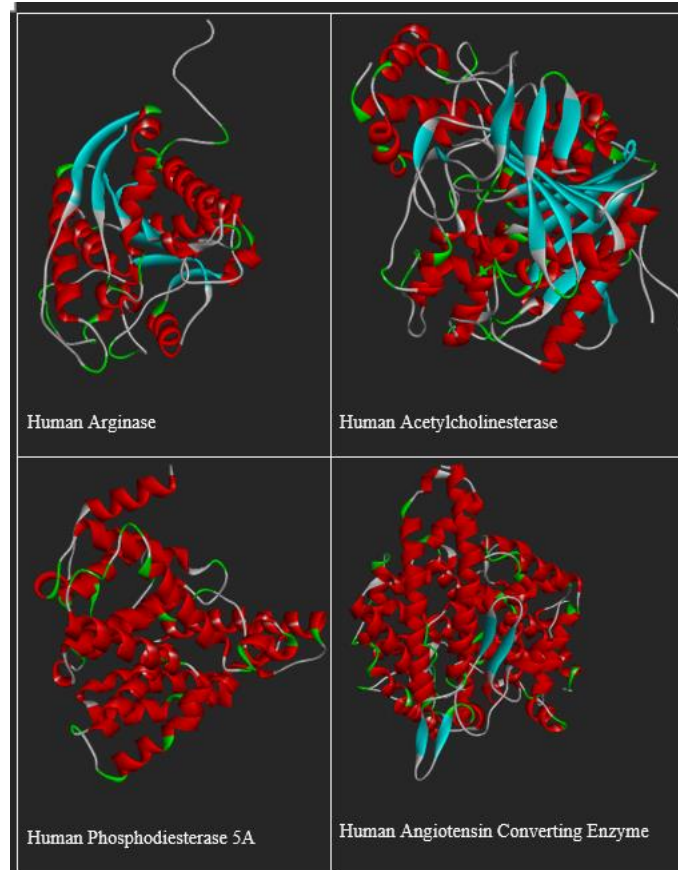


Figure S1. Target Proteins retrieved from the Protein Data Bank (PDB).

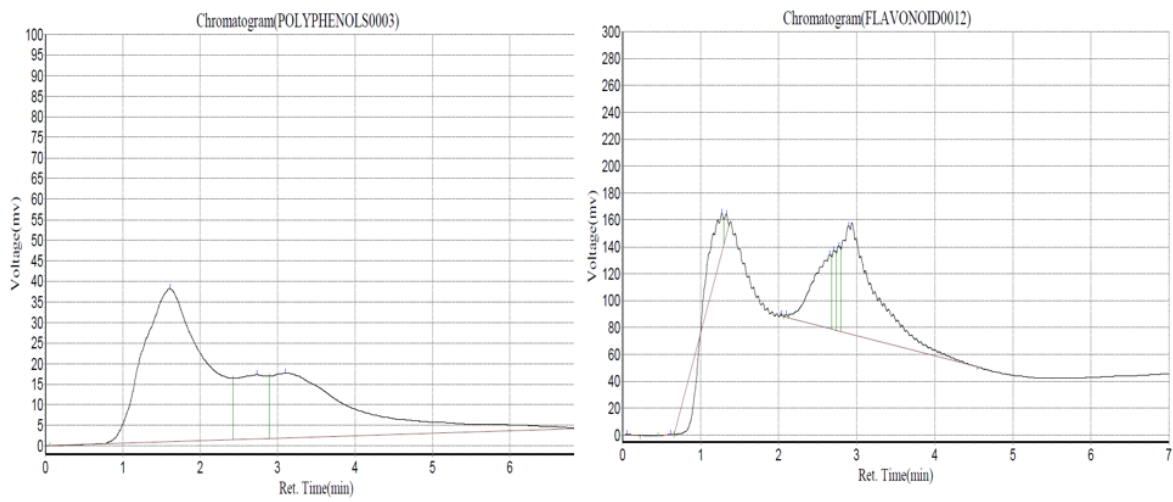


Figure S2. HPLC chromatogram of polyphenolics and flavonoids in EtOHFSL.

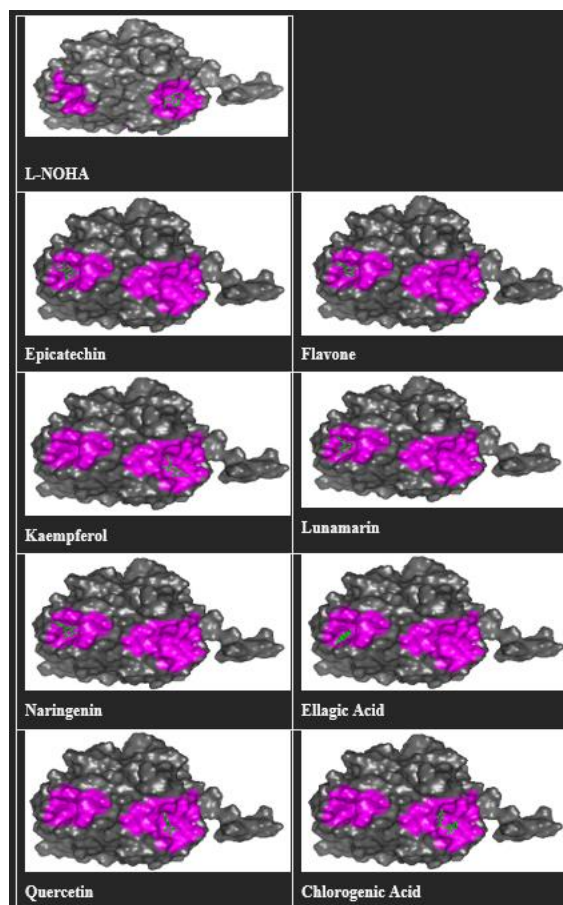


Figure S3a. 3D (surface) view of Arginase – Ligand Interaction.

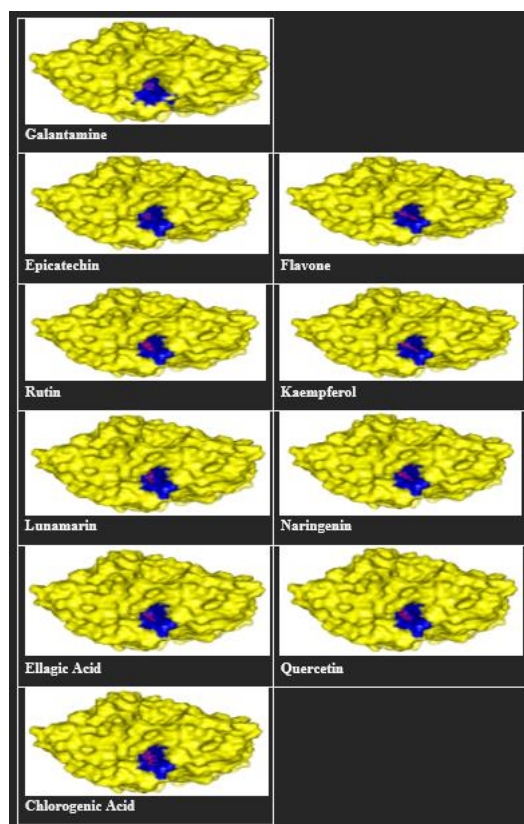


Figure S3b. 3D (surface) view of Acetylcholinesterase – Ligand Interaction.

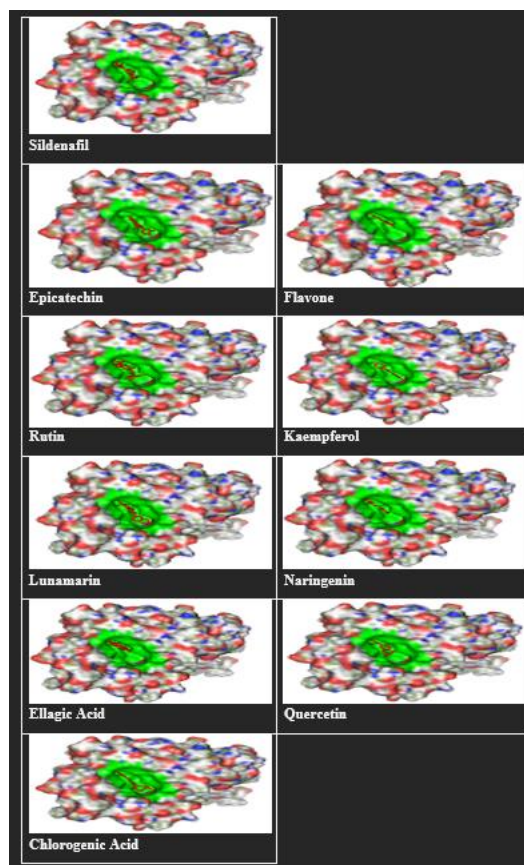


Figure S3c. 3D (surface) view of Phosphodiesterase – Ligand Interaction.

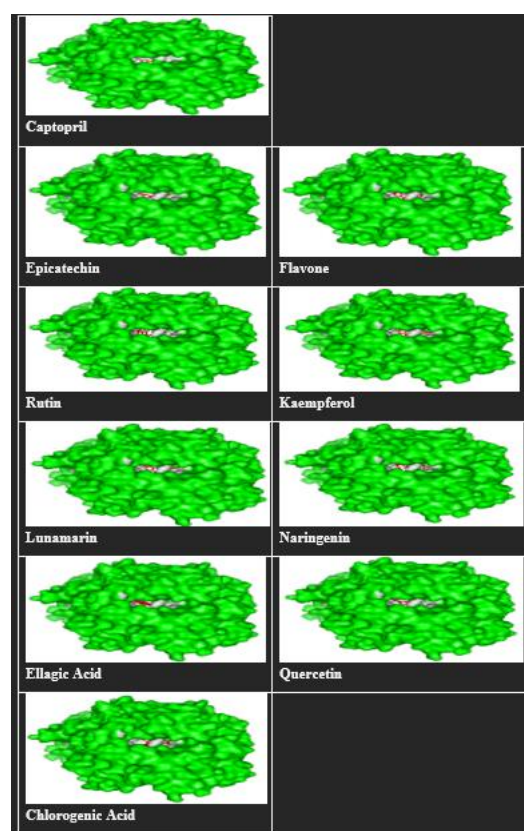


Figure S3d. 3D (surface) view of ACE– Ligand Interaction.