ARTICLE

Gas chromatography, molecular docking analysis, and insecticidal activity assessment of Kamamba (*Piper umbellatum* L.) crude leaf extract against maize weevil (*Sitophilus zeamais*)

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ABSTRACT

Kamamba (*Piper umbellatum* L.) is a tropical shrub native to Central and South American countries. It is known in other tropical countries outside America for its various culinary and folk medicinal applications. Several researchers are exploring other use of the different plant parts of *P. umbellatum* L., such as in pest management. In this study, an ethanolic extract of *P. umbellatum* L. leaves was analyzed through GC-MS for identification of compounds and tested through contact/residual bioassay for mortality against maize weevil (*Sitophilus zeamais*). Further evaluation using molecular docking with AutoDock 4 was performed to better understand the probable mode of action and explain the mortality observed in the bioassay. GC-MS results showed the presence of phenols, esters, sugar derivatives, and other volatile organic compounds. LC_{50} of 478.60 mL/L (volume of extract per liter of treatment solution) was calculated through probit analysis, indicating relatively low potency under the assay conditions. The result of molecular docking indicates possible mode of action of the compounds through inhibition of acetylcholinesterase or agonism of octopamine receptor. 2,3-dihydrobenzofuran was noted as the promising compound responsible for the insecticidal activity observed for its promising interactions with both acetylcholinesterase and octopamine receptor. These results suggest neurotoxic activity of the *P. umbellatum* L. leaf extract to *S. zeamais.*

Piper umbellatum L. is a South American tropical shrub now well-distributed in most humid areas such as the forests in the Philippines. It has been introduced in the Philippines and became part of the culture, in the form of its culinary and folklore medicinal applications. *P. umbellatum* L. is locally called Kamamba, Kumamba or Kabamba in the Southern Tagalog localities and is commonly used as a vegetable complement to fish and coconut in a dish in the province of Quezon. Topical application of the leaves of Kamamba is known to relieve skin swellings and rashes.

Several chemical analyses such as phytochemical screenings and proximate analysis were performed by researchers to determine the compounds present in the different parts of *P. umbellatum* L. in the hopes of developing novel utilizations such as in pest management. Analysis of the *P. umbellatum* L. using modern analytical techniques has been reported in both South America and Africa using the local *P. umbellatum* L. grown in those areas. Phytochemical screening and analysis of the hydroethanolic extract of the *P. umbellatum* L. leaves from Brazil using liquid chromatography by da Silva et al. (2014), showed presence of around 16.5% flavonoids of which rutin and quercetin are the major compounds while the remaining percent is attributed to alkaloids and terpenes. GC analysis of the local *P. umbellatum* L. will enable us to determine whether the local P. umbellatum L. is chemically comparable to samples from Africa or from South America based on the major chemical compounds present. This will give an idea of what other possible application can be developed from the locally sourced *P. umbellatum* L.

The practices in pest infestation management and resistance minimization were developed as part of several alternative farming systems. One practice is the use of biopesticides, specifically, the use of compounds or active ingredients derived from natural

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KEYWORDS

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sources. Biopesticides can either come from microorganisms (microbial), plant material (biochemical), or chemicals released by organisms (semiochemical) (Chandler et al., 2011). These compounds and the products developed address pest infestations with less risk of significant harm to human health and the environment as compared to the conventional synthetic pesticides. In the study of Abe et al. (2019), *P. umbellatum* L. components were found to be biologically active against certain pest insects, suggesting future applications of *P. umbellatum* L. part of pest management strategies. This activity can be verified through bioassay testing and utilization of modern screening tools such as molecular docking. Molecular docking and other *in silico* techniques are commonly encountered in drug discovery and screening procedures. Most of the time, they are utilized in determining possible bioactivities and binding affinity assessment for novel lead compounds to be used in drug design.

This study primarily determined the compounds present in the leaf extract of local *P. umbellatum* L. from the foothills of Mt. Banahaw using GC-MS. The extract of *P. umbellatum* L. was studied for possible toxic activity against storage pest insect *Sitophilus zeamais* through contact/residual bioassay. The result was expressed in terms of LC_{50}. Further understanding of the activity was done using molecular docking.

MATERIALS AND METHODS

Leaf Sample Processing and Extraction

Fresh samples of *P. umbellatum* L. were collected from the upland barangays of the towns of Sariaya and Lucban, in the province of Quezon. These places are situated at the foot of Mt. Banahaw. Because of this, the areas were considered relatively remote and receive low anthropogenic disturbances. The plant of interest was identified and verified by the University of the Philippines Los Baños Museum of Natural History (UPLB-MNH) herbarium where proper identification and certification were provided by the museum taxonomist. The leaves of the samples gathered were removed from the stem and were washed in running water to remove physical contaminants. The washed leaves were air-dried under normal laboratory conditions away from direct sunlight for three days. The leaves were further dried using a forced-air circulation oven at 40 °C for six hours. Oven-dried leaves were milled into powder using an electric Wiley mill. In this modified extraction method following Arunachalam et al. (2020), one liter of 70% (v/v) ethanol solution together with 100 g of the powdered leaves was placed into an amber bottle. The macerate setup underwent 30 min of manual agitation twice daily for seven days. After seven days, the content of the amber bottle was filtered using a cheesecloth and Whatman filter paper 1 under the fume hood. The extract obtained was concentrated by removing the ethanol through rotary evaporator operating at temperature of 40-50° C at reduced pressure. The resulting extract was placed in a small screw-capped amber bottle. The cap's inner surface was lined with aluminum foil, and the outer surface sealed with Parafilm. The bottle was properly labeled and was stored in a refrigerator under 4°C to prevent any possible degradation.

Gas Chromatography Analysis

The extract obtained was subjected to analysis using gas chromatography coupled with mass spectrometry for phytochemical identification. The analysis was performed by a third-party analytical facility using an Agilent 8890 GC coupled to a 5977B MSD located at Dr. George Ty Central Instrumentation Facility, De La Salle University Laguna Campus, City of Biñan, Laguna. The sample preparation prior to the analysis was performed by drying 500 µL of the sample in a vacuum centrifuge concentrator at 12,000 rpm for 3 hours at 25 ºC. 1000 µL of methanol was added to the sample after drying to reconstitute the solution. The resulting solution was sonicated for 5 minutes and dried over anhydrous sodium sulfate. Then, the solution was centrifuged for another 5 minutes at 12,000 rpm. The resulting supernatant was obtained for the GC-MS analysis. A method blank was prepared by drying 1000 µL of methanol using anhydrous sodium sulfate and centrifugation at 12,000 rpm for 5 minutes. The GC system is coupled to a 5977B Mass Selective Detector (MSD), a quadrupole mass spectrometer, as its detector and uses electron ionization as its ionization method. The analysis performed utilized a HP-5MS column with dimensions of 30 m × 0.250 mm and film thickness of 0.25 µm. Data were processed using the Agilent MassHunter Qualitative Analysis 10.0. Compound identification was conducted by comparing the acquired mass spectra with entries in the NIST MS Search (version 2.3) spectral library. Only peaks with acceptable spectral matching criteria were considered for tentative identification. The resulting chromatograms and peak assignments were reviewed and verified in consultation with the analytical service provider to ensure consistency and accuracy of interpretation.

Bioassay for Insecticidal Activity

An initial population of *S. zeamais* was obtained from the Entomology Division of the Institute of Plant Breeding (IPB), College of Agriculture and Food Science. These insects were collected from infested corn cobs and kernels from insecticide-free corn variety "lagkitan". One hundred individual insects were collected and placed in a glass jar as a rearing cage with a metal screen lid as cover that is strong enough to keep the weevils from chewing out of the container but still allows exchange of gases. The jar was placed inside a plastic container covered with cloth to keep foreign contaminants from compromising the setup. The rearing cage is provided with 100 g of insecticide-free corn kernels and was maintained at 29°C, 70% relative humidity, and a 16:8 h light:dark photoperiod. After 72 hours, all the adults were removed from the rearing cage while kernels infested with eggs were kept and incubated until they reached adulthood. Contact/residual bioassay was performed where the test insects are exposed to the test pesticide or compound through direct contact with a surface that has residual amounts of the compound. The experiment was performed using 300 mL glass jars as test containers and the different extract concentrations were prepared by serial dilution using pipette and volumetric flasks with distilled water as solvent. These solutions along with the control were applied on a Whatman filter paper and were allowed to dry under room conditions to leave a residual amount of the extract on the filter papers. The dried filter papers were placed onto the glass containers after which 20 insects were introduced. The insects are allowed to have direct contact with the paper and the glass. Mortality was determined based on visual observations such as lack of coordinated movement or lack of reaction from physical stimulus using forceps and a toothpick (Heinrichs et al., 1981). The mortality in each test container was observed and recorded after 24, 48, and 72 hours from the initial exposure. All the experiments in this bioassay test were implemented through completely randomized design with three replicates per treatment to make sure each test unit has equal chance of receiving any of the treatment. The calculated percent mortalities were corrected with respect to the negative control using Abbott's formula. Probit analysis was performed to determine the toxicity of the test extract using the procedure described by Heinrichs et al. (1981). The final LC_{50} is presented as LC_{50} ± fiducial limits. The platform used in this analysis was Microsoft Excel, under the Microsoft Office 365 suite with the Data Analysis Pack addon installed.

Molecular Docking

To further evaluate and understand the effects of the *P. umbellatum* L. leaf extract to the *S. zeamais*, an *in-silico* study was conducted using molecular docking to evaluate the compounds present in the leaf extract as acetylcholinesterase (AChE) inhibitors or octopamine receptor (OctR) agonist. The structures of the compounds identified from the chromatograph were retrieved from the NCBI PubChem compound database and were downloaded in SDF format. These compounds were converted to PDB format

using PyMOL software and were prepared for docking in AutoDock 4 through AutoDock MGL Tools. For the target proteins, they were chosen based on the literature studies on the molecular docking in insects such as that of Ocampo et al. (2023). Most insect docking studies target the acetylcholinesterase of insects due to their connection with organophosphate and carbamate insecticides. These studies aim to utilize the rapid neurotoxic effect of disrupting the insect AChE. In addition, the availability of the amino acid sequence was vital in choosing the target protein. The limited availability of the amino acid sequence protein targets such as GABA receptor, limits exploration of additional targets. The three-dimensional structures of the target proteins were prepared using AlphaFold, an emerging AI tool for computationally creating protein 3D structures from sequences. The retrieved 3D protein structure of the acetylcholinesterase and octopamine receptor were downloaded as PDB files and were imported into AutoDock 4 where they were prepared for docking. Blind docking was performed using the actual substrates of the targets: acetylcholine for acetylcholinesterase and octopamine for octopamine receptor which also functioned as control ligands. Known compounds that bind to the target proteins and produce the desired outcome activities can be docked to gather information on the active site of the target proteins as described by Bennion et al., (2013). To identify the putative active site for acetylcholine binding/hydrolysis, acetylcholine was used as a ligand and was docked to the receptor protein using blind docking with a grid size of 120 Å × 120 Å × 120 Å, spaced by 0.531 Å, covering the entire protein. A similar procedure was done on octopamine receptor. Upon analysis of the docking results, specifically the binding energies, the putative binding site was identified to be centered near TRP117, TRP485, and SER250 for the AChE and around HIS8, LEU190, PRO408, CYS407, and MET400 for OctR. After identifying the most putative binding site, site-specific dockings were performed using the ligands and the control substrate one at a time utilizing the Lamarckian genetic algorithm (GA) option in the AutoDock 4. The best binding pose of each ligand corresponding to the lowest binding energy that fits within the docking grid was recorded for each ligand. All docking results were repeated at least twice to ensure minimal input errors and independence of each run. Protein-Ligand Interaction Profiler (PLIP) was used for visualization of the results.

RESULTS AND DISCUSSION

Extraction and Identification

The extract obtained after the maceration was yellowish to brown in color with relatively low viscosity as observed in its free-flowing behavior inside the container. The extract has high miscibility in water. The yield from the extraction was calculated to be **13.7%**. This extraction yield is relatively high as expected for extractions through maceration. The presence of nonvolatile co-extractives accounts for the high extraction yield. Changes in transport and storage are needed in the handling of leaf samples due to their vulnerability. The freshness of the leaf sample is also important to obtain consistent and reliable amounts of extract. Further concentration after rotary evaporation can be done. Also, it should be noted that the use of 70% ethanol may have resulted in the extraction of non-volatile compounds that are not amenable to GC–MS analysis. Thus, the identified compounds represent only the fraction of the extract that is volatile or can be thermally desorbed under GC–MS conditions.

The GC-MS analysis of the extract, particularly the mass chromatogram (Figure 1), reported a total of 20 peaks. Of these peaks, eleven (11) were tentatively identified through spectral matching in the NIST MS Search 2.3 database. Identification was based on comparison of mass spectra and consideration of spectral similarity indices, with only peaks meeting acceptable matching criteria included in the reported assignments. This analysis suggests that the unique compounds in the extract are mainly plant-derived metabolites and volatile constituents (Table 1). In addition, the compounds constituting the ethanolic extract of *P. umbellatum* L. leaves are predominantly esters, sugar derivatives, and volatile organic compounds. Some of these compounds were identified by other researchers from other plant sources such as pepper, pineapple, and grapes (Stefanini, 2017). They are studied for their possible contribution to some of the biological activities that these plants exhibit. Excluding the unknown peaks, the major known compounds in the sample are the ethyl α-D-glucopyranoside (51.4%), Benzofuran, 2,3-dihydro- (13.1%), and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (12.2%) based on their peak area. It should be emphasized that compound identification in this study is tentative and based solely on library matching of mass spectra. Such assignments are subject to limitations, including potential co-elution and similarity of fragmentation patterns among structurally related compounds. Confirmation of compound identity and accurate quantification would require the use of authentic analytical standards and complementary techniques. The chromatographic data and corresponding peak assignments were reviewed and verified in consultation with the analytical service provider to ensure consistency of interpretation.

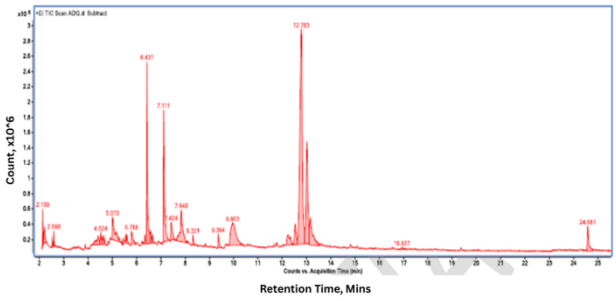
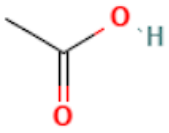
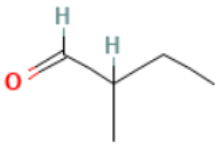
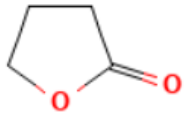
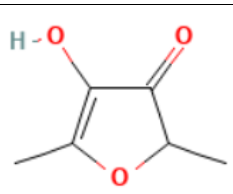
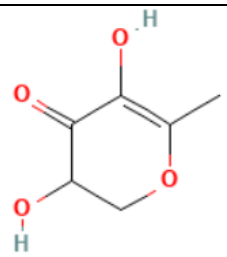
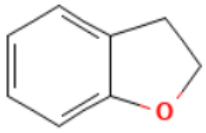
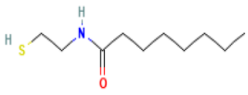
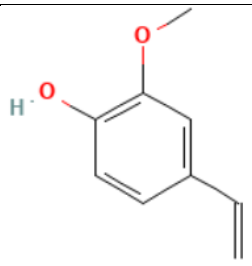
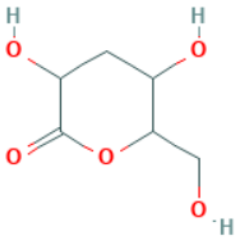
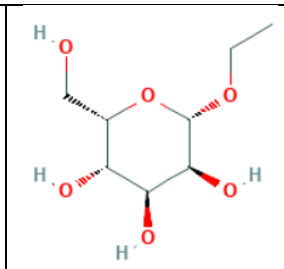
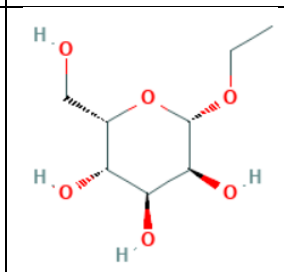
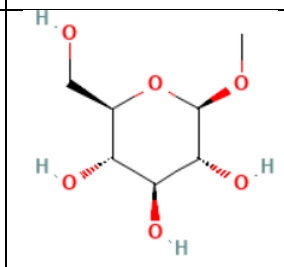


Figure 1: GC-MS chromatogram of the Kamamba leaf extract.

Table 1: List of identified compounds present in the GC-MS analysis of the extract.

COMPOUND NAME	STRUCTURE	MATCH	R. MATCH	AREA	RETENTION TIME (min)
Acetic Acid		901	929	628332.9	2.195

Butanal, 2-methyl-		880	888	253539.1	2.590
Butyrolactone		858	970	516241.4	4.524
Furaneol		NA	NA	269282.5	5.599
4H-Pyran-4-one, 2,3-dihydro-3,5-dyhydroxy-6-methyl-		829	899	4955627.0	6.430
Benzofuran, 2,3-dihydro-		NA	NA	5305185.0	7.111
Octanamide, N-(2-mercaptoethyl)-		NA	NA	3127135.0	7.840
2-methoxy-4-vinylphenol		932	933	326666.9	8.321
3-Deoxy-d-mannoic lactone		866	870	1170172.0	12.243

Ethyl α-D-glucopyranoside		NA	NA	1353347.0	12.546
Ethyl α-D-glucopyranoside		633	634	19581599.0	12.783
B-D-Glucopyranoside, methyl		829	922	3164355.0	13.156

The compounds identified are notably plant metabolites that are also key components of other plant species. Database searching of these compounds in a dedicated natural product database LOTUS (https://lotus.naturalproducts.net/) gave information about the other plant species where these compounds were identified. Butanal, 2-methyl- is short-chain aldehyde that is known to be volatile oil compound found in dandelion, pepper, eggplant, and yam. Butyrolactone is a lactone that is commonly found in pepper, pineapple, and blackberries. Furaneol is a furan famously found in fruits such as pineapple, strawberries, and durian. 4H-Pyran-4-one, 2,3-dihydro-3,5-dyhydroxy-6-methyl- is a compound of the class dihydropyranose and has been found in small amounts in pepper and pineapple. Benzofuran, 2,3-dihydro- is a compound under the family coumaran and is a benzofuran derivative commonly found present in grapes, fenugreek, cogon grass, and frog fruit. Octanamide, N-(2-mercaptoethyl)- is another natural volatile compound found in grapes. 2-methoxy-4-vinylphenol is a methoxyphenol found in several natural sources such as coffee, apricot, daisy, wheat, hops, buckwheat, and corn. 3-Deoxy-d-mannoic lactone is a lactone bioactive compound found in flowering plants such as hill glory bower and pink nodding orchid. Ethyl α-D-glucopyranoside has been detected in small amounts in legumes such as horse gram (Rutz et al., 2022). The presence of small amounts of acetic acid can be attributed to the plant metabolite indole-3-acetic acid, a plant regulatory hormone that is present in the leaves of most plants (Sundberg, 1986). The compounds identified from the sample are also identified in other plant sources and are studied for their biological properties and possible beneficial applications from their activities. Butyrolactone has been reported for its neurotoxic and depressant activity through inhibition of the GABA receptors in insects and humans. 2-methoxy-4-vinylphenol has been noted for its role as pheromone in some insects such as palm weevil and as flavoring agent in food. 3-Deoxy-d-mannoic lactone has been studied recently for its antibacterial activity (Rutz et al., 2022).

Bioassay and Probit Analysis

The bioassay data followed Abbott's rule and showed control percent mortality of 3.33 %. This is lower than 20 % and is considered acceptable (Yu, 2011). The effect of the extract can be examined in terms of comparison of the mortality of a given treatment with respect to time. This gave the dose-response curve at different time points (Figure 2). The dose-response curve is a very useful tool which also helps identify the toxicity and the lethal concentration. It follows a typical logarithmic function behavior where there is a rapid increase on the y value as the value of x increases, but plateaus at a certain point. This signifies that the effect of the extract to the insects observed follows the conventional logarithmic behavior of the dose-response relationship of most biological organisms to a xenobiotic. The logarithmic dose-response curve offers better graphical and analytical assessment of the response and is convenient in understanding the relationship of the response at low concentrations. At 72-hour, the Pearson correlation coefficient is at 0.9538. This indicates the direct relationship between the logarithm of the extract concentration with the percent mortality. The result of the Chi-squared test of goodness-of-fit also supports goodness-of-fit of the logarithmic dose-response curve.

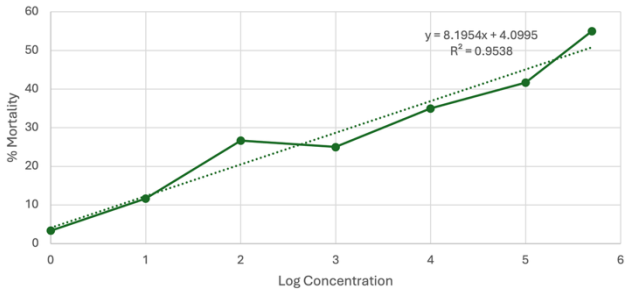


Figure 2: The logarithmic dose-response curve and the linear regression trendline for 72-hour time of the varying logarithm of *P. umbellatum* extract concentrations to mortality of *S. zeamais*.

Pairwise analysis of the treatments as a post hoc test was done using Bonferroni test which determined the pairwise significant differences between every combination of treatments. Based on the result, significant difference of the mortality starts at the

concentration of 0.1 mL of extract per liter of treatment solution (100.0 ppm) as compared to the negative control. Examination of the mortality of a single treatment through the three different times gave an idea of when the extract exerts the greatest effect. This analysis suggests that there is a drastic increase in mortality between 48 and 72 h. Further investigation incorporating longer exposure periods, increased sample size, and inclusion of a standard insecticide as a positive control is recommended to strengthen interpretation of the extract's efficacy.

The extract caused mortality in *S. zeamais* as observed in the contact/residual bioassay. Probit analysis estimated the lethal concentration to be 478.60 ± 1.54 mL/L (**4.79×10^5 ppm**). The dose-response curve generated at 72 h of exposure showed mortality increase correlation to log of dose. To contextualize the observed toxicity, the LC_{50} of the extract was compared with values reported in the literature for commonly used insecticides such as malathion and cypermethrin, which have reported LC_{50} values of 6.54 ppm and 65.19 ppm, respectively (Iqbal et al., 2012). These comparisons are presented solely for general reference and do not represent a direct experimental positive control, as differences in assay conditions (e.g., exposure duration, formulation, and insect population) limit strict comparability. Relative to these compounds, the extract exhibits lower potency, as indicated by its higher LC_{50}.

The LC_{50} obtained in this study is, however, within the range reported for other plant-derived extracts tested against *S. zeamais*. This is shown in the study by Suleiman et al. (2017) where they performed bioassay on *S. zeamais* using extracts from various plant sources including balsam spurge (*Euphorbia balsamifera*), Henna tree (*Lawsonia inermis*), tropical girdlepod (*Mitracarpus hirtus* L.), and sicklepod (*Senna obtusifolia*). They determined the LC_{50} of these extracts after 1 week of exposure and calculated a range of LC_{50} ranging from 1.41×10^4 ppm to 1.651×10^5 ppm. While these comparisons provide a general framework for interpreting the activity of the extract, they should likewise be considered qualitative due to methodological differences across studies.

The calculated lethal concentration can still be considered for its applicability in pest management. Post-harvest application could be explored through application of the extract to infected corn bags/sack by spraying using appropriate equipment. Without the dilution, the extract can exhibit significantly greater insecticidal effect for potential post-harvest applications with less safety and environmental concerns regarding its residue as compared to commercial pesticides. The observed insecticidal activity can be explained by analyzing the components of the extract in molecular docking.

***In Silico* Analysis**

The 3D protein structures from AlphaFold are presented below (Figures 3 and 4). The dockings were performed using Lamarckian genetic algorithm using 50 GA runs and a population size of 300.

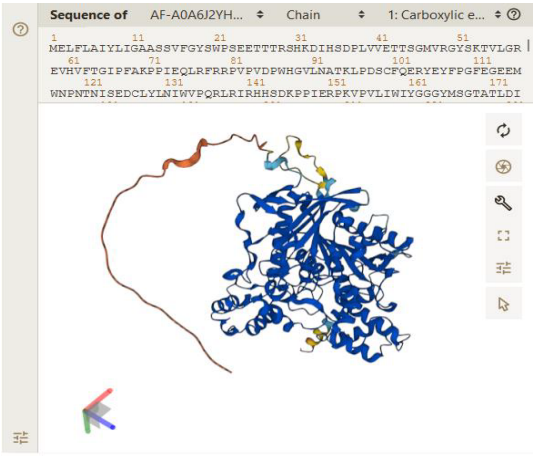


Figure 3. 3D model of acetylcholinesterase of the target insect retrieved from AlphaFold.

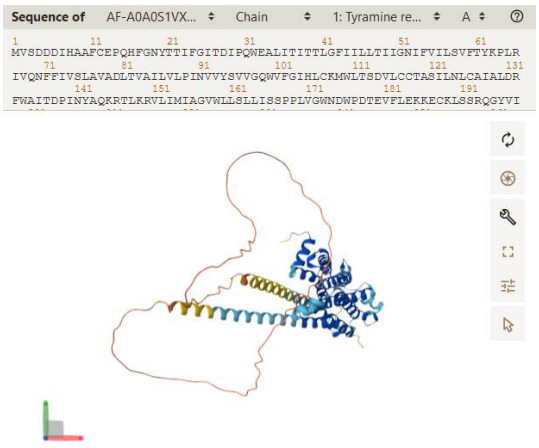
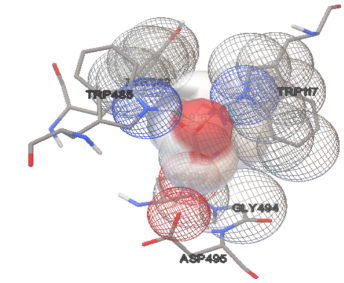
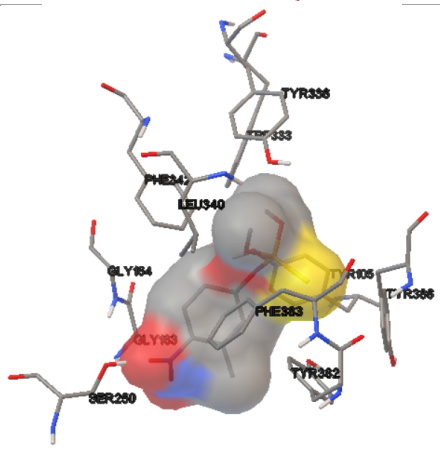
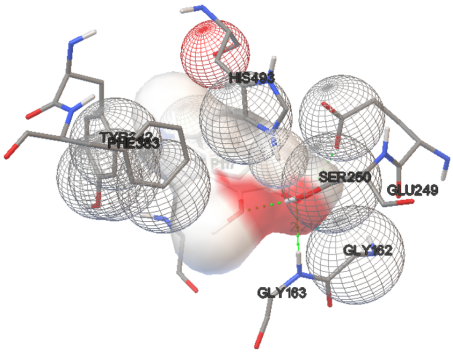
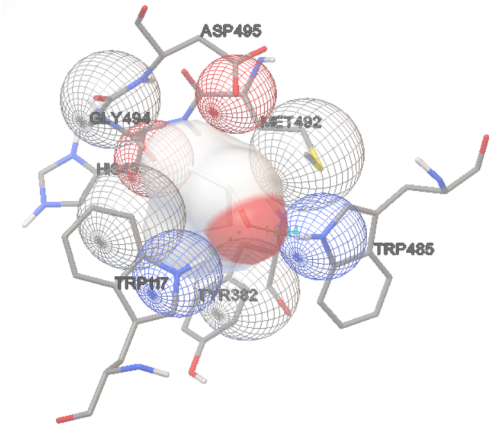


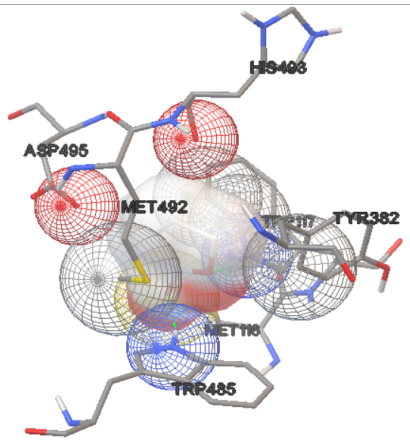
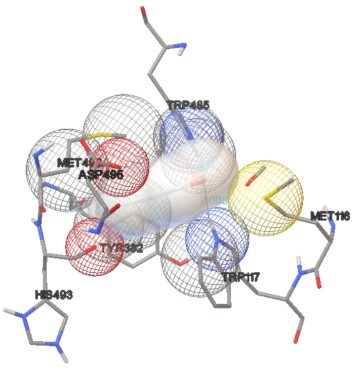
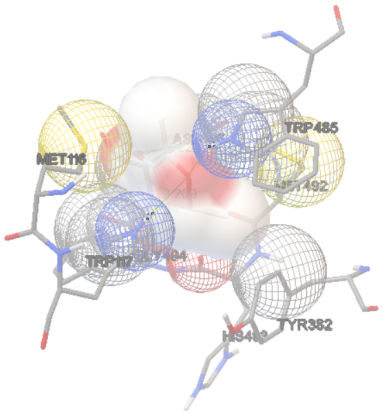
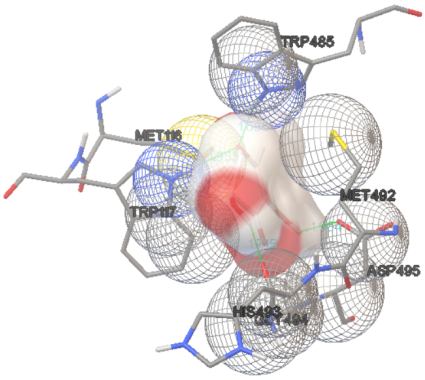
Figure 4. 3D model of octopamine receptor of the target insect retrieved from AlphaFold.

The best docking conformations were determined by their binding energies. The same procedure was done on the determination of the binding site of the octopamine receptor. The grid center coordinates were (x, y, z) = (0.284, -0.679, -2.156). The run time of the molecular docking ranges from 13 to 19 minutes per ligand. It was determined that most of the compounds interact with the target through hydrogen bonding, van der Waals interaction, desolvation, and electrostatic interaction.

The binding energy and the inhibition constant presented (Table 2 for the AChE and Table 3 for OctR) are the values calculated for the best ranked conformation out of the 50 runs of 300 population. The binding energies, inhibition constants, and the residues interacting with the ligand suggest that that 2-Methoxy-4-vinylphenol, octanamide, N-(2-mercaptoethyl)-, and benzofuran, 2,3-dihydro- are among the compounds that have significant interaction with the insect AChE that might be related to insecticidal activity. On the other hand, the compounds that show favorable interaction with the OctR are the following: benzofuran, 2,3-dihydro-, 3-deoxy-d-mannoic lactone, and B-D-glucopyranoside, methyl.

Table 2: Molecular docking results of ligands-AChE visualized in AutoDock 4.

COMPOUND IDENTITY	BINDING ENERGY (KCAL/MOL) AD4	INHIBITION CONSTANT (μM)	INTERACTION	COMPLEX VISUALIZED
Acetylcholine (control)	-6.08	35.21	TRP117, TRP485, SER250	
Fenitrothion (OP control)	-5.2	153.22	SER250, TYR105,	
2-Methoxy-4-vinylphenol	-5.65	71.96	GLY163, SER250, GLU249, HIS493, TRP117, TYR382, PHE383	
Butanal, 2-methyl-	-3.97	1.23	TRP117, TRP485	

Butyrolactone	-4.28	732.36	TRP117, TRP485	
Benzofuran, 2,3-dihydro-	-5.27	192.98	TRP117, TRP485	
Furaneol	-4.34	662.61	TRP117, TRP485, ASP495	
4H-Pyran-4-one, 2,3-dihydro-3,5-dyhydroxy-6-methyl-	-4.81	298.93	TRP117, TRP485, ASP495, HIS493	

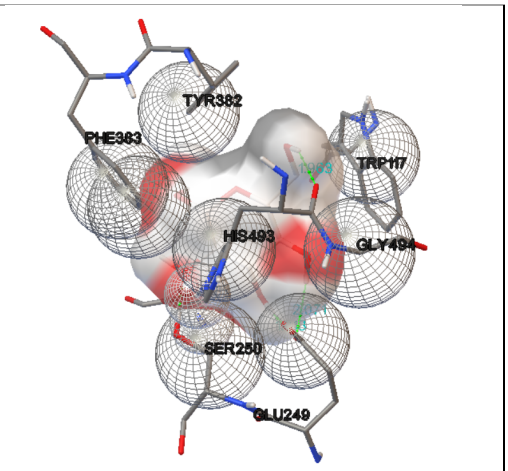
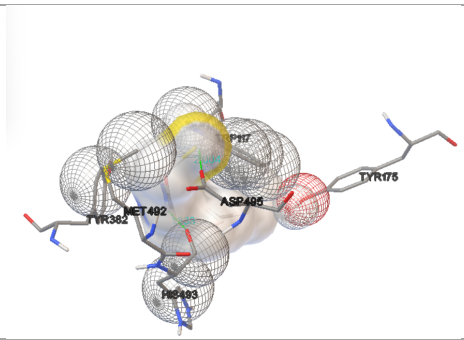
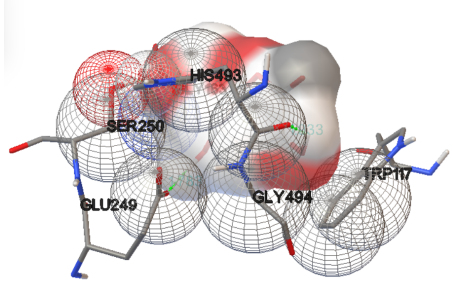
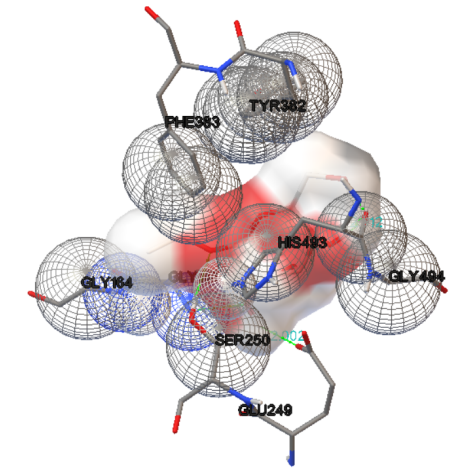
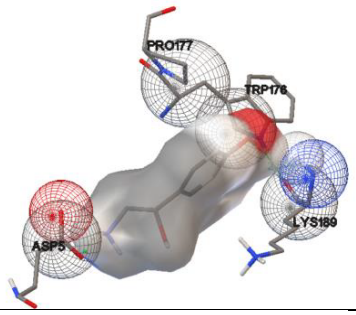
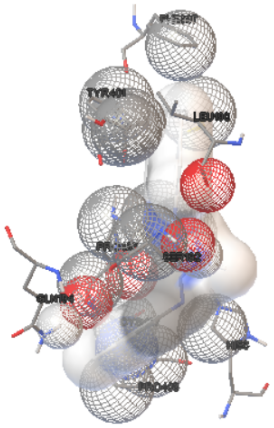
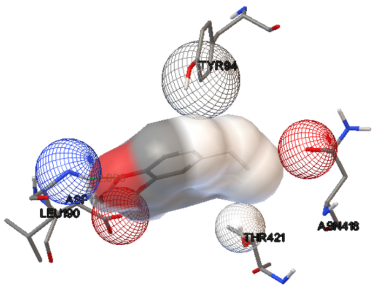
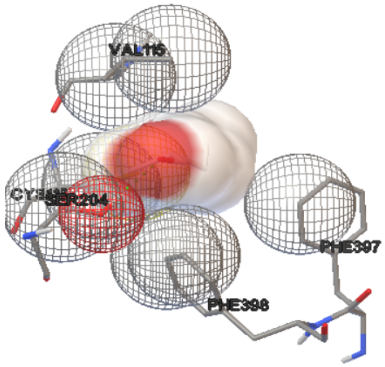
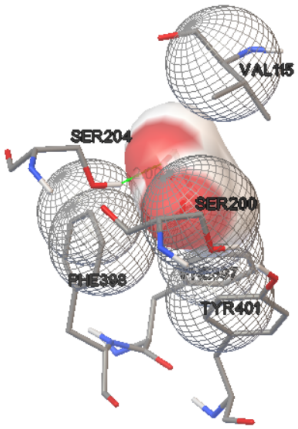
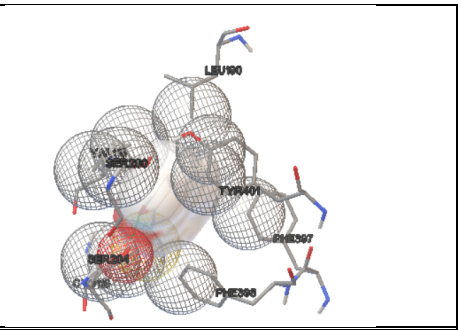
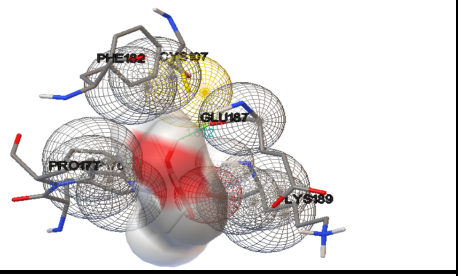
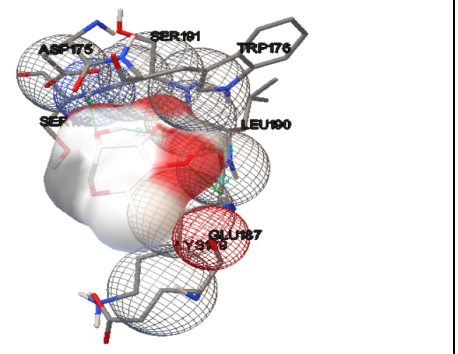
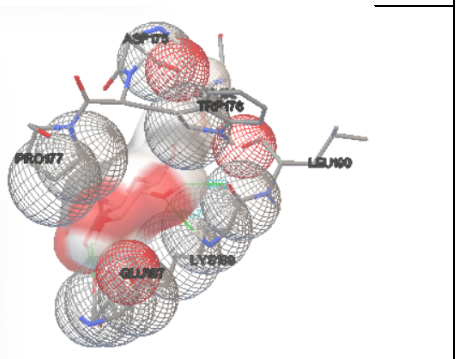
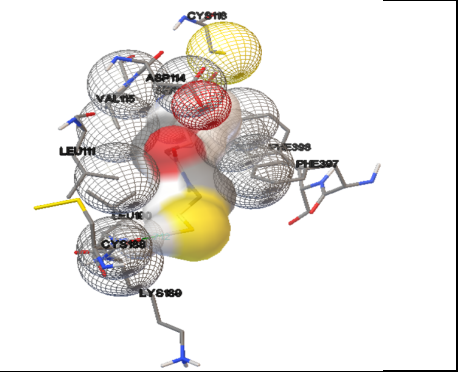
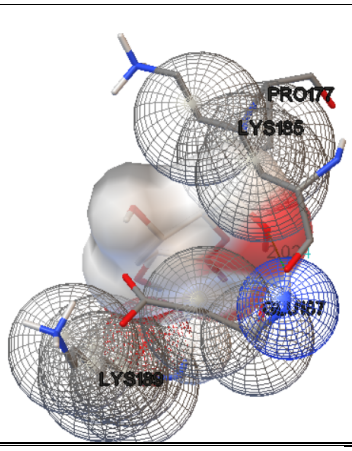
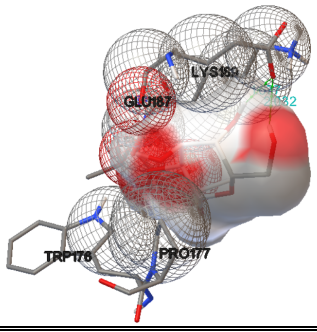
B-D-Glucopyranoside, methyl	-4.65	390.91	SER250, GLY494, GLU249	
Octanamide, N-(2-mercaptoethyl)-	-5.24	144.21	TYR382, TRP117, HIS493	
3-Deoxy-d-mannoic lactone	-4.71	354.38	SER250, GLU249, HIS493	
Ethyl α-D-glucopyranoside	-4.84	285.09	GLY163, SER250, HIS493	

Table 3. Molecular docking results of ligands-OctR visualized in AutoDock 4.

COMPOUND IDENTITY	BINDING ENERGY (KCAL/MOL) AD4	INHIBITION CONSTANT (μM)	INTERACTION	COMPLEX VISUALIZED

Octopamine (control)	-4.67	375.35	ASP5, LYS189, TRP176, PRO177	
Amitraz (control)	-7.5	3.16	HIS8, LEU190, PRO408, CYS407, MET400	
2-Methoxy-4-vinylphenol	-3.47	2.85	LEU190	
Butanal, 2-methyl-	-3.65	2.12	SER204	
Butyrolactone	-3.79	1.68	SER204	

Benzofuran, 2,3-dihydro-	-5.29	133.15	SER204, VAL115, LEU190, PHE397, PHE398, TYR401	
Furaneol	-4.38	616.79	GLU187	
4H-Pyran-4-one, 2,3-dihydro-3,5-dyhydroxy-6-methyl-	-4.55	460.98	LYS189, SER192, LEU190	
B-D-Glucopyranoside, methyl	-4.96	232.54	TRP176, GLU187, LYS189, LYS189	
Octanamide, N-(2-mercaptoethyl)-	-4.33	699.91	CYS188	

3-Deoxy-d-mannoic lactone	-5.09	184.6	GLU187, LYS189	
Ethyl α-D-glucopyranoside	-4.28	731.83	LYS183	

Among the compounds with promising interaction with the insect protein targets, benzofuran, 2,3-dihydro- is the only compound that has favorable interaction with both AChE and OctR. This indicates that the *P. umbellatum* L. leaf extract may demonstrate insecticidal activity to *S. zeamais* in several modes of action, primarily neurotoxic activity through inhibition of the acetylcholinesterase and the octopamine receptor.

To better understand the ligand-receptor interaction formed between the five promising ligands to their targets, analysis using Protein-Ligand Interaction Profiler (PLIP) was employed. 2-Methoxy-4-vinylphenol (Figure 5) was resolved to interact with the AChE binding site through H-bonding of the ligand O lone pair and the amine H of the GLY163, H-bonding of the ligand -OH with the GLU249 side chain oxygen, H-bonding of the ligand O with the SER250 -OH side chain, and the H-bonding of ligand O with the HIS493 -NH. Hydrophobic interactions were also observed with the TRP117, TYR382, and PHE383. T-shaped/perpendicular π-stacking was also observed between the ligand and the HIS493 residue. Octanamide, N-(2-mercaptoethyl)- interaction with AChE (Figure 6) was determined to be dominated by hydrophobic interactions between TRP117 and TYR382. H-bonding was observed between the ligand -NH and the HIS493 O. Analysis of benzofuran, 2,3-dihydro- in PLIP revealed the interactions with AChE (Figure 7) which include H-bonding of the ligand O with the -NH of the TRP117 and TRP485, hydrophobic interaction with TYR382, and the T-shaped/perpendicular π-stacking of the ligand aromatic ring with the TRP117. For the OctR (Figure 8), H-bonding of ligand O with SER204 -OH and hydrophobic interactions with VAL115, LEU190, PHE397, PHE398 and TYR401 were observed. The docking of 3-Deoxy-d-mannoic lactone with the OctR (Figure 9) showed interactions which include H-bonding of ligand -OH and GLU187 O, H-bonding of ligand O with GLU187 -OH, H-bonding of ligand O with GLU187 -NH, H-bonding of ligand -OH with GLU187 O, H-bonding of ligand O with LYS189 -OH, and H-bonding of ligand O with LYS189 -NH. Hydrophobic interactions of the ligand with GLU187 and LYS189 is also present. The target-ligand complex of B-D-Glucopyranoside, methyl with OctR (Figure 10) showed solely H-bonding interactions with TRP176, GLU187, and LYS189 due to the presence of multiple -OH in the ligand.

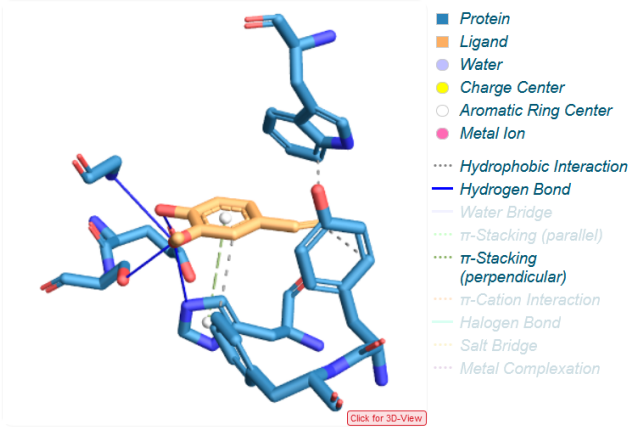


Figure 5. 3D model of the complex created by docking 2-methoxy-4-vinylphenol to acetylcholinesterase.

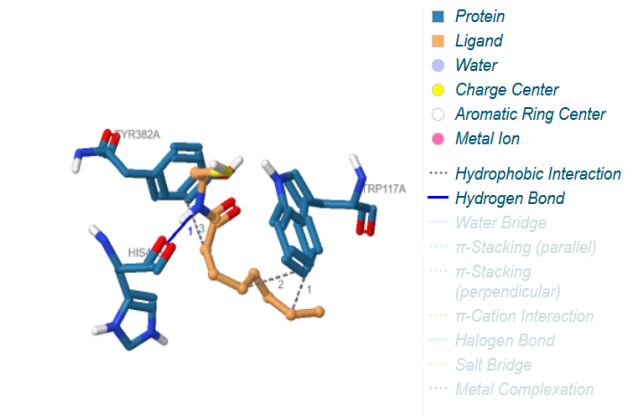


Figure 6. 3D model of the complex created by docking octanamide, N-(2 mercaptoethyl)- to acetylcholinesterase.

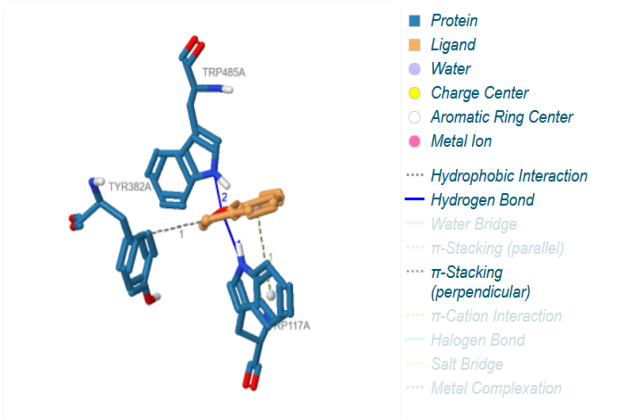


Figure 7. 3D model of the complex created by docking benzofuran, 2,3-dihydro- with acetylcholinesterase.

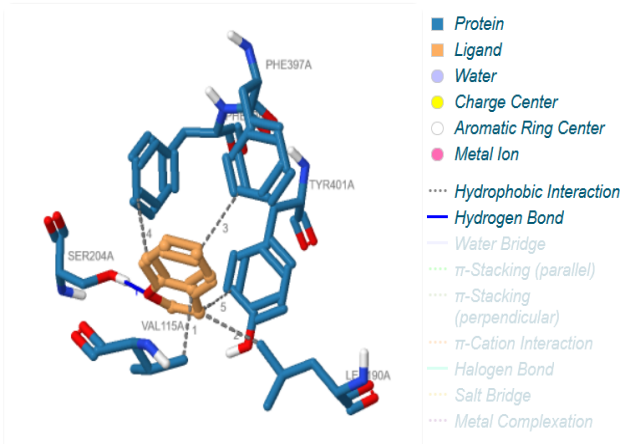


Figure 8. 3D model of the complex created by docking benzofuran, 2,3-dihydro- to octopamine receptor.

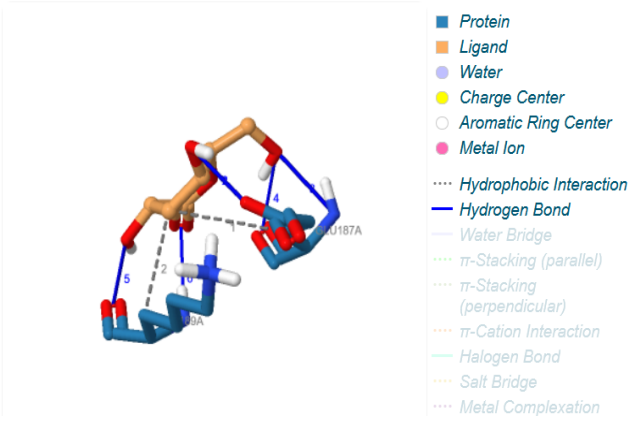


Figure 9. 3D model of the complex created by docking 3-Deoxy-d-mannoic lactone to reg receptor.

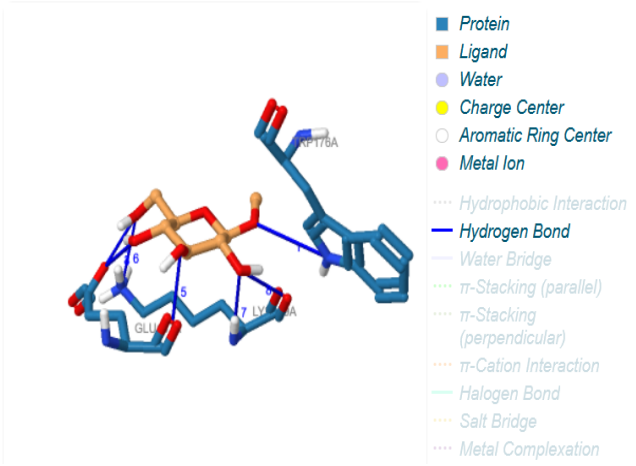


Figure 10. 3D model of the complex created by docking beta-D-Glucopyranoside, methyl to octopamine receptor.

The result of the molecular docking, specifically the binding energy is comparable to literature journals that performed the molecular docking of certain ligands to the protein target. In the study where that focused on the molecular docking and simulation of mammalian acetylcholinesterase by Bennion et al. (2013), they determined the active site and binding energy of mammalian acetylcholinesterase when they docked the natural substrate acetylcholine and the nerve toxins used as chemical weapon agents such as sarin, tabun, and soman to the enzyme. They determined the active site to be at the TRP86 and adjacent amino acids such as SER203, GLY120, GLY121, and ALA204. Their study also presented the binding energies that ranges from -6.83 to -5.87 kcal/mol using AutoDock Vina. Although both Autodock 4 and Autodock Vina are versions of the same software by Scripps Research, Vina is preferred for its speed and ease of use for virtual screening procedures while Autodock 4 is preferred for accurate calculation of binding energies (Nguyen et al., 2020). In a separate study, Ocampo et al. (2023) was able to perform molecular docking on insect octopamine and tyramine receptors of insects with the natural substrates octopamine (both R and S enantiomers) and tyramine together with well-known monoterpenes. They determined that molecular docking of natural substrates to the octopamine receptor results to binding energies ranging from -5.57 to -5.30 kcal/mol and the binding site to involve the VAL404, ASP114, VAL115, CYS118, SER204, LYS189, LEU190, TRP394, PHE397, and THR421.

The result of the molecular docking study helped identify the candidate compounds most likely to contribute to the observed toxicity of the extract. It showed five promising compounds have noteworthy interactions at the active sites and residues of the target proteins. Overall, this supported the observed bioassay trends. Although two protein targets were studied for probable mode of action, further confirmatory studies must be done to determine the complete mode of action of the compounds identified.

CONCLUSION

The GC-MS analysis demonstrated the presence of unique natural products with measurable insecticidal activity on *P. umbellatum* L under the assay conditions. The GC-MS TIC chromatogram also showed a difference of the *P. umbellatum* L. sample obtained as compared to those from other countries based on the compounds present in the leaf extract as reported by Arunachalam et al. (2020). The GC-MS analysis also showed that the ethanolic leaf extract of *P. umbellatum* L. has compounds previously found in other biological sources and were acknowledged for their insecticidal activity for application in pest management. The presence of compounds with known insecticidal activity such as the

benzofuran, 2,3-dihydro- has shown by the GC-MS result. The contact/residual bioassay evaluation demonstrated a statistically significant mortality against standardized *S. zeamais* with an LC_{50} of 478.60 mL/L. This was supported by the molecular docking study conducted to known insect target proteins. Benzofuran, 2,3-dihydro- was found to bind favorably to the insect acetylcholinesterase and octopamine receptor, giving rise to potentially favorable interactions with acetylcholinesterase and octopamine receptor. However, future studies and modification of the methodology can be made to provide better understanding of the mode of action of the compounds. The insecticidal activity of the extract can be improved through modifying the extraction method in this study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Anthony Joperson B. De Guzman: Formulated the study and experimental design, conducted the experiments, gathered and analyzed data, and interpreted results into the manuscript. Amelia B. Hizon-Fradejas: Provided necessary equipment and facility where analytical experiments were conducted. In addition, responsible for the review, checking, aligning, and supervising of the methodology, study framework, and manuscript. Karen B. Alviar: Provided necessary equipment and facility for the bioassay and rearing procedures together with the supervision, review and correction of experimental design, *in silico* processes, and manuscript. Ruel C. Nacario: Provided supervision, alignment, and critique on the theoretical framework and experimental design. In addition, provided problem-solving assistance, and important revisions for the study workplan and the manuscript.

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