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In Vitro and In Silico Docking Techniques on Molluscicidal Activity of Tuba-Tuba (*Jatropha Curcas*) and Santol (*Sandoricum Koetjape*) Seed Against Golden Apple Snail (*Pomacea Canaliculata L.):* A Potential Treatment for Preventing Crop Infestation in Rice Fields

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ABSTRACT

The Golden Apple Snail (Pomacea canaliculata) threatens rice production, necessitating eco-friendly molluscicidal alternatives. This study evaluated the molluscicidal effects of Jatropha curcas and Sandoricum koetjape seed extracts through in vitro bioassays and in silico molecular docking. In vitro results showed J. curcas had higher efficacy, with 76.67% mortality after 24 hours compared to S. koetjape at 46.67%. One-way ANOVA confirmed significant differences among treatments ($p < 0.05$). Molecular docking revealed strong interactions with acetylcholinesterase (PDB ID: 1ACJ), with J. curcas compounds Vitexin (-8.2 kcal/mol) and Stigmasterol (-7.8 kcal/mol) showing notable binding, while S. koetjape compounds Quercetin (-9.8 kcal/mol) and Luteolin (-10.1 kcal/mol) exhibited even stronger affinities. These findings highlight J. curcas as a potent molluscicide and S. koetjape as a promising enzyme inhibitor, supporting their potential use in sustainable pest management.

KEYWORDS: Golden Apple Snail, Molecular Docking, acetylcholinesterase, jatropha curcas, sandoricum koetjape, PDB ID: 1ACJ

I. INTRODUCTION

Rice serves as the staple food for over half of the global population and stands as the most crucial crop in developing countries. A researcher found that any insect infestations in rice fields have the potential to significantly impact both our environment and livelihoods (Williams, 2017). *Pomacea canaliculata*, sometimes known as the Golden apple snail (GAS), is a big freshwater snail that is native to tropical and subtropical South America. It eventually expanded to Taiwan, Japan, the Philippines, and the southeastern People's Republic of China. According to IRRI Rice Knowledge Bank, Golden apple snails pose a significant threat to rice cultivation as they feed on young and emerging rice plants. By cutting the rice stem at the base, they effectively destroy the entire plant, leading to considerable crop loss.

The Golden Apple Snail (*Pomacea canaliculata*) is a major agricultural nuisance, especially in rice fields, where it feeds on the rice plants and destroys them ("Golden Apple", 2016). The conventional chemical molluscicides employed to manage these pests pose threats of polluting the environment and harming other organisms. In this regard, natural molluscicides from plant origin are being considered as a more sustainable option. Of these, Tuba-tuba (*Jatropha curcas*) and Santol (*Sandoricum koetjape)* have been found to possess potential because of their bioactive compounds (Dong et al., 2012). Nevertheless, there are few studies that have been conducted to determine the effectiveness and mode of action of these extracts on the GAS. This research will seek to determine the molluscicidal potential of Tuba-tuba (*Jatropha curcas*) and Santol (*Sandoricum koetjape*) seed extracts using molecular docking to determine the bioactive compounds and in vitro studies to evaluate the efficacy against *P. canaliculata* specifically targeting its hepatopancreas as this is a crucial organ for snails that is particularly vulnerable to injuries (He et al., 2017). The results could be useful in the creation of eco-friendly and sustainable molluscicides for pest control in agriculture (Brito et al., 2016).

Santol (*Sandoricum koetjape*) is a fruit commonly found in Southeast Asia. This fruit belongs to the family Sapindaceae, order Sapindales. It is valued for its wood and edible fruits. Fruits are eaten raw and processed into marmalades and other preserves. The medicinal uses of this plant in folklore medicine include inflammatory and stomach disorders. Phytochemical studies of *S. koetjape* have reported that it contains flavonoids, limonoids, and terpenoids, which contribute to its health-promoting properties and pharmacological activities (Bailly, 2022). The seeds of the *S. Koetjape* are toxic, containing insecticidal limonoids that make them a potential source of natural pest control for the potential chemical control of Golden apple snails in rice fields. Properties illustrating positions of *S. koetjape* as an especially promising source of botanical molluscicides, capable of combating parasitic

diseases with ecological benefits (Bailly, 2022b). *S. koetjape* is valued not only for its fruit and timber but also for general medicinal uses and its potential to control agricultural pests. Indeed, the research identified the bioactive compounds present in the roots, leaves, stems, and fruits of *S. koetjape*, which consisted of flavonoids, limonoids, and terpenoids. The seeds are toxic and contain insecticidal limonoids. This makes the seed extract a potential solution for the control of GAS in rice fields, as the plant was already found to fight parasiticdiseases effectively. Thus, it positions *S. koetjape* as one of the promising sources for botanical and organic molluscicides.

Tuba-tuba (*Jatropha curcas*) is a plant that shows promise as a natural molluscicide against the Golden apple snail (*Pomacea canaliculata*). It contains various bioactive compounds such as saponins, steroids, alkaloids, phenols, flavonoids, and terpenes, which have been found to have potent cytotoxic and molluscicidal properties (Maruni et al., 2022). The saponin compounds in particular are reported to be the main contributors to *Jatropha*'s molluscicidal effect (Ruma & Sanchez, 2016). These compounds can interfere with the nutrition, development, and respiration of cold-blooded animals like mollusks. Studies have shown that the toxicity of *J. curcas* extracts, especially from the leaves, is high enough to effectively kill golden apple snails. Several researchers have advised conducting field experiments to further evaluate the efficacy of *J. curcas* as a natural and environmentally friendly molluscicide against this invasive rice pest (Prabhakaran et al., 2017). The high reproductive capacity and adaptability of the Golden apple snail make it a challenging pest to control, necessitating the development of alternative control methods like botanical molluscicides.

This study aims to evaluate the potential of natural compounds from *S. koetjape* and *Jatropha curcas* seeds as inhibitors of the acetylcholinesterase (AChE) enzyme through both In vitro assays and molecular docking analysis. By combining experimental and computational approaches, the study seeks to explore their potential as alternative bioactive agents for pest control and neurotoxicity management. To achieve this, the researchers have set the following objectives:

In Vitro

1. **Sample Collection and Preparation.** Collect mature seeds of *Sandoricum koetjape* and *Jatropha curcas* and subject them to proper cleaning and drying
2. **Extraction of Bioactive Compounds.** Prepare seed extracts using ethanol extraction methods to isolate potential bioactive compounds.
3. **Statistical Analysis.** Use one-way ANOVA to determine significant differences in AChE inhibitory activity among different concentrations of the extracts (Discovery, n.d.).

In Silico

4. **Assessment of Drug-likeness.** Conduct a drug-likeness evaluation of the main phytochemical constituents from *S. koetjape* and *J. curcas* using the Lipinski Rule of Five via SwissADME (Daina et al., 2017).
5. **Molecular Docking Simulations.**

Perform molecular docking simulations to assess the binding affinity and interaction between selected phytochemicals and the acetylcholinesterase (AChE) enzyme (Madeddu et al., 2022).

6. Analysis of Binding Interactions: Analyze and visualize the strength, stability, and nature of the binding interactions between the phytochemicals and AChE to identify potential lead compounds for further study.

II. METHODOLOGY

This section describes the experimental approaches employed in this study, consisting of both In vitro and In silico analyses to evaluate the molluscicidal activity of *Jatropha curcas* and *Sandoricum koetjape* seed extracts. The in vitro bioassay details the laboratory procedures, including the preparation of plant extracts, exposure of *P. canaliculata* to varying concentrations, and observation of their mortality to assess the extracts' potential as molluscicides. Meanwhile, the In silico study involved computational simulations to evaluate the interaction between selected phytochemicals from *Jatropha curcas* (such as Phorbol esters and Jatrophone) and *Sandoricum koetjape* (such as Sandorinic acid and Meliacin compounds) with the target enzyme Acetylcholinesterase (AChE). Molecular docking and drug-likeness assessments were conducted to predict the binding affinity and interaction strength of these compounds, providing insights into the potential AChE-inhibiting mechanism that contributes to the molluscicidal activity of the plant extracts at the molecular level.

1.1 Collection of J. curcas and S. koetjape seeds. *J. curcas* seeds were collected from a nearby garden located in Mintal, Davao City, Philippines. On the other hand, *S. koetjape* are bought from Bankerohan, Davao City, Philippines. Both seeds are rinsed with distilled water to remove dust and unwanted materials. Then, damaged seeds will be discarded and healthy and clean seeds will be automatically selected. The seeds should be stored in a well-ventilated area for sometime to remove any remaining moisture. According to Trajković et al. (2019), proper preparation and cleaning of seeds are crucial steps in optimizing germination rates and reducing contamination risks.

Fig 1.*Collection of J. curcas seed and S. keotjape seeds.*

1.2 Preparation of Jatropha curcas and Sandoricum koetjape seed extract. The seeds of *J. curcas* and *S. koetjape* were cracked, and the shells were completely removed. The dried seeds were then crushed into a fine powder using a grinder or mortar and pestle since seed preparation methods like cracking, shell removal, and grinding are essential to maximize extraction efficiency and improve in vitro propagation outcomes as stated in a study by Koné et al., (2015).

Fig 2. *J. curcas and S. keotjape seeds after grinding*

The ground powder was placed in a container and fully immersed in 1 liter of ethanol, which served as the solvent. The container was sealed properly to prevent evaporation and contamination. The mixture was allowed to soak for 72 hours, during which it was occasionally shaken or stirred to enhance extraction efficiency. After the soaking period, the mixture was filtered to separate the liquid extract from the solid plant material.

Fig 3. *Maceration process of J. curcas and S. koetjape seeds*

Once the maceration process was complete, the mixture was subjected to rotary evaporation, a technique used to effectively remove ethanol as a solvent by lowering the pressure and gently heating the mixture. According to Nichols (2021) overview of rotary evaporation techniques, this method is preferred in laboratories for solvent removal due to its efficiency, as it reduces the boiling point of solvents under vacuum and increases the surface area for evaporation through rotation. As a result, the ethanol evaporated, leaving behind a concentrated pure extract of the plant material. The collected pure extract was then stored in an amber-colored container to protect it from light and preserve its potency as stated in a study conducted by Abubakar & Haque (2020).

Fig 4. *J. curcas and S. koetjape seed extracts after the rotary evaporation process*

1.3 Collection of Golden Apple Snails (P. canaliculata). At least 100 Golden apple snails (*Pomacea canaliculata*) with shell sizes ranging from 40 mm to 60 mm were handpicked along the edges of ponds and rice fields in Tugbok District, Davao City, where the snails were most abundant. Out of the collected snails, only 90 were selected and used in the laboratory for testing.

1.4 Acclimatization of GAS (P. Canaliculata). After collecting the snails, they were acclimated to new environmental conditions. The snails were divided into two groups, with fifty individuals placed in each container. They were housed in a slightly opened container measuring 20 x 10 x 12 inches. To mimic their natural habitat and minimize stress, pieces of rice plants and water collected from the rice field were added to each container based on an article by Blackpool Reptiles & Aquatics (2024). The snails were maintained under these conditions to ensure proper acclimatization before the

experiment since maintaining snails under controlled conditions, such as specific light-dark cycles and water conditions, is crucial for acclimating them to laboratory settings, which helps in stabilizing their physiological state according to Gomez & Anacta (2020).

Fig 5. *Collection and Acclimatization process of P. canaliculata*

2. In Vitro Bioassay

2.1 Laboratory Set-up. For the actual laboratory experiment conducted at Davao City National High School Laboratory, three groups were prepared: Extract A (*Jatropha curcas),* Extract B (*Sandoricum koetjape*), and the Control Group using the commercial molluscicide "Parakuhol" commonly used to kill golden apple snails (GAS). Each group consisted of three beakers, making a total of nine beakers. The beakers were clearly

labeled to avoid mix-ups during the experiment. The extracts of *J. curcas* and *S. koetjape* were prepared in advance through maceration. After setting up the extracts in the beakers, ten Golden apple snails (GAS) were placed into each beaker.

Fig 6. *Laboratory setup showing the P. canaliculata in their respective beakers*

Every beaker was set up with 500 ml of distilled water and a 1 ml droplet of their assigned treatment.

Fig 7. *Application of 1 mL of Extract A, Extract B, and the control extract in each beaker*

The experiment was closely monitored within a specified period, observing changes in behavior, survival rate, and other significant factors in the test subjects. According to El-Wahed (2024) on molluscicidal assays, such methods are essential for evaluating the efficacy of molluscicides, as they provide accurate and reproducible data on lethal concentrations and behavioral changes in snails, aiding the development of effective control strategies.

Fig 8. *Storing and monitoring of P. canaliculata every 3 hours over a 24 hour period.*

Statistical Analysis

3.1 Raw mortality rate. The number of dead golden apple snails per replicate after 24 hours of exposure was recorded. The total and mean mortality for each treatment group—*Jatropha curcas*, *Sandoricum koetjape*, and Control were computed and tabulated as the raw data for analysis.

3.2 Computation using one-way ANOVA. The data were analyzed using One-Way ANOVA to determine if there were significant differences in the mortality rates among the three groups. The following formulas were used:

Each mortality value was squared, then summed per group to compute ΣX^2. This is used to find the total scalculatedum of squares (SST).

SSB was computed to measure the variation between the treatment groups

SSW was to measure the variation within each group

$$SST = \sum X^2 - \frac{(\sum X)^2}{N}$$

Mean Square Between (MSB) and Mean Square Within (MSW) were computed by dividing the sum of squares by their respective degrees of freedom

F-value determines if there is a significant difference between the treatments.

$$SSB = \frac{T_1^2}{n_1} + \frac{T_2^2}{n_2} + \frac{T_3^2}{n_3} - \frac{(\sum X)^2}{N}$$

4. In Silico Analysis

$$SSW = SST - SSB$$

4.1 Screening and Preparation of phytochemicals from Jatropha Curcas and Sandoricum Koetjape. A total of 21 primary phytochemicals were identified in *Jatropha curcas* seeds (Ramadan, 2022),

$$MSB = \frac{SSB}{df_b} \qquad MSW = \frac{SSW}{df_w}$$

while 13 primary phytochemicals were found in *Sandoricum koetjape* seeds (Wijaya, 2022).

$$F = \frac{MSB}{MSW}$$

These 34 compounds were then evaluated using SwissADME (http://www.swissadme.ch) to assess their drug-likeness based on Lipinski's rule of five. Out of the 34 compounds, 10 from *Jatropha curcas* and 6 from *Sandoricum koetjape* seeds passed the Lipinski test and were selected for molecular docking studies using AutoDock Vina. Ligands were initially downloaded from PubChem as SDF conformer files, which were subsequently converted into PDB format using PyMOL. The compounds were then prepared for docking in MGL AutoDock Tools, where Gasteiger charges and torsional degrees of freedom were set automatically. The optimized ligands were finally saved as PDBQT files and used in the docking process with AutoDock Vina.

The ligands used include Vitexin (PubChem CID: 5280441), Apigenin (PubChem CID: 5280443), Isovitexin (PubChem CID: 162350), Xylose (PubChem CID: 135191), Gallic acid (PubChem CID: 370), Pyrogallol (PubChem CID: 1057), Quercetin (PubChem CID: 5280343), Luteolin (PubChem CID: 5280445), Sentulic acid (PubChem CID: 70683988), Koetjape acid (PubChem CID: 15513441), Sandorinic acid a (PubChem CID: 70683988), and Sandorinic Acid B (PubChem CID: 70683988). These compounds have passed toxicity evaluations using SwissADME and Protox II, confirming their potential safety for further analysis.

4.2 Preparation of Acetylcholinesterase.

The 3D structure of the target protein (RCSB PDB ID: 1ACJ) was obtained as a 3D model from the RCSB Protein Data Bank (*https://www.rcsb.org/*) in PDB format. It was then opened in MGL Autodock Tools for preparation. The researchers removed water molecules, other small molecules, and any naturally attached ligands (THA), the researchers also added polar hydrogens and Kollman charges, ensuring they were evenly distributed throughout the protein according to the studies conducted by Sharma & Sharma, (2021) and Ali et al., (2018). Finally, the updated protein file was saved as a PDBQT file for use with Autodock Vina

4.3 Receptor Grid Box Manual Generation.

The active binding residues of the LACI protein were predicted using CASTP (Ali et al., 2018). The receptor grid area was defined through Autodock Vina using MGL Autodock Tools. The grid box center was aligned with the active binding site, corresponding to the key residues in the receptor protein's main pocket: PRO229, ASN230, CYS231, PRO232, TRP233, SER235, VAL236, SER237, GLU240, ARG244, LEU282, PRO283, PHE284, SER286, ARG289, PHE290, VAL293, ILE296, SER304, LEU305, GLU306, LEU309, PRO361, HIS362, HIS398, CYS402, PRO403, HIS406, TRP524, ASN525, LEUS28, PRO529, LEUS32, and ASN533. The binding site was reduced to a size of 90×74×80 Angstroms based on Ali et al., (2018) to minimize inaccuracies and irrelevant binding predictions. The grid box's center coordinates (x: 5.903; y: 50.369; z: 60.273) and dimensions (90×74×80) were recorded in a text file for configuration, with the exhaustiveness level set to 8 and the energy range at 3 as per the default1 protocol.

4.4 Molecular Docking Analysis and Simulation

After the protein, configuration, and optimized ligand files were saved in the same location, the docking analysis and simulation took place. The Command Prompt on Windows 10 and 11 PCs was used for the procedure. The computer's Command Prompt was used to launch the Autodock Vina docking software. The destination was changed to the directory of the folder containing the configuration files, ligand, and protein. Then the code:

"C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" --receptor protein.pdbqt --ligand [ligand.pdbqt] --config [config.txt] --log [log.txt] --out [output.pdbqt]"

was written in the prompt for the computation as stated in a study by Sharma & Sharma (2021). Following this docking, the compounds binding affinities to the Active-site Gorge of Acetylcholinesterase (PDB ID: 1ACJ) were provided. For every ligand, this procedure was carried out ten times. The output files in PDBQT format for the simulation and the log files in text format were the product files of this code. The output and protein files (in PDBQT format) were exported to PyMol for the 3D simulation following the computation. The docking mechanism between proteins and ligands was visually shown using PyMOL. and Ligplot.

4.5 Scoring and Analysis.

The docking computation's output was saved as a text file in the same location as the other docking files. The various phytocompounds' binding affinities to the proteins were shown in the log text file. In addition to measuring binding affinity, the degree of interaction between a ligand and a protein-a scoring function assesses the ligand's orientation and conformation inside the protein's binding site. Stronger ligand-protein interaction and a higher chance of effective binding are indicated by a more negative binding affinity score according to Pantsar & Poso, (2018) and Torres et al., (2015). The only results deemed competent were those in which the ligand was located inside the protein's primary pocket. Given that the compound is located inside the primary pocket of the protein, the output with the lowest binding affinity value out of the ten docking attempts was selected as the typical data for the docking Interaction for each ligand. The stronger the connection between the ligand and the protein, the lower the binding affinity value as stated by Ali et al., (2018), as it indicates a higher energy release upon binding, resulting in a more stable complex between molecules as stated by Seo et al., (2021), therefore a higher inhibition potential for protein-protein interactions. The structure-based virtual screening method based on the study by Ali et al., (2018) was used for the visual analysis, and PyMol was used to see the 3D outputs of the protein-ligand docking. The hydrogen bonds and hydrophobic interactions were viewed using Ligplot. These interactions play a crucial role in ligand-protein docking as they determine the specificity and binding affinity of the protein-ligand complex according to Hudait et al, (2019). The interacting residues are also evaluated after docking through Ligplot+.

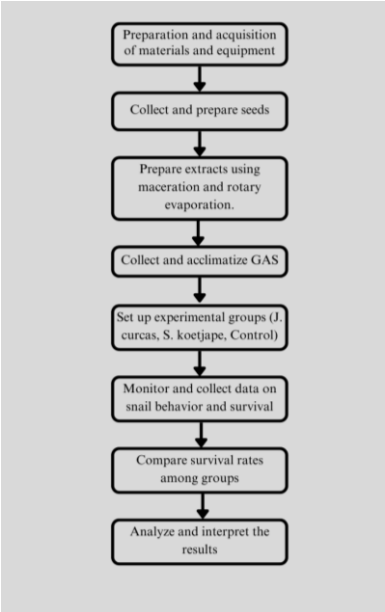

Fig 9. *Methodological Framework of In Vitro Bioassay*

The experiment involves several key steps. It begins with the preparation of Jatropha curcas and Sandoricum koetjape seeds, which are cleaned by removing the flesh, rinsed with distilled water, and checked for damage to ensure only healthy seeds are used. Extracts are then prepared using maceration followed by rotary evaporation with ethanol as the solvent. Golden apple snails (GAS) are collected from a rice field and acclimatized in plastic containers with paddy water and rice plants. The laboratory setup consists of three groups: Group A with Jatropha curcas extract, Group B with Sandoricum koetjape extract, and Group C as a control using Parakuhol, each containing 10 snails in a beaker with 500 ml of distilled water. Data on changes in behavior, survival rates, and other factors are collected every 3 hours. After four hours, the survival rates are compared to assess the impact of the extracts. Finally, the results are analyzed to determine the toxicity or efficacy of the extracts based on the observed effects on the snails.

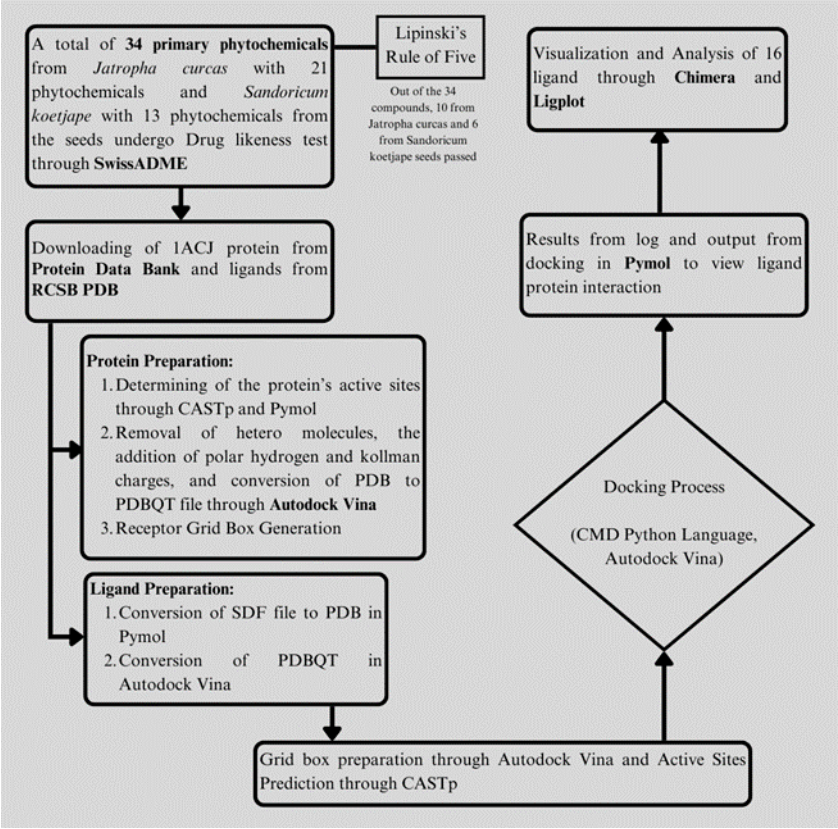

Fig 10. *Methodological Framework of In Silico Analysis*

The evaluation of drug-likeness and molecular docking for phytochemicals from Jatropha curcas and Sandoricum koetjape follows a systematic approach. A total of 34 phytochemicals (21 from Jatropha curcas and 13 from Sandoricum koetjape) are assessed using the SwissADME web tool, with 10 and 6 compounds, respectively, passing the Lipinski Rule of Five for drug-likeness. The receptor protein 1ACJ is downloaded from the Protein Data Bank, and its active sites are identified using CASTp and PyMOL. Protein preparation involves removing hetero molecules, adding polar hydrogens, assigning Kollman charges, and converting the structure into PDBQT format using Autodock Tools. Phytochemical structures are initially converted from SDF to PDB format and then to PDBQT format. A receptor grid box is generated for docking simulations. Molecular docking is conducted using Autodock Vina, employing a Python script to aim for a binding affinity of -7 kcal/mol or better. The docking results are visualized in PyMOL, while detailed interactions are analyzed using Chimera and LigPlot to evaluate the phytochemicals' biological activity and drug-like properties. This comprehensive workflow enables the identification of potential drug candidates based on their binding affinities and molecular interactions.

IV. RESULTS AND DISCUSSIONS

This section presents the statistical results of the In vitro bioassay and In silico analysis conducted to determine the molluscicidal activity of *Jatropha curcas* and *Sandoricum koetjape* seed extracts against Golden Apple Snails. The In vitro bioassay results show the mortality rates of snails exposed to the extracts, while the In silico analysis supports the findings by predicting the potential bioactive compounds responsible for molluscicidal activity. Statistical analysis, including ANOVA, was used to determine the significance of the observed results.

In Vitro Bioassay

In the In vitro bioassay, each treatment group *Jatropha curcas* extract, *Sandoricum koetjape* extract, and the control was tested using three replicates, each consisting of 10 Golden apple snails, totaling 30 snails per group. After 24 hours of exposure, varying mortality rates were recorded.

In te first replicate of *Jatropha curcas*, 9 snails died; in the second replicate, 7 mortalities were observed; and in the third replicate, 7 snails also died. For the *Sandoricum koetjape* treatment, 4 snails died in the first replicate, 5 in the second replicate, and 4 in the third replicate. In the control group, mortalities were recorded as follows: 10 snails in the first replicate, 10 in the second replicate, and 9 in the third replicate.

The detailed mortality data for each treatment group are summarized in the table below:

Table 1. *Raw Data and Mortality Rate of Golden Apple Snails Exposed to Jatropha curcas and Sandoricum koetjape Seed Extracts and the Control Group After 24 Hours of Exposure.*

Treatment	Beaker 1	Beaker 2	Beaker 3	Total	Mean (mortality)
J curcas extract	9	7	7	23	7.67
S koetjape extract	4	5	4	13	4.33
Parakuhol (control)	10	10	9	29	9.67

Table 2. *Summary of One-Way ANOVA Results Showing the Significant Difference in the Mortality of Golden Apple Snails Among the Treatments.*

Source of Variation	SS	df	MS	F-value	F-critical (0.05)
Between groups	43.56	2	21.78	32.52	5.14
Within groups	4	6	0.6		
Total	47.56	8			

The raw mortality data were analyzed using one-way ANOVA to determine if there were significant differences in the molluscicidal effects of *Jatropha curcas* and *Sandoricum koetjape* seed extracts compared to the control. Based on the statistical results, the computed F-value was greater than the F-critical value at a 0.05 significance level. This indicates that there is a significant difference in mortality among the three groups, suggesting that the seed extracts, particularly *Jatropha curcas*, exhibited molluscicidal activity against the Golden Apple Snails.

In Silico Analysis

This study sought to identify and analyze the 21 primary phytochemicals extracted from *Jatropha curcas* seeds and 13 primary phytochemicals from *Sandoricum koetjape*, specifically targeting acetylcholinesterase active binding sites. The 34 phytochemicals were evaluated using SwissADME software, adhering to Lipinski's Rule of Five, which assesses the physicochemical properties of ligands prior to molecular docking analysis. Of the 34 compounds tested, 16 were found to comply with Lipinski's rule, indicating their potential for drug-likeness.

Table 3: *Phytochemicals found in J. curcas*

a	***b***	***c***	***d***	***e***	***f***
Vitexin	432.38	7	10	-2.02	1
Apigenin	270.24	3	5	0.52	0
Isovitexin	432.38	7	10	-2.02	1
Rhoifolin	578.52	8	14	-2.96	3
Glycosides	584.65	8	12	-1.11	3

Xylose	150.13	4	5	-2.32	0
Gallic Acid	170.12	4	5	-0.16	0
Pyrogallol	126.11	3	3	0.18	0
3,4-Dihydroxybenzoic	138.12	2	3	0.99	0
Gentsic Acid	154.12	3	4	0.40	0
Catechol	110.11	2	2	0.79	0
-Sitosterol	414.71	1	1	6.73	1
Stigmasterol	412.69	1	1	6.62	1
Campesterol	400.68	1	1	6.54	1
Jatrophane	699.74	0	14	1.81	2
Squalene	410.72	0	0	1.81	2

Table 4: *Phytochemicals found in S. koetjape*

c	***b***	***c***	***d***	***e***	***f***
Sandoricin	588.64	1	11	1.58	2
Quercetin	302.24	5	7	-0.56	0
Luteolin	286.24	4	6	-0.03	
6-Hydroxy sandoricin	604.64	2	12	0.83	2
Sentulic Acid	470.68	2	4	5.60	1
Koetjapic Acid	470.68	2	4	5.60	1
Sandorinic Acid A	486.68	3	5	4.04	0
Sandorinic Acid B	486.68	3	5	4.04	0

***a** = Ligands; **b** = Molecular Weight (g/mol, <**500 Da**); **c** = Number of Hydrogen bond donors (<**5**); **d** = Number of Hydrogen bond acceptors (<**10**); **e** = M Log $P_{o/w}$ (**≤4.15**); **f** = Number of Violations (<**1**)*

The 16 phytochemicals were then docked with the control variables using PDB 1ACJ. To ensure consistency and accuracy, a total of 10 repetitions were conducted. Following the docking process, ten distinct outputs and their respective reports were produced; for each iteration, the output with the lowest binding affinity value was documented.

Scoring Functions

Table 5. *Phytochemicals with -7.0 and Lower Binding Affinity Values from J. curcas*

a	***b***	***c***	***d***
Vitexin	5280441	-8.2	Output 1
Stigmasterol	5210794	-7.8	Output 1
Isovitexin	162350	-7.7	Output 1
Apigenin	5280443	-7.3	Output 1

Table 6. *Phytochemicals with -7.0 and Lower Binding Affinity Values from S. koetjape*

a	***b***	***c***	***d***
Luteolin	5280343	-10.1	Output 1
Quercetin	5280445	-9. 8	Output 1
Sandorinic Acid A	70683988	-9.1	Output 1
Sandorinic acid B	15513441	-9.0	Output 1
Sentulic Acid	10323147	-7.7	Output 1
Koetjapic Acid	10323148	-7.6	Output 1

***a** = phytochemical name; **b** = CID from PUBCHEM (<**5**); **c** = binding affinity values; **d** = output number*

Overall, the combined docking analysis of phytochemicals from *Sandoricum koetjape* and *Jatropha curcas* against the acetylcholinesterase receptor (PDB ID: 1ACJ) revealed notable binding affinities. Among all the tested compounds, Luteolin exhibited the strongest binding interaction with a binding energy of -10.1 kcal/mol, followed closely by Quercetin at -9.8 kcal/mol, and Sandorinic Acid A at -9.1 kcal/mol. Meanwhile, the other ligands—Sandorinic Acid B, Vitexin, Stigmasterol, Iso-vitexin, Sentulic Acid, Koetjape Acid, and Apigenin—displayed moderate binding affinities ranging from -9.0 to -7.3 kcal/mol.

These results suggest that flavonoid compounds such as Luteolin and Quercetin have a strong potential to interact with acetylcholinesterase (1ACJ), indicating possible inhibitory activity at the molecular level.

Protein and Ligand Interactions between interacting residues

Pomacea canaliculata, commonly known as Golden apple snails, are highly invasive and can cause damage to rice crops. Acetylcholinesterase (PDB ID: 1ACJ) acts as a biomarker for exposure to neurotoxic chemicals, including organophosphate insecticides, in *P. canaliculata*. AChE activity can vary according to the concentration and length of exposure to certain compounds, according to studies.

The interaction between these receptors and AChE occurs through the Active site gorge, thereby engaging AChE largest residues Acetylcholinesterase binding pocket, measuring 950.949 A3. Key within ASN230, CYS231, PRO232, TRP233, the Gorge of namely PRO229, SER235, VAL236, SER237, GLU240, ARG244, LEU282, PRO283, PHE284, SER286, ARG289, PHE290, VAL293, ILE296, SER304, LEU305, GLU306, LEU309, PRO361,

H15362, HIS398, CYS402, PRO403, HIS406, TRP524, ASN525, LEU528, PRO529, LEUS32, and ASN533 are responsible for the interaction with acetylcholinesterase (Tian et al., 2018).

The study of the protein-ligand interactions between phytochemicals and the 1ACJ protein's active residues emphasizes the importance of hydrophobic and hydrogen bonding interactions in stabilizing these complexes. This study examines the specific interactions between the two substances that demonstrated the highest binding affinities: Vitexin from *Jatropha curcas* and Luteolin from *Sandoricum koetjape*. The binding affinities of Luteolin and Vitexin both exceeded the standard cutoff of -7 kcal/mol, indicating a high probability of successful interaction with the protein residues. The combination of hydrogen bonds and hydrophobic interactions contributes to this strong binding. These phytochemicals bind to the 1ACJ protein through a specific binding site that is structurally adaptable, facilitating optimal interactions.

Table 6. *Ligplot Analysis of the Phytochemicals of J curcas*

a	***b***	***c***
Vitexin	**ASN230**	**TRP524 CYS231**
	[2.78]	
	SER235	
	[3.20]	
	PRO232	
	[2.86, 2.91]	
	HIS398	
	[2.69, 3.15]	
Apigenin	**TRP524**	**ASN525**
	[3.13]	**PRO529**
	HIS398	**ASN230**
	[2.70]	**LEU305**
	SER235	**PRO232**
	[3.00]	
Isovitexin	**ASN525**	**HIS406**
	[2.74, 2.99]	**PRO403**
	ASN230	**PRO529**
	[3.13, 2.74]	**PRO232**
	ARG244	**HIS362**
	[3.12]	**ARG289**
Xylose	THR62	TYR96
	[3.03]	PRO69
	ASN59	

	[2.96] LEU31 [3.13] TRP58 [3.22] GLY3 [3.03, 3.10]	
Gallic acid	**ARG289** **[3.01, 2.91**] TYR121 [2.99]	HE287 PHE288 **SER286** TRP279 PHE331 PHE330 **PHE290**
Pyrogallol	**ASN525** **[2.71]** **ASN230** **[3.21]**	**HIS406 TRP524** **HIS398** **PRO232** **PRO403** **CYS402**
3,4-Dehydroxybenzoic	H1S440 [2.84]	GLY 117 GLY118 GLU199 GLY441 HIS440 GLY441 TRP84 PHE330 SER122 HE439
Catechol	TYR334 [2.91]	TRP84 SER81 TYR442 HE439

		HIS440
Stigmasterol	**HIS398**	**TRP524**
	[2.85, 3.02]	**PRO232**
		PRO403
		GLU306
		ASN230
		LEU305
		SER235
		SER304
		HE296
Campesterol	N/A	**LEUS32**
		HIS398
		TRP524
		PRO232
		ASN230
		ASN525
		LEU528
		HIS362
		PRO529

Table 3: Ligplot Analysis of the Phytochemicals of S koetjape

a	***b***	***c***
Quercetin	GLU199	GLY117
	[3.33]	GLY118
	HIS440	GLY441
	[2.70**]**	TYR442
	SER122	HE439
	[2.98]	ASP72
	TYR70	TYR121
	[2.30]	PRO86
	ASN85	VAL71
	[2.97]	
	TRP84	
	[3.11**]**	
Luteolin	GLU199	TYR130
	[2.70]	TRP84

	HIS440	GLY441
	[2.83]	GLY 117
	TYR70	GLY118
	[2.50]	SER122
		ASN85
		PHE330
		TYR121
		VAL71
		ASP72
Sentulic acid	N/A	**SER235**
		LEU305
		GLU306
		PRO232
		ASN230
		HIS398
		TRP524
		LEU528
		HIS362
Koetjapic acid	**TRP524**	**ASN525**
	[2.96]	**ASN230**
		GLU306
		SER304
		HE296
		PRO232
		PRO403
Sandorinic Acid A	N/A	**PRO403**
		PRO232
		ASN525
		HIS398
		HIS406
		TRP524
		ASN230
		GLU306
		SER304
		LEU305
		SER235

		HE296
Sandorinic Acid B	N/A	**TRP524** **HIS362** **HIS398** **LEU528** **PRO529** **ASN230** **PRO232** **GLU306** **LEU305** **SER304** **SER235** HE296

a *= phytochemical name;* ***b*** *= hydrogen bonds with interacting residues;* ***c*** *= Hydrophobic interactions with interacting residues;* ***bold*** *= binding sites of ephrin B2/B3*

Top Three Phytochemicals of S. Koetjape with the most interacting residues. Luteolin, Quercetin, and Sandorinic Acid A all exhibit significant interactions with key residues in the acetylcholinesterase , emphasizing their potential as bioactive compounds. Luteolin forms crucial hydrogen bonds with amino acids such as GLU199 (2.70 A), HIS440 (2.83 A), and TYR70 (2.50 A), which are essential for stabilizing its binding within the active site. Additionally, it engages in multiple hydrophobic interactions with residues, including TYR130, TRP84, and several glycine and serine residues, contributing to the overall stability of the ligand-protein complex. The calculated binding affinity of -10.1 kcal/mol suggests a strong interaction, indicating Luteolin's high potential for effective biological modulation.

Similarly, Quercetin showcases notable interactions within the same enzyme. It forms hydrogen bonds with critical amino acids such as GLU199 (3.33 A), HIS440 (2.70 A), and TYR 70 (2.30 A), along with several others, enhancing its biological effectiveness, Quercetin also participates in hydrophobic interactions with residues like GLY117 and TYR442, further stabilizing its binding. Its binding affinity value of - 9.8 kcal/mol reflects a strong interaction with acetylcholinesterase, reinforcing its potential as a bioactive agent.

On the other hand, Sandorinic Acid A primarily exhibits hydrophobic interactions with various residues, including PRO-403, ASN525, and HIS398, at the enzyme's binding sites. Although no hydrogen bonds are reported for this phytochemical, the presence of multiple hydrophobic interactions suggests a strong affinity for the protein. The binding affinity value of -9.1 kcal/mol indicates a favorable interaction, highlighting Sandorinic Acid A's potential 25 an effective inhibitor of acetylcholinesterase.

Top Three Phytochemicals of J. Curcas with the most interacting residues. Vitexin, Stigmasterol, and Isovitexin all exhibit significant interactions with key residues in the acetylcholinesterase enzyme, underscoring their potential as bioactive compounds. Vitexin forms hydrogen bonds with several important amino acids, including ASN230 (2.78 A), SER235 (3.20 A), PRO232 (2.86 A and 2.91 A), and HIS398 (2.69 A and 3.15 A). These interactions are critical for stabilizing Vitexin's binding within the enzyme's active site. Additionally, it engages in hydrophobic interactions with residues such as TRP524

and CYS231, enhancing the stability of the binding complex. The calculated binding affinity value of -8.2 kcal/mol indicates a favorable interaction between Vitexin and acetylcholinesterase.

Stigmasterol also demonstrates significant interactions with the same enzyme, forming hydrogen bonds with HI5398 at distances of 2.85 A and 3.02 A, which are crucial for stabilizing its binding within the active site. Furthermore, it engages in hydrophobic interactions with several residues, including TRP524, PRO232, PRO403, GLU306, ASN230, LEU305, SER235, SER304, and HE296. Notably, HI5398, ASN230, and SER304 are located within the binding sites of acetylcholinesterase, enhancing the likelihood of effective inhibition. The binding affinity value of 7.8 kcal/mol reflects a favorable interaction between Stigmasterol and the enzyme.

Isovitexin similarly exhibits significant interactions with acetylcholinesterase by forming hydrogen bonds with ASN525 (2.74 A and 2.99 A), ASN230 (3.13 A and 2.74 A), and ARG244 (3.12 A). These hydrogen bonds are essential for stabilizing Iso-vitexin binding within the active site. It also engages in hydrophobic interactions with residues such as HIS406, PRO-403, PRO529, PRO232, HIS362, and ARG289. All these residues-ASN525, ASN230, ARG244, HIS406, PRO403, PRO232, HIS362, and ARG289-are located within the binding sites of acetylcholinesterase, further enhancing the likelihood of effective inhibition. The calculated binding affinity value of -7.7 kcal/mol indicates a favorable Interaction between Isovitexin and the enzyme.

Other Phytochemicals with notable interacting residues. Apigenin exhibits a binding affinity of -7.3 kcal/mol, which is indicative of its strong interaction with the target protein. This binding is stabilized by the formation of hydrogen bonds with specific amino acid residues such as TRP524, HIS398, and SER235. The distances between Apigenin and these residues are 3.13 A, 2.70 A, and 3.00 A, respectively. In addition to hydrogen bonding, Apigenin also engages in hydrophobic interactions with several amino acid residues. These include ASN525, PRO529, ASN230, LEU305, and PRO232.

Hydrophobic interactions are crucial for the overall stability of the protein-ligand complex, as they contribute significantly to the binding energy. While Apigenin may rank lower than the top three ligands, its strong binding interactions highlight its potential as a potent inhibitor. The binding affinity of Sandorinic Acid B is notably high with a number of -9.0 kcal, indicating strong interactions with the target protein. Despite the lack of hydrogen bonds, which are often crucial for the stability of protein-ligand complexes, Sandorinic Acid B relies heavily on hydrophobic interactions to achieve its high binding affinity. These interactions involve key hydrophobic residues such as TRP524, HIS362, HIS398, LEU528, PRO529, ASN230, PRO232, GLU306, LEU305, SER304, SER235, and HE296. The involvement of multiple hydrophobic residues in the binding of Sandorinic Acid B suggests a well-defined hydrophobic pocket within the protein. This pocket is likely to be structurally flexible, allowing for optimal interactions between the ligand and the protein. Its unique binding profile makes it an interesting candidate for further research.

Similarly, Sentulic acid exhibits a strong binding profile to its target protein, mainly due to strong hydrophobic interactions, even in the absence of hydrogen bonds. Nine important hydrophobic residues-SER235, LEU305, GLU306, PRO232, ASN230, HIS398, TRP524, LEUS28, and HIS362-are involved in these interactions. A key component of Sentulic Acid's binding affinity for its target protein Acetylcholinesterase. Sentulic acid binding involves several hydrophobic residues, indicating a distinct hydrophobic pocket in the protein. This pocket probably has structural flexibility, which enables the best possible interactions between the protein and the ligand.

Koetjape Acid, with its notable binding affinity of 7.6 kcal/mol, which is significant, indicating a strong interaction with the target protein. Focusing on the significance of hydrophobic and hydrogen bonding interactions in maintaining the stability of the protein-ligand complex. A key component of this interaction is the formation of a hydrogen bond with the residue TRP524 at a distance of 2.96 A. This hydrogen bond is crucial as it provides a specific and directional interaction that contributes to the overall stability of the complex. In addition to the hydrogen bond, Koetjape Acid engages in multiple hydrophobic interactions with several residues. These include ASN525, ASN230, GLU306, SER304, HE296, PRO232, and PRO-403. The involvement of multiple residues in both hydrogen bonding and hydrophobic interactions suggests well-defined binding sites within the protein. This site is likely to be structurally flexible, allowing for optimal interactions between Koetjape Acid and the protein. Although Koetjape Acid may have a lower ranking compared to other ligands, its binding interactions appear strong.

Visualization of the top three Phytochemicals of S. Koetjape with the most interacting residues

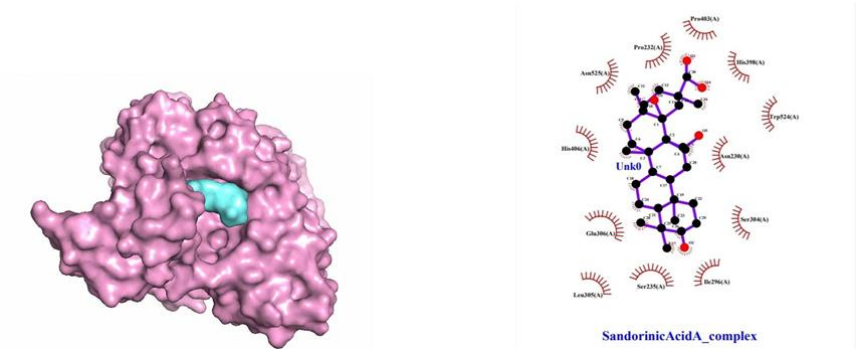

Figure 10. *Luteolin complex. PyMOL 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

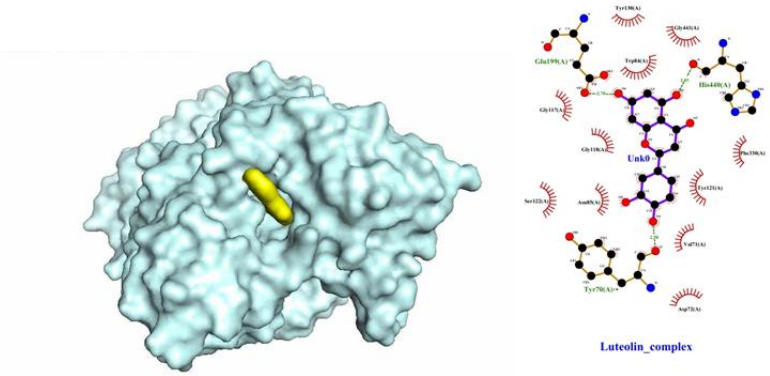

Figure 11. *Quercetin complex. PyMOL 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

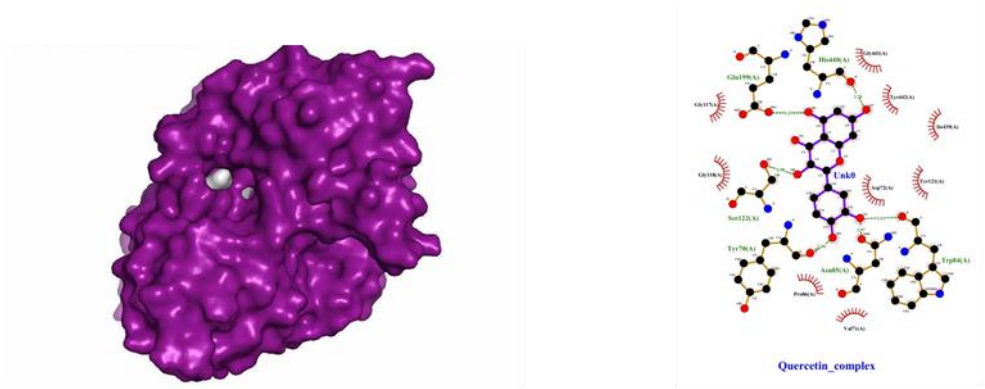

Figure 12. *Sandorinic AcidA complex. PyMOL. 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

Visualization of the top three Phytochemicals of J. curcas with the most interacting residues

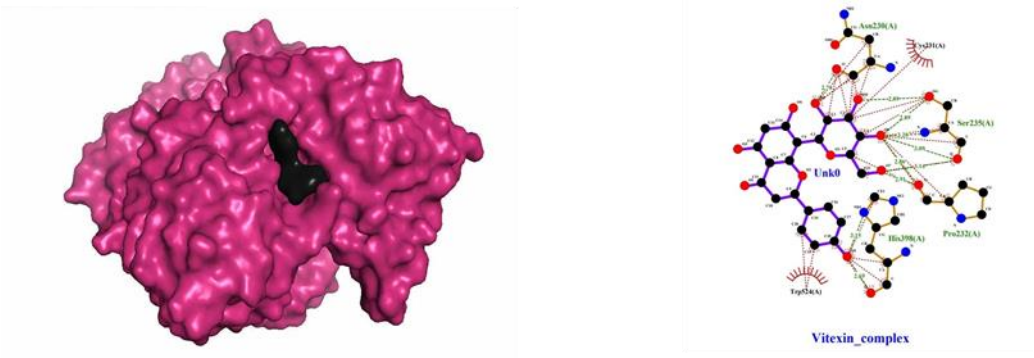

Figure 13.*Vitexin, complex. PyMOL. 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

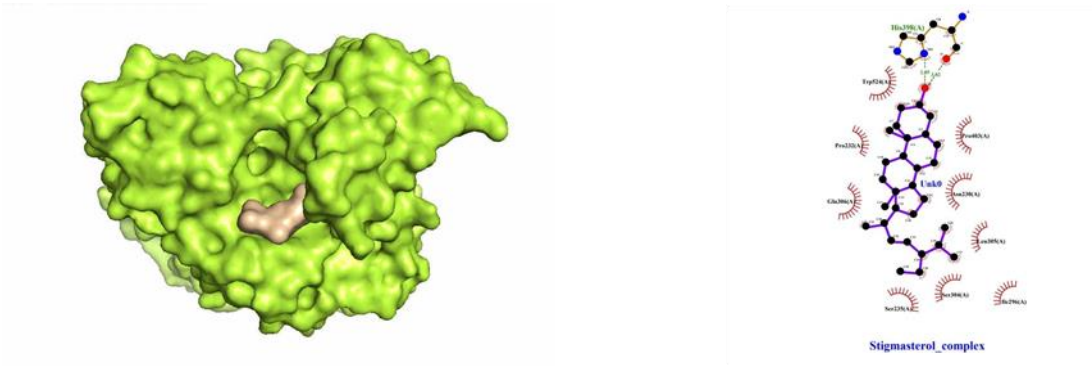

Figure 14. *Stigmasterol complex. PyMOL 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

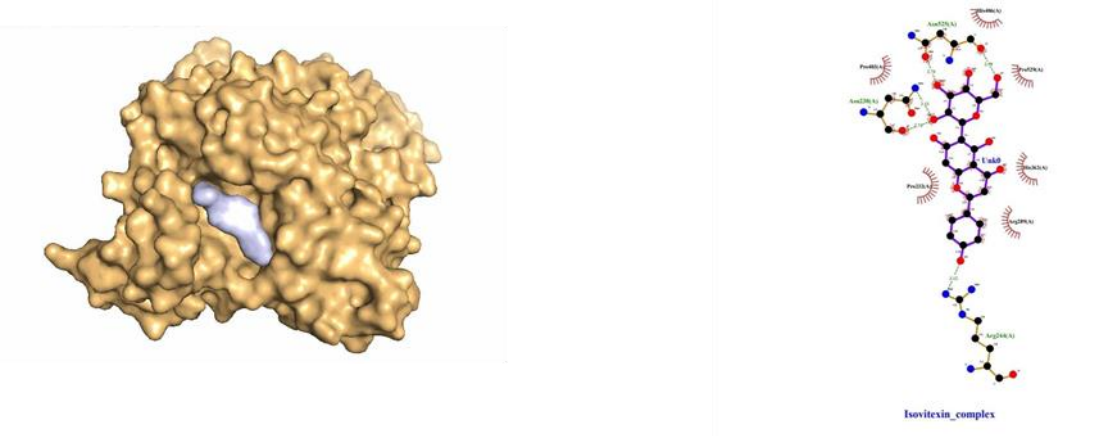

Figure 15. *Isovitexin complex. PyMOL 3D visualization (left). Ligplot hydrogen bonds and hydrophobic interactions 2D visualization (right).*

Conclusion and Recommendations

The study successfully evaluated the molluscicidal potential of *Jatropha curcas* and *Sandoricum koetjape* seed extracts against the Golden apple snails (*Pomacea canaliculata*) through In vitro bioassay and In silico molecular docking.

In the In vitro bioassay, J. curcas showed a higher mortality date among the P. canaliculata compared to S. koetjape and the control group. Across three beakers, J. curcas caused 9, 7, and 7 deaths respectively, indicating consistent molluscicidal activity. *S.keotjape* exhibited lower mortality rates, with 4, 5, and 4 snail deaths in each replicate. Interestingly, the control group recorded unexpectedly high mortality, with 10, 10, and 9 deaths, suggesting potential environmental or procedural factors influencing baseline mortality.

The statistical analysis using one-way ANOVA confirmed a significant difference in mortality rates among the three groups, with the computed F-value exceeding the F-critical value at a 0.05 significance level. The results suggest that Jatropha curcas holds promise as a natural molluscicide against golden apple snails, though the not expected control mortality highlights the need for careful reevaluation of experimental conditions in future studies.

Complementing the bioassay results, the in silico analysis provided insight into the molecular interactions of the top three phytochemicals from each extract with acetylcholinesterase (1ACJ) is crucial in snail neural activity. Phytochemicals from *S. koetjape*, particularly Luteolin and Quercetin, exhibited strong binding affinities of -10.1 kcal/mol and -9.8 kcal/mol, respectively, forming significant hydrogen bonds and hydrophobic interactions with key active site residues. These interactions suggest that *S. koetjape* compounds could potentially disrupt acetylcholinesterase (PDB:1ACJ) function, contributing to snail mortality, albeit to a lesser extent than *J. curcas* in In vitro bioassay.

On the other hand, phytochemicals from J. curcas—Vitexin, Stigmasterol, and Isovitexin—also showed favorable binding affinities, ranging from -8.2 to -7.7 kcal/mol.

Vitexin, in particular, displayed strong molecular interactions, aligning with the significant mortality observed in the In vitro test. The molecular docking results support the hypothesis that these compounds contribute to the molluscicidal activity of *J. curcas*, likely through neurotoxic effects on the snails.

Overall, both the experimental and computational results reinforce the molluscicidal potential of *J. curcas* and, to a lesser degree, *S. koetjape*. The convergence of In vitro mortality data, significant statistical findings, and In silico molecular docking analyses highlight J. curcas as a more effective bio-molluscicide candidate.

These compounds formed multiple hydrogen bonds and hydrophobic interactions with active site residues of acetylcholinesterase.

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