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RESEARCH

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Phytochemical Compounds of Ethanol Extract *Kigelia africana* (Lam.) Benth. from Pericarpium, Fructus, and Semen using LCMS/MS and in Silico Study (Potential of *Phosphodiesterase-5* Inhibitor)

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Abstract

Erectile dysfunction can be treated by pharmacological therapy with *phosphodiesterase-5 inhibitors* (PDE5-I). One plant that has a function as a phosphodiesterase-5 inhibitor is *Kigelia africana* (Lam.) Benth. Research on *Kigelia africana* (Lam.) Benth. An extract as a *Phosphodiesterase type 5* (PDE 5) inhibitor is still lacking. This research aims to determine the phytochemical compounds in the 70% ethanol extract of the *pericarpium*, *fructus* and *semen* of *Kigelia africana* (Lam.) Benth. with LC-MS/MS and to determine the ligand-protein interaction through in-silico studies. In previous studies, no one compared each part of the *Kigelia africana* (Lam.) Benth. as an aphrodisiac. The results of the identification of the 70% ethanol extract of *Kigelia africana* (Lam.) Benth. found several alkaloids, flavonoids, iridoids, phenolics, polyphenols, coumarins, steroids and fatty acids. Based on the results of molecular docking on 70% ethanol extract of *pericarpium*, *fructus* and *semen* of *Kigelia africana* (Lam.) Benth., 18 compounds, 20 compounds and 18 compounds were obtained sequentially, which were analyzed using LC-MS/MS. The sitosterol compound with ($\Delta G = -8.90$ kcal/mol) was identified in the three parts of the 70% ethanol extract sample of kunto bimo fruit, which showed the highest affinity for binding to the target protein compared to sildenafil with ($\Delta G = -8.68$ kcal/mol). Sitosterol compound has the same amino acid residues as the native ligand, namely ILE A:824, TYR A:664, ALA A:779, ILE A:768, ALA A:767, LEU A:765, ASN A:661, HIS A:613, PHE A:786, VAL A:782, LEU A:804, MET A:816, PHE A:820 in hydrophobic bonds. Phytochemical compounds from *Kigelia africana* (Lam.) Benth. It has the potential as an alternative additional therapy and a promising source for the discovery of new drugs as aphrodisiacs targeting PDE5.

Keywords: *Kigelia africana* (Lam.) Benth., Inhibitor of *Phosphodiesterase type 5*, PDE 5, LC-MS/MS, Molecular docking.

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1. INTRODUCTION

Sexual dysfunction (SD) is more common in men with 50-70 years of age will experience a decrease in the concentration of Leydig cells by 40%, and there will be a decrease in Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which results in decreased testosterone levels (Zulkarnain et al., 2020). Sexual dysfunction can be caused by neurological disorders, hormonal imbalances, stress, lifestyle, genetic factors, underlying disease factors (Yafi, et al., 2016).

The chronic inability to get and sustain a penile erection, which leads to inadequate sex, is known as ED (Araujo, et al., 1998). According to earlier research, ROS and oxidative stress play a major role in the pathogenesis of ED (Araujo, et al., 1998; Ritchie, et al., 2021; Sikka, et al., 2001; Jin, et al., 2008). An imbalance between prooxidants, hydroxyl radicals (OH*), nitric oxide (NO*), and free radicals (and antioxidants' capacity to generate excessive reactive oxygen) leads to oxidative stress (Sikka, et al., 2001; Jin, et al., 2008; Sikka, et al., 1995; Kunwar, et al., 2011). Although oxidative stress and ROS have been shown to play a role in the pathophysiological mechanisms of both male and female infertility (Agarwal, et al., 2008), their impact on ED has not been thoroughly investigated. However, early research has demonstrated a strong correlation between the generation of ROS and ED, particularly in animal models of diabetes (Agarwal, et al., 2005).

Therapy to treat erectile dysfunction involves administering phosphodiesterase-5 inhibitor (PDE5-I) drugs such as sildenafil, but long-term use can cause dilatation of blood vessels resulting in headaches, dyspepsia, myalgia, flushing, cyanopsia, angina pectoris, myocardial infarction, arrhythmia, hypertension, priapism, hearing loss, vision loss, nonarteritic arterial ischemic optic neuropathy (NAION) (Huang, et al., 2013).

An alternative to overcome this problem is to use one of the plants which empirically has aphrodisiac activity, namely *Kigelia africana* (Lam.) Benth. from the Bignoniaceae family, or in Indonesia known as Kunto Bimo Fruit. This species is an endemic plant originating from sub-Saharan Africa and mostly comes from tropical Africa. Apart from that, it is also spread in tropical America, Indonesia and Malaysia (Böhlmann, et al., 2016). Empirically, kunto bimo fruit can be used as a treatment for genital infections, infertility, increasing libido, aphrodisiac, skin diseases, cancer and tumors (Halder, et al., 2017).

Kigelia africana plant contains several secondary metabolite compounds that have been studied, including alkaloids, iridoids, naphthoquinones, flavonoids, coumarins, terpenes, terpenoids, steroids and phenylethanoid glycosides, new compounds that have strong antioxidant, antimicrobial and anticancer activity such as verbascoside, verminoside and pinnatal (Bello, et al., 2016; Khairuddin, et al., 2023; Putri, 2014).

The purpose of this study is to evaluate *Kigelia africana* (Lam.) Benth.'s potential as a medicinal aphrodisiac. The phytochemical compounds in the 70% ethanol extract of the pericarpium, fructus, and semen of the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.) will be identified in this instance using LC-MS/MS analysis. The phytochemical compounds thus identified will then be used as ligand compounds in molecular docking to examine the phytochemical compounds' binding affinity with the target protein, phosphodiesterase type 5 (PDE 5) (PDB ID: 1UDT) (Palanichamy, et al., 2022).

The reason this study uses 70% ethanol extract samples of pericarpium, fructus and semen of *Kigelia africana* (Lam.) Benth because in previous studies there is still no research that examines the content of compounds in each part of *Kigelia africana* (Lam.) Benth fruit. This research aims to determine the phytochemical compounds in the 70% ethanol extract of the pericarpium, fructus and semen of *Kigelia africana* (Lam.) Benth. with LC-MS/MS and to determine the ligand-protein interaction through in-silico studies.

2. RESEARCH METHOD

This study employed a true experimental research design, integrating laboratory-based experimentation with computational (in silico) analysis to comprehensively investigate the chemical composition and biological potential of *Kigelia africana* fruit. The experimental approach combined phytochemical profiling using advanced analytical instrumentation with molecular docking simulations to evaluate bioactive compound interactions at the molecular level.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was utilized to identify chemical constituents present in the pericarpium, fructus, and semen of Kunto bimo fruit (*Kigelia africana* (Lam.) Benth.). During chromatographic separation, polar compounds eluted earlier, followed by less polar compounds. Chromatogram peaks were detected using a QToF-MS detector (Xevo G2-S QToF, Waters, USA). Samples were injected using a 5 μ L microsyringe and introduced four times into an ultra-performance liquid chromatography (UPLC) system (Waters, USA) equipped with an ACQUITY UPLC BEH C18 column (1.8 μ m, 2.1 $\times$ 50 mm). Ionization was performed using positive electrospray ionization (ESI+) with a mass detection range of 50–1200 m/z. The source and desolvation temperatures were set at 100 °C and 350 °C, respectively, with collision energies ranging from 4 to 60 eV. The cone gas flow rate was maintained at 0 L/h, while the desolvation gas flow rate was set at 793 L/h. Gradient elution was conducted using solvent A (water with formic acid) and solvent B (acetonitrile with formic acid) at a flow rate of 0.2 mL/min. Chromatographic data acquisition and peak analysis were performed using MestReNova software (Pratama et al., 2023; Roy et al., 2015; Chambers et al., 2014; Eurofins, 2022; Chawla et al., 2016).

The in silico component of this research was conducted using a computational workstation consisting of an Asus X515 laptop equipped with an Intel® Core™ i7 processor, 16.0 GB of 2400 MHz RAM, NVIDIA® GeForce® MX330 graphics, and the Microsoft® Windows® 10 Pro operating system, with internet connectivity. Software tools utilized in this study included Discovery Studio Visualizer®, AutoDock Tools® version 1.5.6, AutoGrid version 4.2.6 (The Scripps Research Institute, USA), PyMOL®, Open Babel®, Marvin Sketch® (ChemAxon), Notepad++, and LigPlot+. Online databases such as the Protein Data Bank, PubChem, SCF Bio, and PCKSM were accessed to retrieve structural and bioinformatics data.

The three-dimensional structure of the phosphodiesterase-5 inhibitor (PDE5-I) protein was obtained from the Protein Data Bank (PDB ID: 1UDT). Ligand structures, including test compounds identified through LC–MS/MS analysis and reference compounds, were retrieved in SDF format from the PubChem database. Ligand structures were prepared and optimized using Marvin Sketch version 20.21 to ensure appropriate geometry and protonation states prior to docking simulations.

Molecular docking studies were performed using the crystal structure of the PDE5-I receptor. Prior to docking, water molecules were removed, and missing residues as well as hydrogen atoms were added to stabilize the protein structure. Energy minimization of ligands was carried out using the MMFF94 force field, while the protein structure was minimized using the GROMOS96 force field. Docking simulations employed a grid box with a spacing of 0.375 Å and dimensions of 60 $\times$ 60 $\times$ 60, centered at coordinates x = 1.676, y = 68.288, and z = 83.841. The Lamarckian Genetic Algorithm was applied with a population size of 150 individuals and a maximum of 2,500,000 energy evaluations across 100 docking runs. The optimal docking pose was determined based on the lowest binding energy (Δ G) and inhibition constant (K_i) values. Key ligand–protein interactions and functional amino acid residues involved in binding were visualized and analyzed using Discovery Studio Visualizer (Gandjar, 2007).

3. RESULTS AND DISCUSSION

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was employed to identify the phytochemical constituents present in the 70% ethanol extracts of the pericarpium, fructus, and semen of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.). This analytical technique was selected due to its superior sensitivity and selectivity compared with conventional liquid chromatography methods, enabling reliable detection of compounds present at low concentrations. The analytical principle of LC–MS/MS involves the injection of a sample solution containing target analytes into a high-pressure liquid chromatography system, where the mobile phase transports the sample through a stationary phase within the LC column. Differential chemical interactions between the analytes, the stationary phase, and the mobile phase result in distinct migration rates, thereby achieving effective separation of individual components (Eurofins, 2022). Following chromatographic separation, analytes are detected based on their mass-to-charge (m/z) ratios.

After elution from the LC column, the analytes enter the ion source of the mass spectrometer, where they undergo ionization. The resulting ions are then separated in the first mass analyzer, which selects a specific m/z value referred to as the parent ion. This parent ion subsequently enters the collision cell, where it is fragmented through collision-induced dissociation. The generated fragment ions are then analyzed in the second mass analyzer, producing ions known as daughter ions with characteristic m/z values. The combined analysis of parent and daughter ion spectra provides a unique molecular fingerprint that enables precise identification of each analyte (Chambers et al., 2014).

Based on the LC–MS/MS identification results, a total of 18 compounds were detected in the pericarpium extract, 20 compounds in the fructus extract, and 18 compounds in the semen extract, as presented in Table 1, Table 3, and Table 2, respectively. The metabolomic profiling revealed a diverse range of secondary metabolites, including alkaloids, flavonoids, iridoids, phenolics, polyphenols, coumarins, steroids, and fatty acids, indicating the rich phytochemical composition of *Kigelia africana* fruit.

Molecular docking analysis was subsequently conducted to evaluate the binding interactions between the identified compounds and the phosphodiesterase-5 inhibitor (PDE5-I) receptor protein (PDB ID: 1UDT). The selection of PDB ID 1UDT was based on its biological relevance as a human PDE5 protein complexed with the native ligand sildenafil, which is widely used as a reference compound in studies related to aphrodisiac and vasodilatory activity. Docking protocol validation was performed by re-docking the native ligand into the protein binding site to confirm the reliability of the docking parameters. The choice of this receptor was further supported by extensive prior evidence regarding its role in aphrodisiac activity, the availability of comprehensive structural information, and the open accessibility of the crystallographic data.

The strength and stability of ligand–receptor interactions were assessed based on free binding energy (ΔG) values and key amino acid interactions within the active site. The docking results for compounds identified from the 70% ethanol extracts of the pericarpium, fructus, and semen of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.) against the PDE5-I receptor are summarized in Table 1, Table 2, and Table 3. Among these, Table 1 demonstrates that the top three compounds from the pericarpium, fructus, and semen extracts exhibited the most favorable binding conformations, characterized by the lowest free binding energy values.

Specifically, the bioactive compound with the lowest binding energy in the 70% ethanol extract of the pericarpium was sitosterol, with a ΔG value of -8.90 kcal/mol. This was followed by 7-hydroxy-7-methyl-1- $\{[3,4,5\text{-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy\}$ 1H,4aH,5H,6H,7aH-cyclopenta[c]pyran-5-yl 4-hydroxybenzoate, which exhibited a ΔG value of -7.40 kcal/mol, and piperine, with a ΔG value of -7.14 kcal/mol. These results suggest that the identified compounds possess strong binding affinities toward the PDE5-I receptor, supporting their potential bioactivity.

Table 1. Compound docking results from LC-MS/MS of 70% ethanol extract of the pericarpium of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.) against IUDT.

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmenta tion (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
11.07	415.2112	415.2108	210.1487; 182.1540; 119.0856; 100.1123	Sitosterol (C ₂₉ H ₅₀ O)	-8.90	301.39 nm
6.18	469.3369	469.3333	354.2403; 337.2113; 228.1595; 182.1538	7-hydroxy-7-methyl-1- $\{[3.4.5$ -trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy $\}$ -1h.4ah.5h.6h.7ah-cyclopenta[c]pyran-5-yl 4-hydroxybenzoate (C ₂₂ H ₂₈ O ₁₁)	-7.40	3.74 μ m
10.44	286.1435	286.1436	245.1723; 154.0861; 135.0431; 115.0537	Piperine (C ₁₇ H ₁₉ NO ₃)	-7.14	5.84 μ m
8.90	297.1121	297.1105	257.0845; 182.1545; 126.0913; 95.0807	5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one (C ₁₈ H ₁₆ O ₄)	-6.91	8.54 μ m
9.49	285.2537	285.2534	228.1613; 210.1488; 182.1543; 100.1121	Dimethylpinocembrin (C ₁₇ H ₁₆ O ₄)	-6.82	10.1 μ m
6.01	329.2260	329.2266	227.1756; 182.1542; 147.0443; 100.1122	3-Hydroxy-7.8.4'-trimethoxyflavone (C ₁₈ H ₁₆ O ₆)	-6.48	17.67 μ m
9.23	511.4213	511.4206	471.3529; 314.2801; 198.1493; 114.0915	8-p-Coumaroylharpagide (C ₂₄ H ₃₀ O ₁₂)	-6.44	18.9 μ m
4.55	245.1862	245.1851	197.1288; 163.0397; 154.0864; 137.0592	2-hydroxy-3-(3-methylbut-2-en-1-yl)-2.3-dihydronaphthalene-1.4-dione (C ₁₅ H ₁₆ O ₃)	-6.34	22.62 μ m
6.46	625.4285	625.4284	454.3370; 309.2631; 228.1602; 114.0915	Verbascoside (C ₂₉ H ₃₆ O ₁₅)	-6.06	36.16 μ m
6.71	453.3391	453.3389	198.1482; 182.1541;	Cinchonain (C ₂₄ H ₂₀ O ₉)	-5.52	89.49 μ m

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmenta tion (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
			140.0704; 83.0857			
5.48	349.2347	349.2360	307.0808; 245.0815; 177.0547; 128.0706	Ajugol (C ₁₅ H ₂₄ O ₉)	-5.34	120.96 μm
4.88	163.0395	163.0393	154.0864; 128.0705; 114.0916; 100.1121	4-Coumarinol (C ₉ H ₆ O ₃)	-4.92	247.11 μm
3.34	151.0752	151.0760	145.0496; 123.0811; 105.0701; 95.0854	hydrocinnamic acid (C ₉ H ₁₀ O ₂)	-3.60	2.31 μm
13.16	496.3406	496.3410	459.2487; 184.0737; 125.0004; 86.0967	(2-{{(2r)-2- hydroxy-3-[(15- methylhexadecyl) oxy]propyl phosphonato]oxy }ethyl)trimethylaz anium (C ₂₅ H ₅₄ NO ₆ P)	-3.54	2.53 μm
12.57	520.3400	520.3411	502.3279; 184.0737; 104.1070; 85.0964	Lysophosphatidyl choline (C ₂₆ H ₅₀ NO ₇ P)	-3.10	5.31 μm
1.21	381.0789	381.0787	360.1513; 343.1226; 325.1129; 163.0600	[4.5-dihydroxy- 2.3- bis(hydroxymethy l)cyclopent-2-en- 1-yl]acetic acid (C ₉ H ₁₄ O ₆)	-2.91	7.35 μm
11.81	597.3054	597.3051	540.3054; 184.0737; 125.0000; 104.1070	(5s)-5-methyl-3- [(2r.8r.11r)- 2.8.11-trihydroxy- 11-[(2r.5r.6r)-5- hydroxy-6- tetradecyloxan-2- yl]undecyl]-5h- furan-2-one (C ₃₅ H ₆₄ O ₇)	-1.80	47.68 mm
5.17	597.3985	597.3982	325.0912; 210.1487; 163.0392; 145.0285	Neoteriocitrin (C ₂₇ H ₃₂ O ₁₅)	2.73	-

The bioactive compound with the lowest free bond energy in the 70% ethanol extract of the fructus of the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.) is sitosterol with ($\Delta G = -8.90$ kcal/mol), followed by piperine with ($\Delta G = -7.14$ kcal/mol), and 5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one with ($\Delta G = -6.91$ kcal/mol).

Table 2 shows that the three best results of molecular docking on 70% ethanol extract of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.), the compound 5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one has been found, which is a flavonoid compound.

This flavonoid compound works by increasing dehydroepiandrosterone levels, which play a role in increasing testosterone hormone levels and encouraging sexual behavior in men (Rusdi, et al., 2018; Wardani, et al. 2017). In addition, flavonoid and alkaloid compounds also have peripheral action, namely by helping to relax the corpus cavernosum, thereby triggering an erection (Chauhan, 2014; Indrisari, 2018; Istiqamah, 2024).

Table 2. Compound docking results from LC-MS/MS of 70% ethanol extract of the fructus of Kunto Bimo fruit (*Kigelia africana* (Lam.) Benth.) against 1UDT.

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmentation (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
11.07	415.2112	415.2108	210.1487; 182.1540; 119.0856; 100.1123	Sitosterol (C ₂₉ H ₅₀ O)	-8.90	301.39 nm
10.37	286.1435	286.1434	245.1723; 154.0861; 135.0431; 115.0537	Piperine (C ₁₇ H ₁₉ NO ₃)	-7.14	5.84 μm
8.84	297.1121	297.1105	257.0845; 182.1545; 126.0913; 95.0807	5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one (C ₁₈ H ₁₆ O ₄)	-6.91	8.54 μm
9.47	285.2537	285.2534	228.1613; 210.1488; 182.1543; 100.1121	Dimethylpinocembrin (C ₁₇ H ₁₆ O ₄)	-6.82	10.10 μm
6.01	329.2260	329.2266	227.1756; 182.1542; 147.0443; 100.1122	3-Hydroxy-7,8,4'-trimethoxyflavone (C ₁₈ H ₁₆ O ₆)	-6.48	17.67 μm
9.23	511.4213	511.4206	471.3529; 314.2801; 198.1493; 114.0915	8-p-Coumaroylharpagide (C ₂₄ H ₃₀ O ₁₂)	-6.44	18.9 μm
4.57	245.1862	245.1864	197.1288; 163.0397; 154.0864; 137.0592	2-hydroxy-3-(3-methylbut-2-en-1-yl)-2,3-dihydronaphthalene-1,4-dione (C ₁₅ H ₁₆ O ₃)	-6.34	22.62 μm
6.46	625.4285	625.4284	454.3370; 309.2631; 228.1602; 114.0915	Verbascoside (C ₂₉ H ₃₆ O ₁₅)	-6.06	36.16 μm
6.71	453.3391	453.3389	198.1482;	Cinchonain (C ₂₄ H ₂₀ O ₉)	-5.52	89.49 μm

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmentation (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
			182.1541; 140.0704; 83.0857			
5.45	349.2347	349.2360	307.0808; 245.0815; 177.0547; 128.0706	Ajugol (C ₁₅ H ₂₄ O ₉)	-5.34	120.96 μm
4.80	163.0395	163.0390	154.0864; 128.0705; 114.0916; 100.1121	4-Coumarinol (C ₉ H ₆ O ₃)	-4.92	247.11 μm
8.04	399.2280	399.2278	238.3459; 210.1497; 174.0917; 159.0678	2-[(4-hydroxypentan-2-yl)oxy]-6- {[(3.4.5-trihydroxyoxan-2-yl)oxy]methyl} oxane-3.4.5-triol (C ₁₆ H ₃₀ O ₁₁)	-4.85	276.66 μm
13.16	496.3406	496.3410	459.2487; 184.0737; 125.0004; 86.0967	(2-{[(2r)-2-hydroxy-3-[(15-methylhexadecyl)oxy]propylphosphonato]oxy}ethyl)trimethylazanium (C ₂₅ H ₅₄ NO ₆ P)	-3.54	2.53 mm
12.57	520.3400	520.3411	502.3279; 184.0737; 104.1070; 85.0964	Lysophosphatidylcholine (C ₂₆ H ₅₀ NO ₇ P)	-3.10	5.31 mm
14.59	524.3713	524.3710	335.2577; 210.1438; 184.0737; 104.1071	1-Stearoyl-sn-glycero-3-phosphocholine (C ₂₆ H ₅₄ NO ₇ P)	-3.07	5.66 mm
1.21	381.0789	381.0787	360.1513; 343.1226; 325.1129; 163.0600	[4.5-dihydroxy-2.3-bis(hydroxymethyl)cyclopent-2-en-1-yl]acetic acid (C ₉ H ₁₄ O ₆)	-2.91	7.35 mm
7.48	155.1430	155.1429	137.0592; 128.0898; 114.0917; 100.1119	Gentisic Acid (C ₇ H ₆ O ₄)	-2.84	8.25 mm
12.86	496.3405	496.3402	397.3925;	1-Hexadecanoyl-	-2.72	10.20 mm

RT	Mass [M-1]H+ (m/z)	Calculated (m/z)	Fragmentation (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
			298.2738; 210.1496; 181.0738	sn-glycero-3- phosphocholin e (C ₂₄ H ₅₀ NO ₇ P)		
11.81	597.3054	597.3051	540.3054; 184.0737; 125.0000; 104.1070	(5s)-5-methyl- 3-[(2r.8r.11r)- 2.8.11- trihydroxy-11- [(2r.5r.6r)-5- hydroxy-6- tetradecyloxan -2- yl]undecyl]- 5h-furan-2-one (C ₃₅ H ₆₄ O ₇)	-1.80	47.68 mm
5.17	597.3985	597.3982	325.912; 210.1487; 163.0392; 145.0285	Neoteriocitrin (C ₂₇ H ₃₂ O ₁₅)	2.73	-

Table 3 show that, the bioactive compound with the lowest free binding energy in the 70% ethanol extract of semen from the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.) is sitosterol with ($\Delta G = -8.90$ kcal/mol), followed by Sterekunthal A with ($\Delta G = -8.04$ kcal/mol), and (2s)-8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)benzo[g]chromene-5,10-dione with ($\Delta G = -7.92$ kcal/mol).

Based on previous research, Beta sitosterol is a natural steroid found in the *Kigelia africana* (Lam.) Benth plant. The presence of secondary metabolite beta sitosterol can increase libido through the mechanism of increasing androgen production and playing a role in the biosynthesis of dihydrotestosterone, thereby increasing testosterone levels in the body (Agyare, et al., 2013). Increased testosterone levels are related to increased libido (Andini, 2014; Wahdaningsih, et al., 2012). Testosterone is synthesized from a cholesterol precursor known as pregnenolone. Pregnenolone will then be converted into progesterone which will act as a precursor in inducing the formation of androgens such as testosterone (Hafez, 2020).

In the three samples of 70% ethanol extract of pericarpium, fructus and semen of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.), piperine compound was obtained which is a compound of the alkaloid group. Alkaloid compounds also play a role in increasing dilation of the blood vessels of the genitals, namely by helping to relaxation the smooth muscles of the corpus cavernosum which triggers an erection (Arifien, 2013).

Table 3. Compound docking results from LC-MS/MS of 70% ethanol extract of the semen of Kunto Bimo fruit (*Kigelia africana* (Lam.) Benth.) against 1UDT.

RT	Mass [M-1]H+ (m/z)	Calculated (m/z)	Fragmentation (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
11.05	415.2112	415.2108	210.1487; 182.1540; 119.0856; 100.1123	Sitosterol (C ₂₉ H ₅₀ O)	-8.90	301.39 nm

RT	Mass [M-1]H+ (m/z)	Calculated (m/z)	Fragmentatio n (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
8.75	339.1227	339.1225	361.1050; 291.0992; 182.1512; 114.0915	Sterekuntha 1 A (C ₂₀ H ₁₈ O ₅)	-8.04	1.28 µm
4.33	325.0923	325.0928	309.0984; 210.1488; 163.0395; 137.0602	(2s)-8- hydroxy-2- methyl-2- (4- methylpent -3-en-1- yl)benzo[g] chromene- 5,10-dione (C ₂₀ H ₂₀ O ₄)	-7.92	1.58 µm
6.18	496.3369	469.3333	354.2403; 337.2113; 228.1595; 182.1538	7-hydroxy- 7-methyl-1- {[3.4.5- trihydroxy- 6- (hydroxym ethyl)oxan- 2-yl]oxy}- 1h.4ah.5h.6 h.7ah- cyclopenta[c]pyran-5- yl 4- hydroxyben zoate (C ₂₂ H ₂₈ O ₁₁)	-7.40	3.74 µm
10.42	286.1435	286.1434	245.1723; 154.0861; 135.0431; 115.0537	Piperine (C ₁₇ H ₁₉ NO ₃)	-7.14	5.84 µm
9.30	511.4213	511.4206	471.3529; 314.2801; 198.1493; 114.0915	8-p- Coumaroyl harpagide (C ₂₄ H ₃₀ O ₁₂)	-6.44	18.9 µm
8.49	323.1273	323.1271	210.1483; 182.1536; 137.0594; 100.1119	2-(1.4- dihydroxyc yclohexyl)e thyl 3-(3.4- dihydroxyp henyl)prop- 2-enoate (C ₁₇ H ₂₂ O ₆)	-6.39	22.68 µm
6.36	625.4285	625.4284	454.3370; 309.2631; 228.1602; 114.0915	Verbascosi de (C ₂₉ H ₃₆ O ₁₅)	-6.06	36.16 µm
6.71	453.3391	453.3389	198.1482; 182.1541;	Cinchonain (C ₂₄ H ₂₀ O ₉)	-5.52	89.49 µm

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmentatio n (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
			140.0704; 83.0857			
5.45	349.2347	349.2360	307.0808; 245.0815; 177.0547; 128.0706	Ajugol (C ₁₅ H ₂₄ O ₉)	-5.34	120.96 μm
9.72	343.1533	343.1531	291.1016; 210.1490; 154.0867; 100.1120	Poacic Acid (C ₁₉ H ₁₈ O ₆)	-4.93	301.39 nm
4.86	163.0395	163.0390	154.0864; 128.0705; 114.0916; 100.1121	4- Coumarinol (C ₉ H ₆ O ₃)	-4.92	247.11 μm
13.16	496.3406	496.3410	459.2487; 184.0737; 125.0004; 86.0967	(2-{[(2r)-2- hydroxy-3- [(15- methylhexa decyl)oxy] propyl phosphonat o]oxy}ethy l)trimethyla zanium (C ₂₅ H ₅₄ NO ₆ P)	-3.54	2.53 mm
12.57	520.3400	520.3411	502.3279; 184.0737; 104.1070; 85.0964	Lysophosp hatidylcholi ne (C ₂₆ H ₅₀ NO ₇ P)	-3.10	5.31 mm
1.21	381.0789	381.0787	360.1513; 343.1226; 325.1129; 163.0600	[4.5- dihydroxy- 2.3- bis(hydrox ymethyl)cy clopent-2- en-1- yl]acetic acid (C ₉ H ₁₄ O ₆)	-2.91	7.35 mm
7.52	155.1430	155.1429	137.0592; 128.0898; 114.0917; 100.1119	Gentisic Acid (C ₇ H ₆ O ₄)	-2.84	8.25 mm
11.81	597.3054	597.3051	540.3054; 184.0737; 125.0000; 104.1070	(5s)-5- methyl-3- [(2r.8r.11r) -2.8.11- trihydroxy-	-1.80	47.68 mm

RT	Mass [M-1]H ⁺ (m/z)	Calculated (m/z)	Fragmentatio n (m/z)	Tentative Compound	Energy Binding (kcal/mol)	Inhibitor constant
				11- [(2r.5r.6r)- 5-hydroxy- 6- tetradecylo xan-2- yl]undecyl] -5h-furan- 2-one (C ₃₅ H ₆₄ O ₇)		
5.17	597.3985	597.3982	325.0912; 210.1487; 163.0392; 145.0285	Neoeriocitr in (C ₂₇ H ₃₂ O ₁₅)	2.73	-

Based on figure 1, the sitosterol compound has the same amino acid residues as the native ligand, namely ILE A: 824, TYR A: 664, ALA A: 779, ILE A: 768, ALA A: 767, LEU A: 765, ASN A: 661, HIS A: 613, PHE A: 786, VAL A: 782, LEU A: 804, MET A: 816, PHE A: 820 in hydrophobic bonds. The compound 7-hydroxy-7-methyl-1- $\{[3,4,5\text{-trihydroxy-6-(hydroxymethyl)oxan-2-yl}]\}$ has the same amino acid residues as the native ligand, namely ILE A:768, ALA A:767, LEU A:765, VAL A:782, PHE A:786, PHE A:820, TYR A:664, LEU A:804, HIS A:613, ALA A:823, MET A:816 in hydrophobic bonds and GLY A:817 in hydrogen bonds. While the piperine compound has the same amino acid residues as the native ligand, namely PHE A: 786, ILE A: 768, ALA A: 767, LEU A: 804, MET A: 816, LEU A: 765, PHE A: 820, VAL A: 782 in hydrophobic bonds and GLY A: 817 in hydrogen bonds. The types of bonds produced mostly occur in hydrophobic bonds, so it is predicted that the sitosterol, 7-hydroxy-7-methyl-1- $\{[3,4,5\text{-trihydroxy-6-(hydroxymethyl)oxan-2-yl}]\}$ and piperine compounds have active sides in hydrophobic bonds.

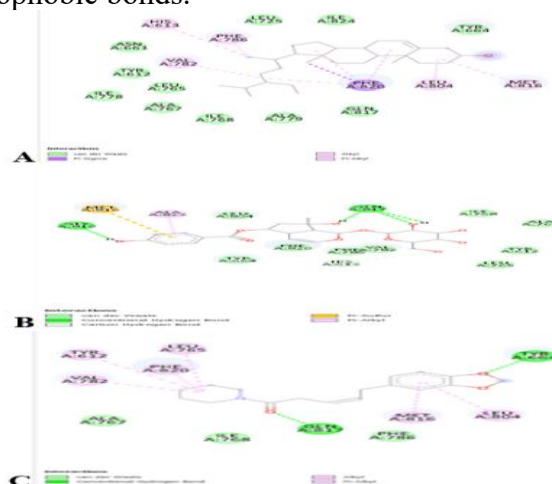
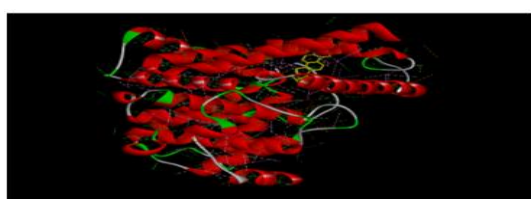


Figure 1. Protein-Ligand Interactions of the three best docking results of 70% ethanol extract samples of the pericarpium of the Kunto Bimo fruit (*Kigelia africana* (Lam.) Benth.) (A) Sitosterol (B) 7-hydroxy-7-methyl-1- $\{[3,4,5\text{-trihydroxy-6-(hydroxymethyl)oxan-2-y}$

Based on figure 2, the sitosterol compound has the same amino acid residues as the native ligand, namely ILE A: 824, TYR A: 664, ALA A: 779, ILE A: 768, ALA A: 767, LEU A: 765, ASN A: 661, HIS A: 613, PHE A: 786, VAL A: 782, LEU A: 804, MET A: 816, PHE A: 820 in hydrophobic bonds. The piperine compound has the same amino acid residues as the native ligand, namely PHE A:786, ILE A:768, ALA A:767, LEU A:804, MET A:816, LEU A:765, PHE A:820, VAL A:782 in hydrophobic bonds and GLY A:817 in hydrogen bonds. While the

5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one compound has the same amino acid residues as the native ligand, namely LEU A:765, ILE A:768, TYR A:664, ALA A:767, VAL A:782, LEU A:804, PHE A:786, PHE A:820, MET A:816 in hydrophobic bonds. The types of bonds produced often occur in hydrophobic bonds, so it is predicted that the compounds sitosterol, piperine and 5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4-one have active sides in hydrophobic bonds.

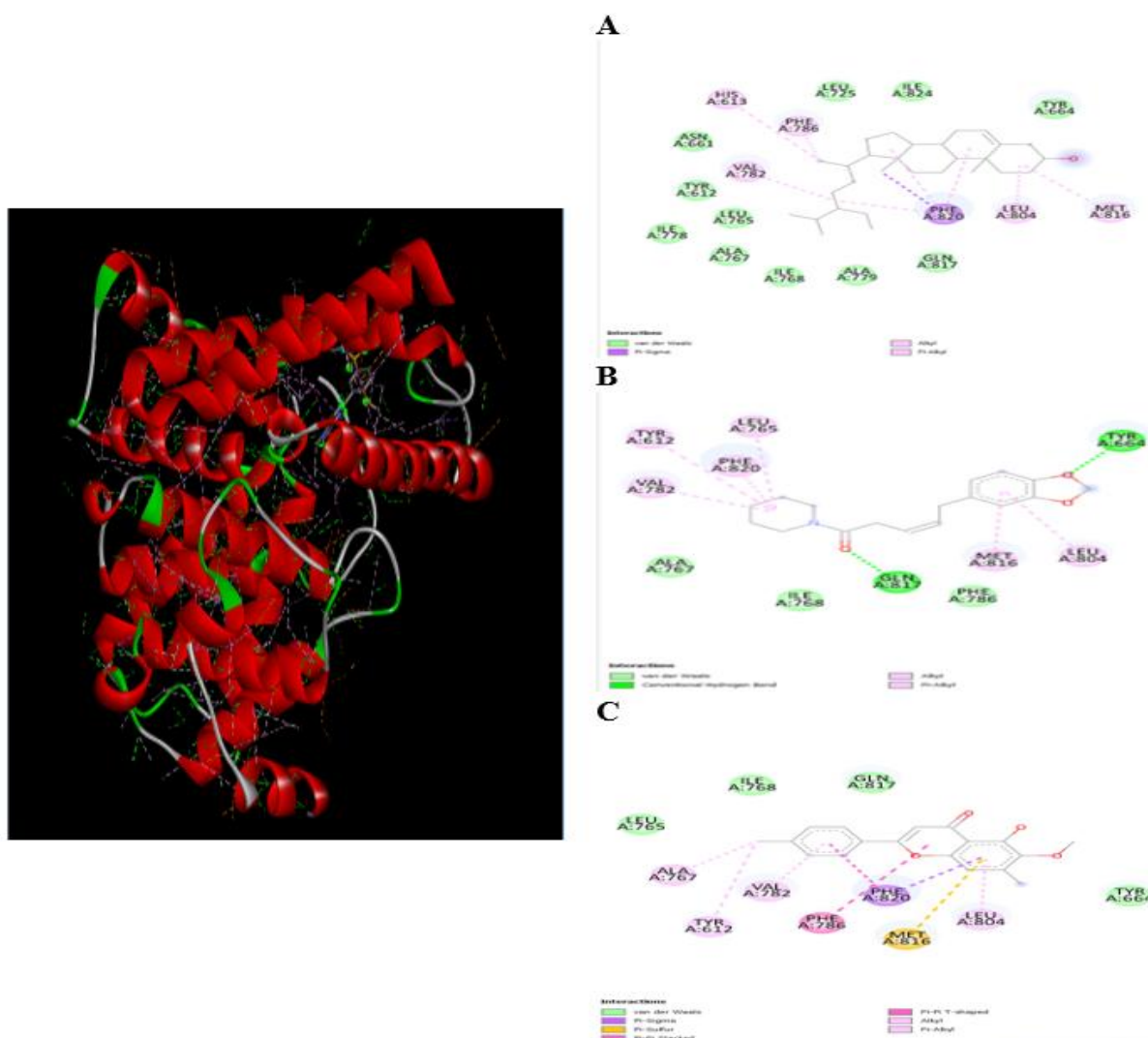


Figure 2. Protein-Ligand Interactions of the three best docking results of 70% ethanol extract samples of the fructus of the Kunto Bimo fruit (*Kigelia africana* (Lam.) Benth.) (A) Sitosterol (B) Piperine (C) 5-hydroxy-6-methoxy-7-methyl-2-(4-methylphenyl)chromen-4

Based on figure 3, the sitosterol compound has the same amino acid residues as the native ligand, namely ILE A: 824, TYR A: 664, ALA A: 779, ILE A: 768, ALA A: 767, LEU A: 765, ASN A: 661, HIS A: 613, PHE A: 786, VAL A: 782, LEU A: 804, MET A: 816, PHE A: 820 in hydrophobic bonds. The compound sterekunthal A has the same amino acid residues as the native ligand, namely ALA A:767, LEU A:765, LEU A:804, MET A:816, PHE A:820, PHE A:786, VAL A:782 in the hydrophobic bond. While the compound (2s)-8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)benzo[g]chromene-5,10-dione has the same amino acid residues as

the native ligand, namely LEU A:765, ALA A:767, PHE A:786, ALA A:783, VAL A:782, LEU A:804, MET A:816 in the hydrophobic bond. The types of bonds produced often occur in hydrophobic bonds, so it is predicted that the compounds sitosterol, sterekunthal A and (2s)-8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)benzo[g]chromene-5,10-dione have active sites in hydrophobic bonds.

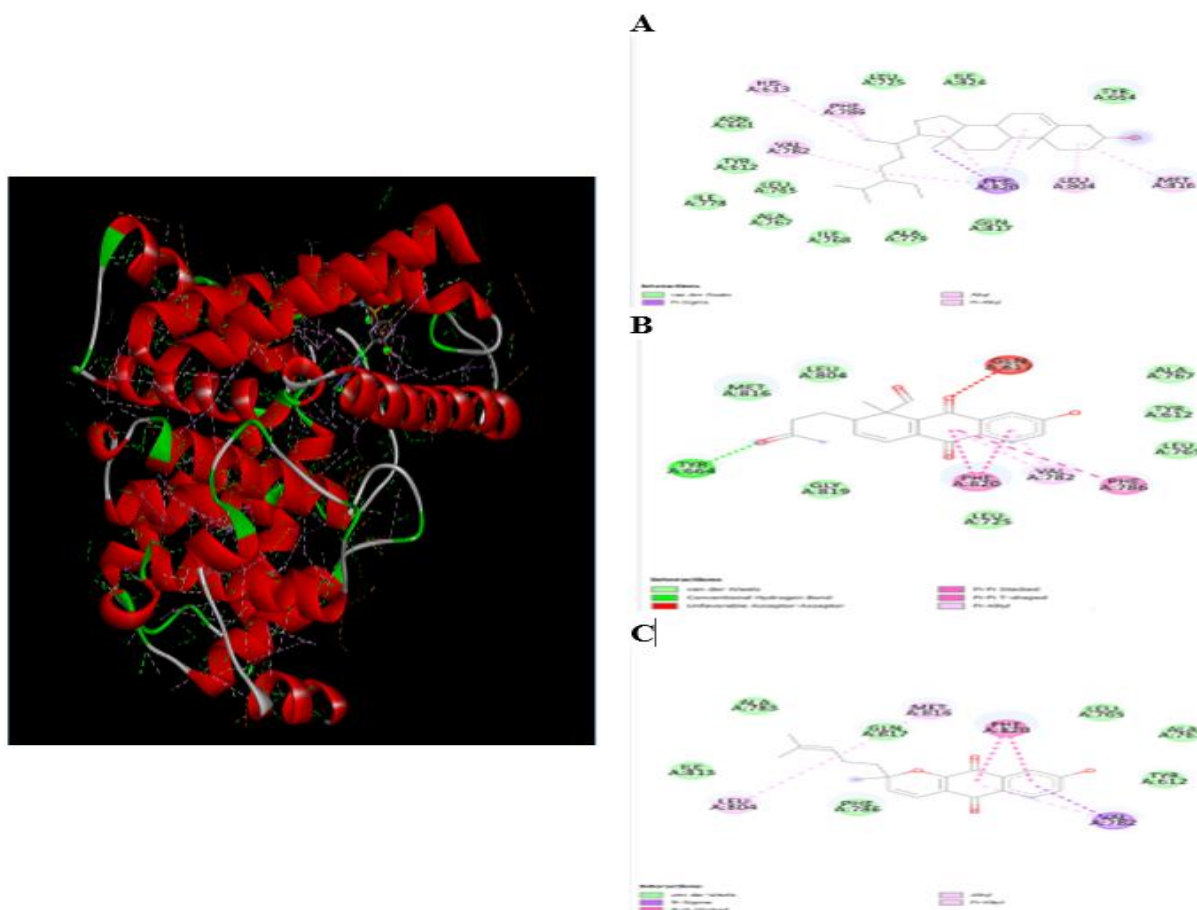


Figure 3. Protein-Ligand Interactions of the three best docking results of 70% ethanol extract samples of semen from the fruit of Kunto Bimo (*Kigelia africana* (Lam.) Benth.) (A) Sitosterol (B) Sterekunthal A (C) (2s)-8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)benzo[g]chromene-5,10-dione.

Kunto bimo fruit (*Kigelia africana* (Lam.) Benth) contains phenol, sterol, flavonoid and terpenoid compounds that are thought to have aphrodisiac effects (Bello et al., 2016). Phenol group compounds can increase spermatogenesis by affecting the hypothalamus-psituitary-godanal axis and increase testosterone by inhibiting the conversion of testosterone to estrogen involving phosphodiesterase inhibition. Sterol group compounds can activate body receptors such as testosterone hormones so that they can increase libido. Flavonoid compounds play a role in increasing testosterone hormones and encouraging sexual behaviour in men by increasing dehydroepiandrosterone levels. Flavonoids and alkaloids increase blood flow to the sexual organs by the mechanism of nitric oxide synthase (Indrisari et al., 2018). In addition, these two classes of compounds also have peripheral action, which helps relax the corpus cavernosum so that it triggers an erection (Wardani & Santoso, 2017). Terpenoid compounds can increase the concentration of testosterone in the blood (Chauhan et al., 2014).

In previous studies, the content of phenol, sterol, flavonoid and terpenoid compounds in *Kigelia africana* (Lam.) Benth plants that are efficacious as aphrodisiacs is mostly found in the

fruit. This research wants to know the compound content of each part of the *Kigelia africana* (Lam.) Benth fruit that has the most compound content that has the potential aphrodisiac activity in silico. Therefore, this study used 70% ethanol extract samples of pericarpium, fructus dan semen of *Kigelia africana* (Lam.) Benth fruit. Based on the results of the study, it can be seen that the semen of *Kigelia africana* (Lam.) Benth fruit contain compounds that have the potential for the best aphrodisiac activity based on in silico tests seen from the free energy binding. In addition, there is still no research that examines the content of compounds in each part of the *Kigelia africana* (Lam.) Benth fruit.

The results of this study can be used as a reference to find alternative plants that have the potential to have aphrodisiac effectiveness, but still need to do further research using molecular dynamic to determine the stability of the interaction between ligands and receptors.

4. CONCLUSION

Alkaloids, flavonoids, iridoids, phenolics, polyphenols, coumarins, steroids, and fatty acids are among the compounds derived from the LC-MS/MS analysis results; 18 compounds were obtained from the LC-MS/MS ethanol extract of 70% of the pericarpium of the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.); 20 compounds were obtained from the LC-MS/MS ethanol extract of 70% of the fructus of the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.); and 18 compounds derived from the LC-MS/MS ethanol extract of 70% of the semen of the kunto bimo fruit (*Kigelia africana* (Lam.) Benth.).

The bioactive compound sitosterol with ($\Delta G = -8.90$ kcal/mol) was identified in all three parts of the 70% ethanol extract sample of kunto bimo fruit (*Kigelia africana* (Lam.) Benth.). In contrast to the positive control, sildenafil ($\Delta G = -8.68$ kcal/mol), the bioactive compound sitosterol has the potential to be a PDE5 inhibitor. The high negative free binding energy (ΔG) value implies the effect of spontaneous protein-ligand binding and stabilizes the protein-ligand interaction. The lowest binding value correlates with the lowest inhibition constant (K_i) value.

REFERENCES

- Agarwal, A., Makker, K., & Sharma, R. (2008). Clinical relevance of oxidative stress in male factor infertility: An update. *American Journal of Reproductive Immunology*, 59(2), 2–11. <https://doi.org/10.1111/j.1600-0897.2007.00559.x>
- Agarwal, A., Nandipati, K. C., Sharma, R. K., Ritslining, C. D., & Raina, R. (2005). Role of oxidative stress in the pathophysiological mechanisms of erectile dysfunction. *Journal of Andrology*, 27(3), 335–347. <https://doi.org/10.2164/jandrol.05136>
- Agyare, C., David, D. O., Yaw, D. B., & Newman, O. (2013). Anti-inflammatory and analgesic activities of African medicinal plants. In *Medicinal Plant Research in Africa* (pp. 725–752). <https://doi.org/10.1016/B978-0-12-405927-6.00019-9>
- Andini, D. (2014). Potential of katuk leaf (*Sauropus androgynus* L. Merr) as aphrodisiac. *Jurnal Majority*, 3(7), 17–22.
- Arifien, A. (2013). Uji efek seduhan daun katuk (*Sauropus androgynus*) terhadap libido tikus jantan (*Rattus norvegicus*) dalam penggunaannya sebagai afrodisiaka dengan alat libidometer. *Jurnal Ilmiah Mahasiswa Universitas Surabaya*, 2(1), 1–18.
- Araujo, A. B., Durante, R., Feldman, H. A., Goldstein, I., & McKinlay, J. B. (1998). Relationship between depressive symptoms and male erectile dysfunction: Cross-sectional results from the Massachusetts Male Aging Study. *Psychosomatic Medicine*, 60(4), 458–465. <https://doi.org/10.1097/00006842-199807000-00011>
- Bello, I., Mustapha, W. M., Mustapha, M., Mohd, Z. A., & Roziathanim, M. (2016). *Kigelia africana* (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of a quintessential African traditional medicinal plant. *Journal of Ethnopharmacology*, 189,

- 253–276. <https://doi.org/10.1016/j.jep.2016.05.049>
- Böhlmann, D., Onyekwelu, J. C., & Stimm, B. (2018). *Kigelia africana* (Lam.) Benth. *Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie*, 1–15. <https://doi.org/10.1002/9783527678518.ehg2017005>
- Chambers, E. E., Fountain, K. J., Smith, N., Ashraf, L., Karalliedde, J., Cowan, D., & Legido-Quigley, C. (2014). Multidimensional LC–MS/MS enables simultaneous quantification of intact human insulin and five recombinant analogs in human plasma. *Analytical Chemistry*, 86(1), 694–702. <https://doi.org/10.1021/ac403055d>
- Chauhan, N. S. (2014). A review on plants used for improvement of sexual performance and virility. *BioMed Research International*. <https://doi.org/10.1155/2014/868062>
- Chawla, G., & Ranjan, C. (2016). Principle, instrumentation and application of UPLC: A novel technique of liquid chromatography. *Open Access Journal of Chemistry*, 3(1), 1–16. <https://doi.org/10.2174/1874842201603010001>
- Eurofins, E. (2022). *Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS)*. Eurofins. Retrieved from: <https://www.eag.com/techniques/gfaas/#>
- Gandjar, I. G. (2007). *Kimia Farmasi Analisis*. Yogyakarta: Pustaka Pelajar.
- Hafez, E. S. E. (2000). *Reproduction in Farm Animals*. USA: Lea and Febiger.
- Halder, S., & Amit, S. (2017). A review on *Kigelia africana*. *World Journal of Pharmaceutical Research*, 6(11), 389–411. Retrieved from: https://www.wjpr.net/abstract_file/7905
- Huang, S. A., & Lie, J. D. (2013). Phosphodiesterase-5 (PDE5) inhibitors in the management of erectile dysfunction. *Pharmacy and therapeutics*, 38(7), 407. Retrieved from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3776492/pdf/ptj3807407.pdf>
- Indrisari, M. (2018). Uji efek afrodisiaka dari ekstrak bawang putih (*Allium sativum*) pada hewan coba mencit (*Mus musculus*). *Akademi Farmasi Kebangsaan*, 2, 140–144.
- Istiqamah, F. (2024). Aktivitas afrodisiak ekstrak dan fraksi akar ginseng borneo (*Rennalia elliptica* Korth.) secara in silico dan in vivo. *Tesis*. Fakultas Farmasi Universitas Airlangga Surabaya.
- Jin, L., Lagoda, G., Leite, R., Webb, R. C., & Burnett, A. L. (2008). NADPH oxidase activation: Mechanism of hypertension-associated erectile dysfunction. *Journal of Sexual Medicine*, 5(3), 544–551. <https://doi.org/10.1111/j.1743-6109.2007.00733.x>
- Khairuddin, A. H. (2023). *Uji aktivitas afrodisiaka seduhan kulit buah kunto bimo (Kigelia africana (Lam.) Benth.) secara in vivo*. *Skripsi*. Fakultas Farmasi Universitas Airlangga Surabaya.
- Kunwar, A., & Priyadarsini, K. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical Allied Sciences*, 1(1), 53–60. Retrieved from: <https://jmas.in/fulltext/154-1449484220.pdf?1766553077>
- Palanichamy, C., Parasuraman, P., Theivendren, P., Sankarganesh, A., Ewa, B., Sureshbabu, R. K. P., ... Selvaraj, K. (2022). Aphrodisiac performance of bioactive compounds from *Mimosa pudica* Linn.: In silico molecular docking and dynamics simulation approach. *Molecules*, 27(12), 3799. <https://doi.org/10.3390/molecules27123799>
- Pratama, R. R., Sholikhah, I., Sahu, R. K., & Widyowati, R. (2023). Phytochemical compounds identification from 70% ethanol extract of *Arcangelisia flava* (L.) Merr stems using LC–MS/MS and in-silico molecular docking approach as inhibitor interleukin-1 β . *Pharmacognosy Journal*, 15(4). <https://doi.org/10.5530/pj.2023.15.114>
- Putri, B. E. (2014). *Kadar polifenol dan efek antioksidan ekstrak etanol buah sosis (Kigelia africana (Linn.) Benth.) serta aplikasinya dalam sabun transparan*. *Skripsi*. Universitas Kristen Satya Wacana.
- Ritchie, C., & Ko, E. Y. (2021). Oxidative stress in the pathophysiology of male infertility. *Andrologia*, 53(1), e13581. <https://doi.org/10.1111/and.13581>
- Roy, K., Kar, S., & Das, R. N. (2015). Understanding the basics of QSAR for applications in pharmaceutical sciences and risk assessment. <https://doi.org/10.1016/B978-0-12->

- 801505-6.00010-7
- Rusdi, N. K., Ni Putu, E. H., & Maifitrianti, Y. S. U. (2018). Aktivitas afrodisiaka fraksi dari ekstrak etanol 70% daun katuk (*Sauropus androgynus* (L.) Merr) pada tikus putih jantan. *Pharmaceutical Sciences and Research*, 5(3), 123–132. <https://doi.org/10.7454/psr.v5i3.4100>
- Sikka, S. C. (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, 8(7), 851–862. <https://doi.org/10.2174/0929867013373039>
- Sikka, S. C., Rajasekaran, M., & Hellstrom, W. J. (1995). Role of oxidative stress and antioxidants in male infertility. *Journal of andrology*, 16, 464-468. <https://doi.org/10.1002/j.1939-4640.1995.tb00566.x>
- Wahdaningsih, H. S., Sartika, D., & Fajriaty, I. (2012). Uji Aktivitas Afrodisiaka Ekstrak Etanol 70% Daun Tapak Liman pada Mencit Putih Jantan Galur BALB/C. *Skripsi*. Pontianak: Fakultas Kedokteran, Universitas Tanjungpura.
- Wardani, I. G. A. A. K., & Santoso, P. (2017). Efektivitas afrodisiaka dari ekstrak etanol jahe merah (*Zingiber officinale* Roscoe) pada tikus (*Rattus norvegicus* L.) putih jantan. *Jurnal Ilmiah Medicamento*, 3(1), 22–28. <https://doi.org/10.36733/medicamento.v3i1.1045>
- Yafi, F. A., Jenkins, L., Albersen, M., Corona, G., Isidori, A. M., Goldfarb, S., ... & Hellstrom, W. J. (2016). Erectile dysfunction. *Nature reviews Disease primers*, 2(1), 1-20. <https://doi.org/10.1038/nrdp.2016.3>
- Zulkarnain, S., Sijid, S. A., Amrullah, S. H., & Rukmana, R. (2022). Keanekaragaman tanaman berpotensi sebagai afrodisiak alami. *Teknosains: Media Informasi Sains dan Teknologi*, 16(2), 255–260. <https://doi.org/10.24252/teknosains.v16i2.28752>