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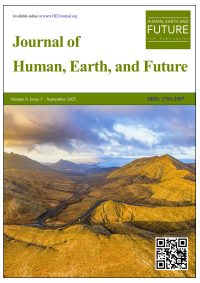

# Anti-Fertility Potential of Aceh Areca Nut Seed Extract: Phytochemical Analysis and Molecular Docking Study

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**Abstract**

Areca nut seeds are rich in alkaloids, terpenoids, flavonoids, phenolics, and tannins, which exhibit antibacterial, anti-oxidant, anti-inflammatory, and anti-fertility properties. However, the specific active constituents of Aceh areca nut seeds and their impact on anti-fertility effects remain unknown. This study aims to identify the active compounds in the ethanolic extract of locally obtained Aceh areca nut (*Areca catechu*) seeds and assess their potential as inhibitors of Follicle Stimulating Hormone (FSH), testosterone, Bone Morphogenic Protein 4 (BMP4), Mothers Against Decapentaplegic Signaling 1 (SMAD1), and protamine 1 by molecular docking approach. The composition of the ethanolic extract of Acehnese areca nut seeds was determined through phytochemical analysis, 2,2-diphenyl-1picrylhydrazyl (DPPH) assay, Fourier Transform Infra-Red (FT-IR), Gas Chromatography-Mass Spectrometry (GC-MS) analysis while the biological activity of its compounds was evaluated through molecular docking. Phytochemical test results showed the presence of alkaloids, terpenoids, flavonoids, phenolics and tannins in the ethanolic extract of Aceh areca nut seeds. The antioxidant activity was very strong with an $IC_{50}$ value of 28.215 ppm. GC-MS analysis identified the compound *n*-hexadecanoic acid (32.29%) as the main component in the ethanolic extract of Aceh areca nut seeds, along with *cis*-vaccenic acid (14.75%) which is a fatty acid. Molecular docking analysis showed that lupeol and stigmasta-3,5-diene demonstrated superior binding energies compared to a standard anti-fertility agent. These findings suggest that compounds from the ethanolic extract of Acehnese areca nut seeds have potential as inhibitors of FSH, testosterone, protamine 1, BMP4, SMAD1 based on their binding energies for further testing as new anti-fertility agents.

*Keywords:* Lupeol; Stigmasta-3,5-Diene; Areca Nut Seeds; BMP4; SMAD1; Protamine 1.

## 1. Introduction

*Areca catechu*, referred to as the betel nut, is a tropical plant with a longstanding cultural and medicinal significance in South and Southeast Asia. The seeds of *A. catechu* are rich in bioactive components, including alkaloids, flavonoids, polyphenols, tannins, and saponins, which exhibit various pharmacological properties, such as antioxidant, antibacterial, anti-inflammatory, and antifertility effects [1-3]. Metabolomic analysis of *A. catechu* showed that the

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seeds contained lipids, nucleosides and nucleotides, organic acids and derivatives, organic oxygen compounds, organoheterocyclic compounds, phenylpropanoids, and polyketides [4]. Despite its widespread use, the pharmacological effects of its ethanolic extracts, particularly their potential for addressing global health concerns, are still limited.

The ethanolic extraction procedure is essential for isolating a wide range of bioactive chemicals and increasing their therapeutic potential. Recent research has discovered the antifertility properties of *A. catechu*, with significant influences on spermatogenesis, testosterone levels, and sperm viability in animal models, indicating its potential as a natural contraceptive agent [5, 6]. The antifertility effects of *A. catechu* are mediated by several mechanisms that involve alterations in testicular function, sperm production, and hormonal balance. Studies using male rats demonstrate that *A. catechu* induces significant testicular damage, including reduced testis weight, degeneration of seminiferous tubules, and necrosis of germinal cells. These structural changes are associated with reduced sperm count and motility, as well as impaired progressive movement of spermatozoa [7]. The biochemical profile of the tests indicates decreased protein content and elevated cholesterol levels, suggesting disrupted steroidogenesis. Histopathological assessments reveal atrophy and cytolysis within the seminiferous tubules, with some studies noting reductions in secondary spermatocytes, spermatids, and fluid accumulation in testicular tissues at higher doses of *A. catechu* [8]. Despite these findings, the precise molecular mechanisms underlying the antifertility effects of *A. catechu* remain inadequately understood.

Follicle-stimulating hormone (FSH) is crucial for spermatogenesis in males, stimulating the Sertoli cells in the testes, which support sperm production. Elevated FSH levels are often associated with impaired spermatogenesis and conditions like azoospermia (absence of sperm in semen) [9]. Testosterone, produced by Leydig cells in response to luteinizing hormone (LH), is essential for the development of male secondary sexual characteristics and the maturation of sperm. Studies indicate that testosterone levels can influence FSH secretion; however, the exact relationship can vary depending on individual health conditions [10]. Protamine 1 is a protein that replaces histones in sperm cells during spermatogenesis, playing a significant role in sperm chromatin packaging and stability. The expression of protamine 1 can be affected by hormonal levels, including testosterone and FSH, indicating a potential link between these hormones and sperm quality [10, 11]. Bone Morphogenetic Protein 4 (BMP4) is involved in various developmental processes, including those related to reproductive tissues. It has been shown to play a role in the differentiation of germ cells. The interaction between BMP4 and gonadotropins like FSH may influence spermatogenesis and testicular function [11]. SMAD1 is a signaling protein that mediates BMP signaling pathways. It plays a critical role in cellular responses to BMPs, including those involved in reproductive development. Changes in SMAD1 activity could potentially affect how FSH and BMP4 influence spermatogenesis and overall testicular health [11]. There is a complex interplay where FSH stimulates Sertoli cells to support spermatogenesis, which is dependent on adequate testosterone levels for optimal function. Higher FSH levels may correlate with increased protamine expression, impacting sperm quality. BMP4 signaling through SMAD1 may modulate the effects of FSH on germ cell development. While direct correlations among these factors require further empirical investigation, existing studies suggest significant interactions that are crucial for understanding male fertility and spermatogenesis. Future research could provide clearer insights into these relationships and their implications for reproductive health.

The primary objective of this study is to examine the bioactive compounds that are contained in the ethanolic extract of young *A. catechu* (betel nut) seeds and to evaluate the potential antifertility effects of these compounds using an in-silico methodology. To be more specific, the research focuses on critical reproductive-regulating molecules such as follicle stimulating hormone (FSH), testosterone, bone morphogenetic protein 4 (BMP4), SMAD1, and protamine 1 in order to provide light on the molecular pathways that are responsible for the extract's antifertility characteristics. These proteins play pivotal roles in spermatogenesis and overall male fertility. The focus of this attention is in line with the worldwide need for natural contraceptives that are derived from plants and that reduce the negative effects that are typically linked with synthetic alternatives. Through the utilization of molecular docking, this study fills a significant void in our understanding of the ways in which the ethanolic extracts of *A. catechu* alter reproductive pathways. This research also provides insights into the possibility of these extracts as non-invasive fertility inhibitors.

## 2. Material and Methods

### 2.1. Plant Sampling

Areca nut (*A. catechu*) seeds were collected from Lam Teuba sub-district, Aceh Besar, Indonesia, with coordinates 5°29'35.7 "N and 95°36'49.1 "E (Figure 1). Plant specimens were collected and taxonomically identified at the Biosystematics Laboratory, Faculty of Mathematics and Natural Sciences, Department of Biology, Syiah Kuala University, Banda Aceh, Indonesia.

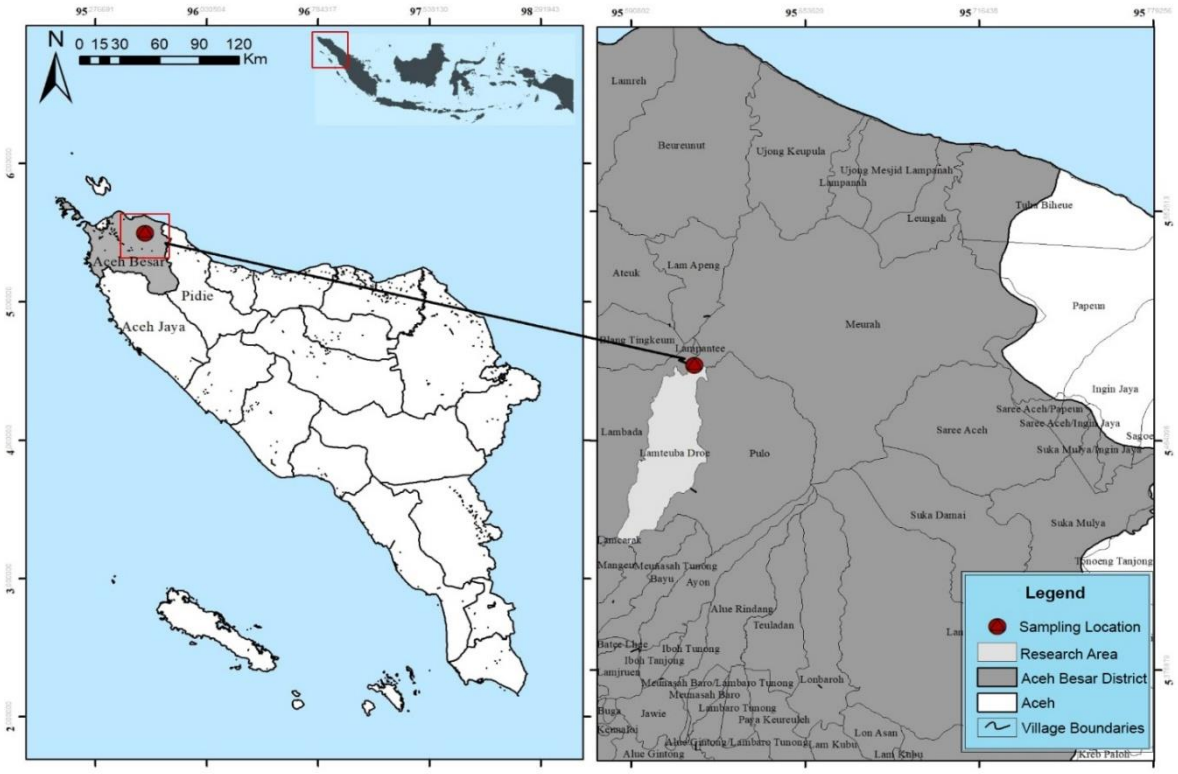



**Figure 1. Sampling point of this research in Lam Teuba sub-district, Aceh Besar, Indonesia**

### 2.2. Extraction Process

Fifteen kilograms of fresh areca nut seeds (*A. catechu*) were meticulously cleaned with tap water, separating the seeds from the skins. Subsequently, the seeds were cut with a knife and dried for a duration of 6 to 7 days. The sample was subsequently mashed with a blender until it transformed into a powdered form of areca nut dried samples. Next, the dried samples were hydrated with 10% ammonia for one night. Macerated in a macerator tube with technical ethanol at a ratio of 1:10 for 48 hours. Maceration was conducted for 2 days, followed by an additional 2 days of maceration in a light-protected environment, with stirring occurring once per 24 hours. The initial macerate extract was filtered with flannel fabric. Subsequently, maceration was performed. The outcomes of the initial and secondary macerates were gathered and evaporated with a rotary evaporator at 45°C to yield a concentrated extract.

### 2.3. Phytochemical Screening

This procedure is conducted according to the standard approach delineated by Harborne (1987) [12]. Screening was performed on seven categories of secondary metabolites: alkaloids, flavonoids, saponins, tannins, phenolics, and terpenoids [13].

### 2.4. Fourier Transform Infra-Red (FT-IR) Analysis

Fourier transform infra-red spectroscopy (FT-IR) is a high-resolution analytical method used to detect bioactive chemicals by analyzing their functional groups and elucidating their structures. In FT-IR molecules show absorption in a specific frequency range. Organic compounds are predominantly absorbed within the range of 4000-400 $cm^{-1}$, which is crucial for the identification and characterization of the chemicals present in the respective extracts. Ten milligrams of the dried extract powder were encapsulated in one hundred milligrams of KBr pellets. A powdered sample of each plant species was placed in an FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan), with scans conducted from 400 to 4000 $cm^{-1}$ at a resolution of 4 cm.

### 2.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was conducted at the Chemical Instrument Laboratory, Universitas Syiah Kuala, utilising a non-polar TraceGOLD TG-5MS column (30 m × 0.25 mm × 0.25 µm) from Thermo Fisher Scientific Inc., USA. The carrier gas employed was helium, with a column flow rate of 0.5 mL/min. One microlitre of ethanol extract from areca nut seeds was fed into the gas chromatography equipment for analysis by the mass spectrometry detector. The outcomes of this compound separation were then examined utilising Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2 [14].

### 2.6. Antioxidant Activity

The test begins with the sample solution being prepared by weighing 2 grammes of dry powder and depositing it in a 75 mL beaker. Next, freshwater is boiled and brewed at 70°C, then poured into the beaker containing the sample, covered, and left for 6 minutes. Following that, 2,2-diphenyl-1picrylhydrazyl (DPPH) solution (0.5 mM) is made by weighing 9.8 mg of DPPH powder and dissolving it in ethanol to a volume of 50 mL. Following the preparation of the sample solution, the antioxidant activity of radical scavenging is determined using the DPPH technique, which is modified from Molynuex (2003) [15]. Specifically, 0.5 mL of the dry powder infusion at the prescribed concentration is mixed with 2 mL of 0.1 mM DPPH, the combination is vortexed, and the solution is incubated at room temperature for 30 minutes in the dark before being measured at the maximum lambda of 516 nm. The same process is followed for the blank solution (DPPH solution without the test material), which contains 2 mL of DPPH and 1 mL of ethanol.

### 2.7. Molecular Docking Analysis

The selected target proteins include FSH, BMP4, SMAD1, protamine 1, and testosterone. The three-dimensional structures of BMP4, SMAD1, and Protamine 1 are available for download from the Protein Data Bank (PDB) by Berman et al. [16]. The downloaded proteins were dehydrated, optimised, and designated to the active sites of BMP4, SMAD1, and Protamine 1. Target proteins were subsequently processed with BIOVIA Discovery Studio software and saved in .pdb format [17]. The active component in the target protein is the active chemical derived from the ethanol extract of seeds. Dimethandrolone Undecanoate (DMAU) served as the control ligand. Ligands can be obtained from the PubChem database [18]. Molecular docking of receptors (FSH protein, BMP4, SMAD1, protamine 1, testosterone) utilising Autodock Vina Software in conjunction with PyRx [19]. The ligand molecule will bind to the active site of the receptor, thereby inhibiting its function, and ultimately, the ligand may serve as a pharmaceutical agent. The examination of docking data involved examining the nature of molecular bond interactions between proteins and ligands. Figure 2 illustrates the flowchart's methodology in detail.

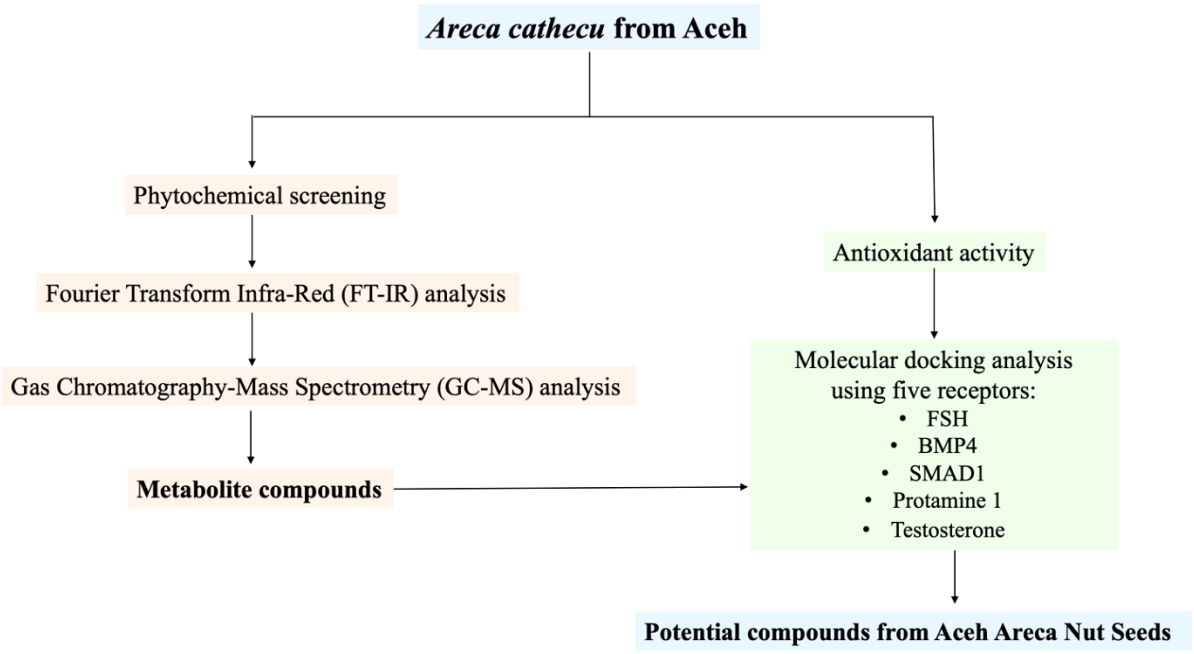



**Figure 2. Methodological Flowchart of the Study**

## 3. Results and Discussions

The qualitative phytochemical screening results presented in Table 1 indicate that the ethanol extract of *A. catechu* seeds has secondary metabolite components such as alkaloids, triterpenoids, flavonoids, phenolics, and tannins. This phytochemical profile aligns with previous research indicating that areca nuts contain a variety of bioactive compounds, including alkaloids, flavonoids, tannins, fatty acids, triterpenes, and steroids [1]. The ethanol extract of *A. catechu* seeds is rich in secondary metabolites such as alkaloids, triterpenoids, flavonoids, phenolics, and tannins, each contributing to the plant's diverse pharmacological properties [20]. However, the majority of current research on areca nuts focuses on complex extracts, while investigations of natural compounds are often limited to alkaloids or polyphenols. Consequently, numerous specific bioactive compounds warrant further investigation.

**Table 1. Phytochemical screening of ethanol extract of areca nut (*A. catechu*) seeds**

| Metabolite Content | Reagents | Results | Description |
|---|---|---|---|
| Alkaloids | Mayer | + | White precipitate formed |
| | Wagner | + | There is brown sediment |
| | Dragendorff | + | There is red sediment |
| Steroids | Liebermann-Buchard Test | - | No green color formed |
| Terpenoids | Liebermann-Buchard Test | + | Formed red color |
| Saponins | Shuffling | - | No foaming |
| Flavonoids | HCl and Metal Mg | + | Purple color formed |
| Phenolic | $FeCl_3$ | + | Blue color formed |
| Tannins | Gelatin+$H_2SO_4$ | + | White precipitate formed |

Descriptions: (+) detected and (-) not detected in ethanol extract of *A. catechu*

The Figure 3 appears to be an FT-IR spectrum of a sample ethanolic extract od *A. catechu*. FT-IR (Fourier Transform Infra-Red) spectroscopy is used to identify functional groups and analyze molecular structures based on their infra-red absorption patterns. Higher wavenumbers correspond to higher energy vibrations (e.g., stretching of bonds), while lower wavenumbers correspond to lower energy (e.g., bending vibrations) [21]. The FT-IR spectrum of Areca nut reveals the presence of various functional groups associated with phytochemicals and bioactive compounds. Key peaks and their functional groups, included 3398 $cm^{-1}$. A strong and broad peak around 3398 $cm^{-1}$ confirms the presence of phenolic and alcoholic compounds. These are likely from tannins and flavonoids, which contribute to the nut's antioxidant properties. This broad peak suggests O-H stretching, commonly associated with a hallmark of hydroxyl groups in both alcohols and phenols, abundant in plant extracts [22]. Fatty acids and lipids also detected in this study. The carbonyl stretch is sharp and prominent, indicating the presence of compounds like fatty acids or esterified phytochemicals. Evident from C=O (~1728 $cm^{-1}$) and C-H stretches (~2925 and 2851 $cm^{-1}$). Peaks around 1612 $cm^{-1}$ (C=C stretching) and 789 $cm^{-1}$ (C-H out-of-plane bending) suggest the presence of aromatic compounds like flavonoids and polyphenols, key contributors to the nut's bioactivity. Next, vibrations in the range 1100–1000 $cm^{-1}$ indicate the presence of C-O bonds, which are typical of alcohols, ethers, or carbohydrates. This might reflect glycosides or polysaccharides in the sample. These findings align with the known phytochemical profile of Areca nut, which includes antioxidants, alkaloids, flavonoids, and tannins, making it a potential candidate for further pharmaceutical studies.

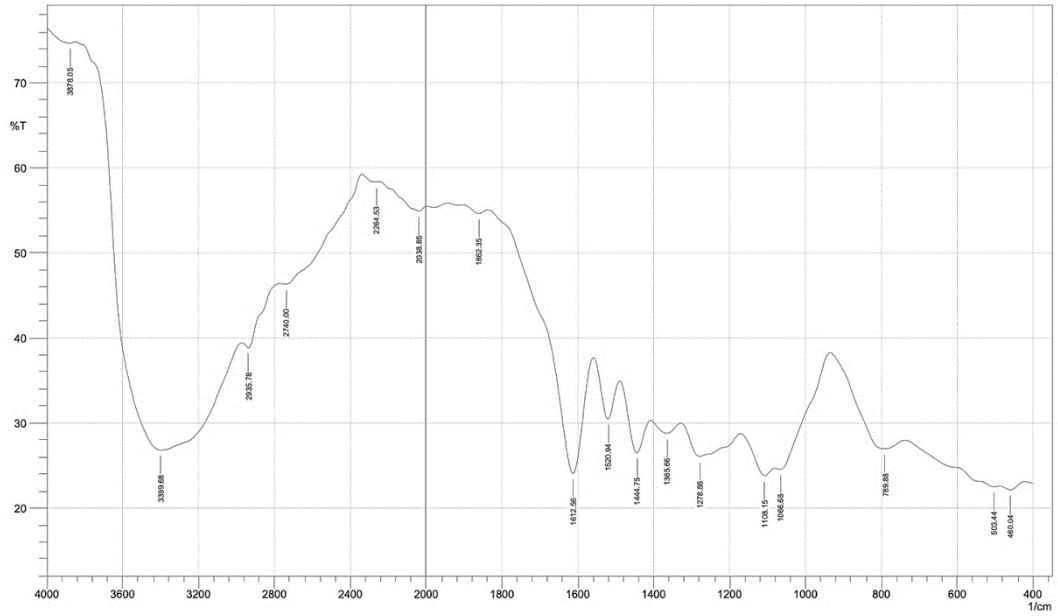



**Figure 3. FT-IR analysis of ethanolic extract *A. catechu***

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis is a type of analysis that is carried out on chemicals under conditions of high vacuum and low pressure when they are heated. This GC-MS study was carried out on the ethanol extract of the seeds of the *A. catechu* plant. The chromatogram profile of the ethanol extract of *A. catechu* seeds is displayed in Figure 4. The GC-MS analysis revealed 19 bioactive compounds in the ethanolic extract of *A. catechu* seeds, categorized into various chemical groups based on their structure and function (Table 2).

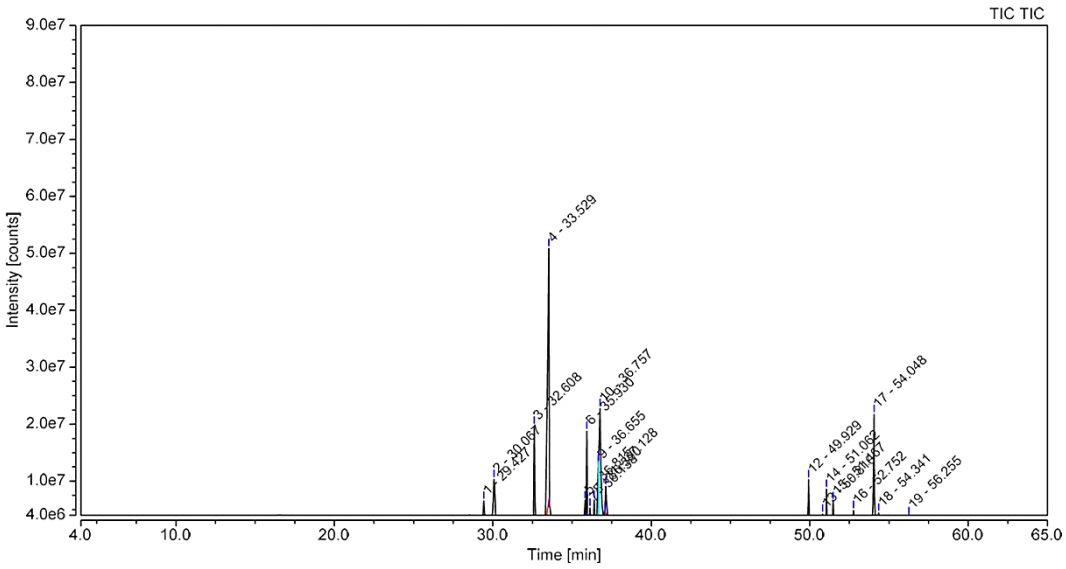



**Figure 4. Total ion chromatogram of GC-MS analysis from ethanolic extract *A. catechu***

The extract of *A. catechu* contains a diverse array of bioactive compounds, predominantly fatty acids and their derivatives, alongside significant contributions from steroids, terpenoids, and lipophilic compounds (Table 2). Fatty acids and their esters dominate the extract, accounting for a significant portion of the area. These compounds are well-known for their biological roles in metabolism, membrane fluidity, and anti-inflammatory activity. *n*-Hexadecanoic acid (palmitic acid) with retention time: 33.52 min; area: 32.29%; SI: 99.55% is the most abundant compound, highlighting its potential as a primary bioactive component. *cis*-Vaccenic acid (RT: 36.75 min; Area: 14.75%; SI: 99.32%) contributes significantly, known for cardiovascular benefits. Other fatty acids include tetradecanoic acid (RT: 29.42 min), octadecanoic acid (RT: 37.12 min), and various methyl esters like hexadecanoic acid, methyl ester (RT: 32.60 min).

**Table 2. Information about phytocompounds from GC-MS analysis**

| No. | Compounds Name | Retention Time (min) | Area (%) | Similarity Index (% ) | Compound Category |
|---|---|---|---|---|---|
| 1 | Tetradecanoic acid | 29.42 | 2.56 | 96.12 | Fatty Acids |
| 2 | 5,5,8a-Trimethyl-3,5,6,7,8,8ahexahydro-2H chromene | 30.06 | 6.08 | 94.63 | Aliphatic heteropolycyclic compounds (Benzopyrans) |
| 3 | Hexadecanoic acid, methyl ester | 32.60 | 7.37 | 98.89 | Fatty Acids |
| 4 | *n*-Hexadecanoic acid | 33.52 | 32.29 | 99.55 | Fatty Acids |
| 5 | 9,12-Octadecadienoic acid, methyl ester | 35.81 | 1.66 | 95.14 | Fatty Acids |
| 6 | *trans*-13-Octadecenoic acid, methyl ester | 35.93 | 5.60 | 99.23 | Fatty Acids |
| 7 | Phytol | 36.13 | 1.29 | 95.19 | Terpenoids (Acyclic diterpenoids) |
| 8 | Methyl stearate | 36.39 | 1.62 | 97.46 | Fatty acid methyl esters |
| 9 | (Z)-18-Octadec-9-enolide | 36.65 | 5.26 | 99.05 | Phenylpropanoids and polyketides (Macrolides and analogues) |
| 10 | *cis*-Vaccenic acid | 36.75 | 14.75 | 99.32 | Fatty Acids |
| 11 | Octadecanoic acid | 37.12 | 2.10 | 98.55 | Fatty Acids |
| 12 | Farnesyl bromide | 49.92 | 2.91 | 94.70 | Terpenoids (Sesquiterpenoids) |
| 13 | 1-Heptatriacotanol | 50.81 | 0.88 | 95.61 | Fatty alcohols |
| 14 | Stigmasta-3,5-diene | 51.06 | 2.27 | 99.13 | Steroids |
| 15 | Vitamin E | 51.46 | 1.55 | 97.26 | Lipophilic compounds |
| 16 | Campesterol | 52.75 | 1.17 | 98.20 | Steroids |
| 17 | *y*-Sitosterol | 54.04 | 8.56 | 99.43 | Steroids |
| 18 | Lupeol | 54.34 | 1.04 | 98.18 | Terpenoid (Triterpenoids) |
| 19 | Stigmast-4-en-3-one | 56.25 | 1.02 | 84.70 | Steroids |

The secondary metabolites classed from GC-MS can be seen into eight classes based on their chemical properties, with their corresponding totals representing the frequency of occurrence. These classes include fatty acids, steroids, terpenoids, lipophilic compounds, phenylpropanoids, aliphatic heteropolycyclic compounds, fatty acid methyl esters, and fatty alcohols (Figure 5). The majority of secondary metabolites belong to the fatty acids class, comprising 43.8% (7/16) of the total. This dominance highlights the significant presence and potential bioactive roles of fatty acids in the analyzed samples, often linked to membrane stability and signaling mechanisms in biological systems [23]. Steroids (4 occurrences, 25%) and terpenoids (3 occurrences, 18.8%) are also prominent. Steroids are essential biomolecules involved in various physiological processes, while terpenoids are known for their wide range of therapeutic applications, including antimicrobial, anti-inflammatory, and antioxidant activities [24, 25].

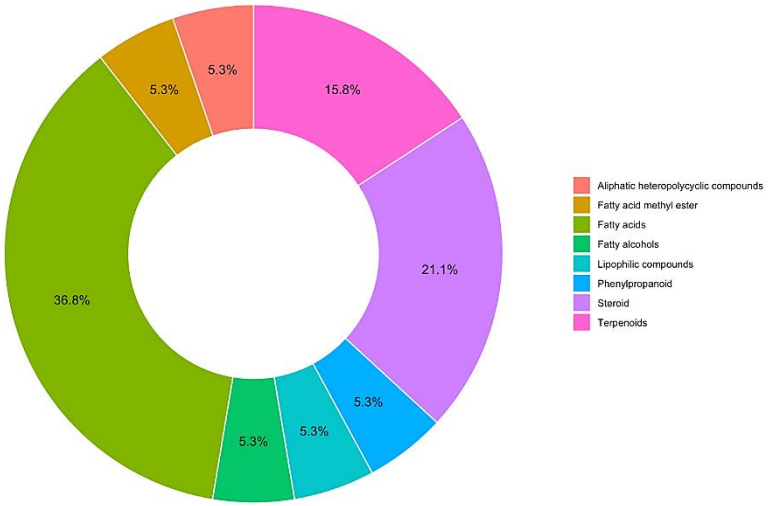



**Figure 5. Proportion of secondary metabolite classes from GC-MS analysis**

Antioxidant activity of plant extracts provides clues to identify the active compounds that play a role in plants [26]. The importance of antioxidant activity is directly or indirectly related to the many treatments for various neurodegenerative diseases, cardiovascular, asthma, diabetes, and cancer [27]. Antioxidant activity is classified into several categories including very strong (<50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and weak (150-200 ppm) [28]. Based on the antioxidant test data from Table 3, the ethanol extract of *A. catechu* seeds showed an $IC_{50}$ value of 28.215 ppm with a very strong category with an $IC_{50}$ value of <50 ppm. Oxidative stress significantly influences male and female fertility by impacting sperm functionality, oocyte quality, and hormonal regulation. Some studies suggest that antioxidants could protect reproductive cells, but others suggest that elevated amounts could interfere with normal reproductive signalling [29, 30]. To evaluate these potential interactions, in vitro and in vivo studies are necessary to examine the extract's direct effects on reproductive cells and hormone levels. In this case, it shows that *A. catechu* seeds have great potential as a major source of antioxidants for future drug development.

**Table 3. Antioxidant activity of ethanolic extract *A. catechu* compare with ascorbic acid**

| Sample | Concentration (ppm) | Absorbance | Percent inhibition (%) | Linear regression | $IC_{50}$ (ppm) |
|---|---|---|---|---|---|
| Ethanolic extract of *A. catechu* | 100 | 0.199 | 79.67 | y = 0.4723x + 36.674 | 28.215 |
| | 50 | 0.323 | 67.01 | | |
| | 25 | 0.462 | 52.81 | | |
| | 12.5 | 0.529 | 45.97 | | |
| | 6.25 | 0.691 | 29.42 | | |
| Ascorbic acid | 15 | 0.085 | 90.70 | y = 3.8509x - 39.397 | 2.753 |
| | 12 | 0.109 | 88.87 | | |
| | 9 | 0.217 | 77.83 | | |
| | 6 | 0.284 | 70.99 | | |
| | 3 | 0.569 | 41.88 | | |

Understanding the molecular interactions among follicle-stimulating hormone (FSH), testosterone, protamine 1, bone morphogenetic protein 4 (BMP4), and SMAD1 is crucial in reproductive biology and hormonal signaling. FSH is essential for gametogenesis, stimulating follicular growth in females and spermatogenesis in males by binding to the

FSH receptor (FSHR) and activating downstream signaling pathways. Testosterone, a key androgen hormone, influences reproductive functions by interacting with androgen receptors, thereby regulating genes involved in spermatogenesis and overall reproductive health. These molecular interactions underscore the complex regulatory mechanisms in reproductive biology and highlight potential avenues for therapeutic intervention. Phytochemical compounds like lupeol and stigmasta-3,5-diene demonstrate stronger binding affinities with values as low as -9.6, indicating potentially higher stability in binding interactions (Figure 6). These ligands may have greater potential for therapeutic effectiveness or receptor modulation. Dimethandrolone undecanoate (DMAU) serves as a positive control for binding affinities, with moderate binding ranging from -5.2 to -6.7 Kcal/mol across receptors. Dimethandrolone undecanoate, also known as 7a,11b-Dimethyl-19-nortestosterone 17b-undecanoate, is an experimental male hormonal contraceptive that has great potential. DMAU is an oral experimental male hormonal contraception that functions as a prodrug, rapidly converting to its active metabolite, dimethandrolone (DMA). The inadequate and inconsistent oral bioavailability of DMA following DMAU administration poses a significant obstacle to its development as an oral medication [31]. Consequently, there is significant opportunity to investigate possible candidates for anti-fertility drugs.

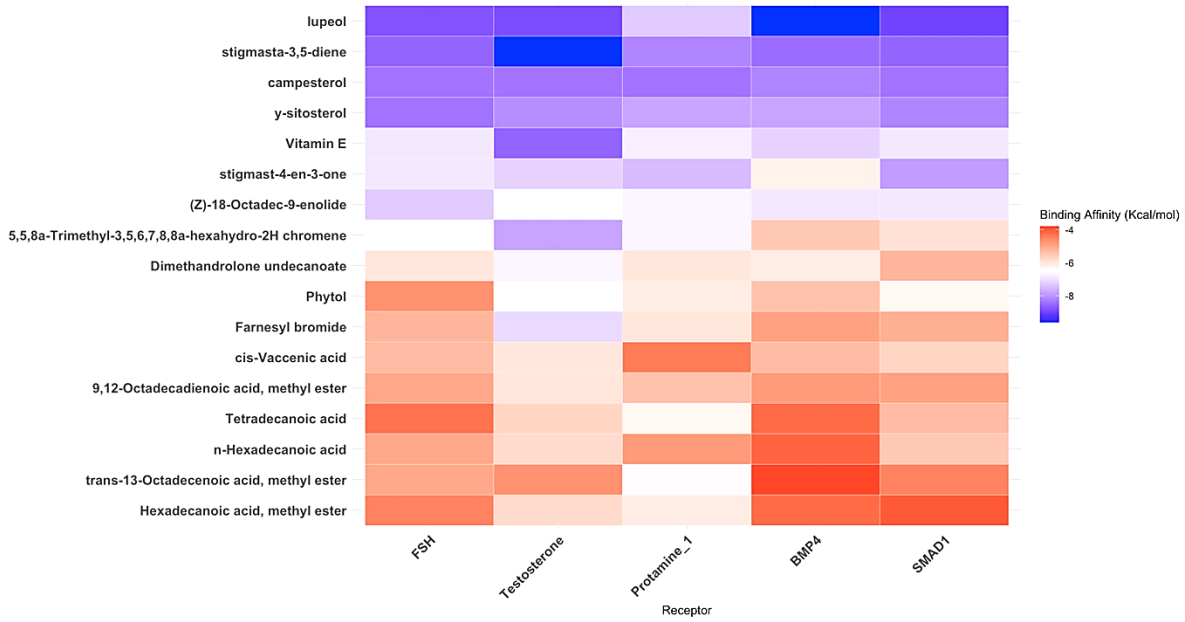



**Figure 6. Molecular docking results of active compounds from *A. catechu* extract**

Lupeol (−8.8 kcal/mol) demonstrated stronger binding affinity toward the FSH receptor than DMAU (−6.0 kcal/mol). Similarly, stigmasta-3,5-diene (−9.6 kcal/mol) bound more tightly to the testosterone receptor compared to DMAU (−6.7 kcal/mol). Compesterol (−8.4 kcal/mol) also showed higher affinity for the Protamine 1 receptor than DMAU (−6.0 kcal/mol). In addition, lupeol displayed stronger interactions with both BMP4 (−9.6 kcal/mol) and SMAD1 (−9.0 kcal/mol) receptors relative to DMAU (−6.1 kcal/mol and −5.2 kcal/mol, respectively) (Table 4). Overall, certain receptors—particularly the testosterone receptor—exhibited consistently lower (more negative) binding energy values across several ligands, suggesting a more stable and robust binding profile. This aligns with the biological role of testosterone-androgen receptor binding as a key mechanism supporting spermatogenesis [32].

Molecular docking studies have elucidated that lupeol exhibits significant interactions with various protein targets, primarily through hydrophobic forces and hydrogen bonding. For instance, docking analyses of lupeol with cancer-related proteins such as BCL-2, topoisomerase, PTK, mTOR, and PI3K revealed favorable binding energies and inhibition constants, underscoring its potential as a therapeutic agent. Research on animals has shown that lupeol is safe to use [33]. There were no documented side effects or deaths in rats or mice after 96 hours of monitoring when 2 g/kg of lupeol was given orally. The cumulative results of these investigations strongly suggest that lupeol are an effective and safe for human use [34, 35].

While studies directly connecting lupeol or other compounds to FSH or BMP4 are limited, these phytosterols have demonstrated broad biological effects, including anti-inflammatory and antioxidant properties, which could indirectly influence protein signaling pathways [36]. Metabolite compounds with strong affinity for testosterone receptors can stabilise this hormone-receptor interaction, which is crucial for sperm production and the maintenance of male reproductive health. In contrast, BMP4 shows varied affinities, with some ligands binding more strongly than others,

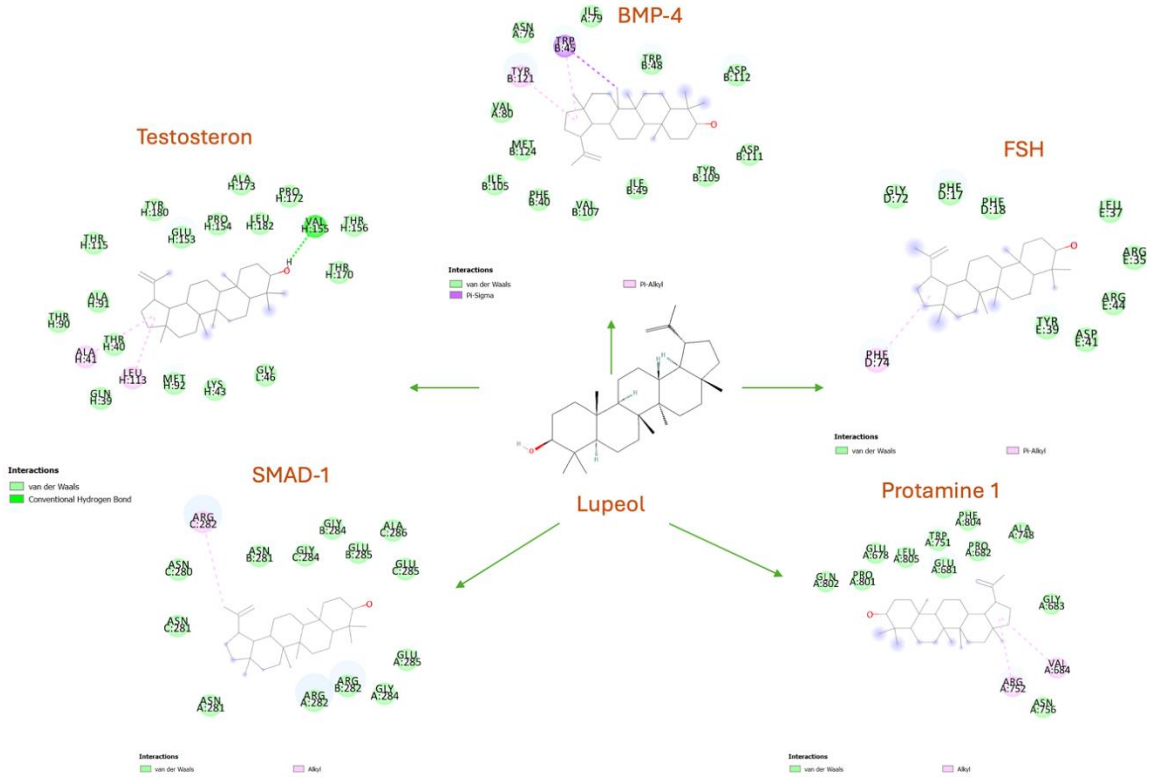
which might reflect selective binding preferences in structural compatibility or biochemical interactions. BMP4 is influential in testicular development and function. BMP signalling in the testes regulates the differentiation of germ cells, which are the precursor cells to mature sperm. BMP4's high affinity for SMAD pathways promotes cellular changes necessary for spermatogenesis and fertility. BMP4 works in tandem with FSH to support testicular health. By enhancing FSH and SMAD1 signalling, BMP4 may help balance testosterone levels, ensuring optimal conditions for sperm maturation and production [37]. The high-affinity interactions observed in BMP4, SMAD1, and FSH receptors are tightly linked to male fertility through their roles in spermatogenesis, hormone regulation, and cellular support functions. Metabolite compounds that influence these pathways especially by enhancing FSH and testosterone stability could hold therapeutic promise for improving fertility outcomes in men.

**Table 4. Comparative of ligand control and the best five binding free-energy value from phytocompounds of ethanolic extract *A. catechu***

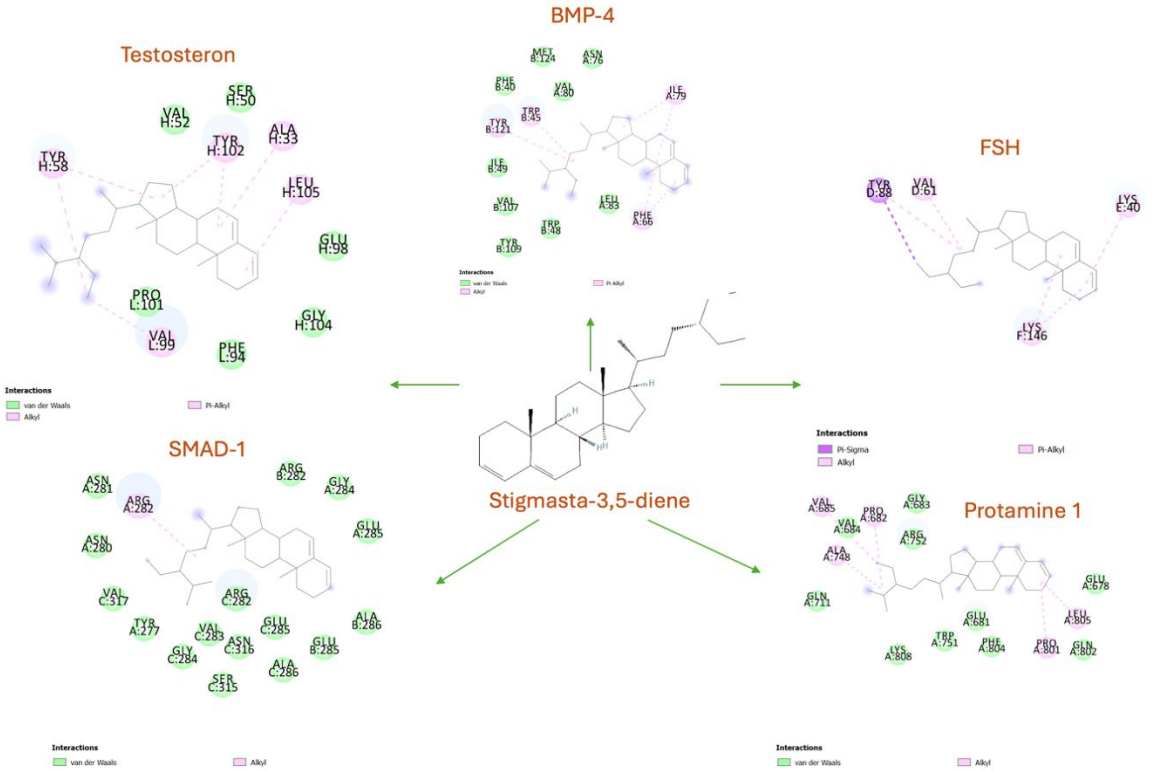
| No. | Ligan | Binding free-energy value (Kcal/mol) | | | | |
|---|---|---|---|---|---|---|
| | | **FSH 1XWD** | **Testosterone 1I9J** | **Protamine 1 1E3G** | **BMP4 2R53** | **SMAD1 1KHU** |
| Control | **Dimethandrolone undecanoate** | **-6** | **-6,7** | **-6** | **-6.1** | **-5.2** |
| 1 | Lupeol | -8.8 | -8.9 | -7.3 | -9.6 | -9 |
| 2 | Stigmasta-3,5-diene | -8.6 | -9.6 | -8.2 | -8.5 | -8.6 |
| 3 | Campesterol | -8.4 | -8.4 | -8.4 | -8.2 | -8.4 |
| 4 | *y*-sitosterol | -8.4 | -8.1 | -7.8 | -7.8 | -8.2 |
| 5 | Vitamin E | -6.9 | -8.6 | -6.8 | -7.2 | -6.9 |

Molecular docking analysis provides insights into the binding interactions between bioactive compounds and target proteins, highlighting the key forces that contribute to their stability and specificity. In this study, both hydrophilic and hydrophobic interactions were observed in the docking results for lupeol and stigmasta-3,5-diene with fertility-related proteins, playing a crucial role in their binding affinity. As illustrated in Figure 7a and 4b, these interactions include conventional hydrogen bonds, which are characteristic of hydrophilic interactions, and a range of hydrophobic interactions that contribute to molecular stability [14]. Specifically, hydrogen bonds are formed between the hydroxyl (-OH) groups of the compounds and polar amino acid residues in the binding pocket of the proteins, enhancing specificity and binding strength [38]. These hydrogen bonds are critical for molecular recognition and stabilization within the active site. In addition to hydrogen bonding, the interactions also involve various hydrophobic forces, including Van der Waals interactions, alkyl, π-alkyl, and π-sigma interactions. Van der Waals forces occur due to temporary dipoles, stabilizing the ligand-protein complex in a subtle yet significant manner. Alkyl and π-alkyl interactions involve nonpolar amino acid side chains forming favorable contacts with the hydrophobic areas of lupeol and stigmasta-3,5-diene, further reinforcing the binding. The presence of π-sigma interactions, where aromatic rings in the ligand interact with electron-dense regions of amino acid residues, suggests additional stabilization through π-electron interactions [17]. These findings suggest that the binding of lupeol and stigmasta-3,5-diene to fertility-related proteins is facilitated by a combination of hydrophilic and hydrophobic forces, indicating strong molecular interactions that could influence their biological activity. Such interactions may play a role in their potential antifertility effects by disrupting or modulating the function of key fertility-related proteins, warranting further experimental validation.

Our findings are in line with earlier research demonstrating the anti-fertility potential of areca nut extracts. For instance, a study on male rats showed that areca nut extract administration resulted in decreased sperm count and motility, as well as alterations in testicular histology [8]. Similarly, in female rats, areca nut extracts have been reported to exhibit anti-ovulatory and abortifacient effects, indicating potential applications in fertility regulation [39]. By integrating phytochemical analysis with molecular docking and comparative studies, this research seeks to elucidate the potential of *A. catechu* as a source of novel antifertility agents and provide insights into their mechanisms of action at the molecular level. Although this study offers insights into the anti-fertility potential of Aceh areca nut seed extract, it is crucial to note its limitations. The molecular docking results are indicative and necessitate validation through in vitro and in vivo research to corroborate the biological activity of the discovered drugs. Subsequent investigations need to concentrate on isolating distinct bioactive compounds, clarifying their precise mechanisms of action, and assessing their efficiency and safety in animal models.




(a)




(b)

**Figure 7. Two-dimensional visualisation of (a) lupeol and (b) stigmasta-3,5-diene with five receptors**

## 4. Conclusion

The ethanolic extract of young Aceh areca nut seeds showed significant antioxidant activity and comprises a variety of bioactive chemicals with potential pharmacological properties. The presence of alkaloids, flavonoids, terpenoids, phenolics, and tannins, as observed by phytochemical screening and GC-MS analysis, indicates a complex interaction of chemicals that contribute to the observed biological activity. Molecular docking studies specifically revealed lupeol

and stigmasta-3,5-diene as significant chemicals with high binding affinities for fertility-related proteins, such as FSH, testosterone, BMP4, SMAD1, and protamine 1. The findings suggest that the extract may disrupt reproductive processes, indicating its potential application as a natural anti-fertility agent. The extract's strong antioxidant activity ($IC_{50}$ 28.215 ppm) highlights major concerns regarding its biological implications. Although antioxidants often offer protective advantages, their influence on reproductive health is intricate, necessitating additional research to investigate potential connections between antioxidant processes and fertility suppression. Future study must prioritize in vitro and in vivo validation studies to verify the anti-fertility effects, evaluate potential synergistic or antagonistic interactions among bioactive compounds, and verify the safety of the extract prior to clinical application. These findings offer significant insights into the potential of Aceh areca nut seeds as a source of innovative anti-fertility drugs and establish a basis for further pharmacological investigation.

## 5. Declarations

### 5.1. Author Contributions

Conceptualization, N.N., S.S., and M.A.; methodology, N.N., S.S., and N.S.; validation S.S., M.A., and N.S.; investigation, N.N. and M.A.; writing—original draft preparation, N.N., S.S., M.A., and N.S.; writing—review and editing, S.S. and M.A.; supervision, S.S., M.A., and N.S. All authors have read and agreed to the published version of the manuscript.

### 5.2. Data Availability Statement

The data presented in this study are available in the article.

### 5.3. Funding and Acknowledgments

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### 5.4. Institutional Review Board Statement

Not applicable.

### 5.5. Informed Consent Statement

Not applicable.

### 5.6. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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