

Identification of flavonoids 4'methoxypelargonidin and Luteolinidin from Parthenium hysterophorus L. and their investigation as an acetylcholinesterase inhibitor against Phlebotomus papatasi using in silico study

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Abstract

Phlebotomus papatasi is a vector of various health risk diseases, such as sandfly fever and leishmaniasis. Chemical-based insecticides seem effective against the vector but are harmful to the non-targeted organisms and the environment. Plant metabolites are an excellent source of natural insecticides without showing any adverse effects. To the environment, plant-based flavonoids are emerging compounds that can be used against human disease vectors. In the present investigation, flavonoids were partially isolated from the leaf and flower of *Parthenium hysterophorus* and characterized using column chromatography. After their identification as 4'-methoxypelargonidin and luteolinidin from LC-MS analysis, flavonoids were further used in the functional analysis through the *in-silico* study against acetylcholinesterase (AChE), a key enzyme for neurotransmission in sand flies. The docking studies revealed that the binding affinity of 4'-methoxypelargonidin (-9.31 Kcal/mol), and luteolinidin (-9.1 Kcal/mol) were significantly more than the reference compound DEET (-6.4 Kcal/mol) against the AChE. Based on the computational study, the findings may provide a leading novel, natural, and effective insecticide against *Phlebotomus papatasi*, contributing to the control and prevention of *Chandipura vesiculovirus*.

Introduction

Vector-borne illnesses threaten public health globally, especially in tropical and sub-tropical areas. Their effects harm the economies and limit the growth in rural and urban areas. In addition, vector-borne diseases increase the chances of unknown future risks (Sutherst, 2004). *Phlebotomus papatasi*, commonly known as sandfly, is a primary vector of various infectious diseases, including Leishmaniasis, Pappataci fever, and Chandipura virus (*Chandipura vesiculovirus*), widely called CHPV (Dantas-Torres et al., 2012). Insect vector resistance to synthetic insecticides is becoming a significant problem in pest control and public health. This has led scientists to consider sustainable and effective eco-friendly alternatives (Pathak et al., 2022). Naturally, plant-derived compounds, particularly flavonoids, have attracted researchers due to their diverse biological properties, such as antioxidant, anti-inflammatory, neuroprotective, antibacterial, antifungal, and antiviral activities (Ullah et al., 2020). Luteolinidin is a bioactive compound with prospective medicinal benefits and serves as a stable marker in botanical taxonomy (Tsukasa Iwashina et al., 2010). Luteolin, a similar flavonoid, also possesses antiulcer and anti-inflammatory activities (I.U. Asuzu 2014). Moreover, luteolin derived from *Junicus acutus* has demonstrated antiviral activity against the coronavirus HCoV-229E (Asma Hakem et al., 2023). Pelargonidin, a flavonoid derived from certain medicinal plants, has been examined for antioxidant activity (Li et al., 2022). Flavonoid-based antiviral activities have been proven against severe acute respiratory syndrome (SARS-CoV), chikungunya, and Japanese encephalitis (JEV) (Zakaryan et al., 2017). Flavonoids extracted from the different plants showed significant insecticidal activity against human vectors (Gautam et al., 2013). Acetylcholinesterase (AChE) is crucial for terminating nerve impulses in insects. Several studies reported that flavonoids can inhibit AChE and impair brain activity, causing paralysis and ultimately leading to the death of insects (Pereira et al., 2024). *P. hysterophorus*, an invasive species from Mexico, has been spread worldwide due to its remarkable adaptation and

durability in varied climatic circumstances. It causes skin rashes, respiratory disorders, and impotence in humans. However, this plant has negatively impacted humankind, but the entire plant has traditionally been employed for its therapeutic characteristics, encompassing anti-dysenteric, febrifuge, and emmenagogue. (Patel, 2011). Employing in-silico studies to investigate the insecticidal activity of certain flavonoids helps to understand the interaction between isolated compounds and the targeted protein. It may provide an affordable method of checking for possible insecticidal screening (Rodrigues et al., 2021). Our study aims to characterize and identify the natural flavonoids luteolinidin and 4'-methoxypelargonidin from the leaves and flowers of *P. hysterothorus* and to assess their potential as acetylcholinesterase inhibitors against *P. papatasi* as an eco-friendly pest control option.

Methods

Chemicals and reagents

Quercetin, ferric chloride, sodium hydroxide, chloroform, methanol, and acetonitrile (HiMedia) were of analytical grade. Column, silica gel, and precoated aluminum sheets with a thickness of 0.25mm were utilized for the analytical work.

Experimental section

Collection of plant samples

Fresh leaves and flowers of wild-growing *P. hysterothorus* were collected from Indira Gandhi National Tribal University (IGNTU), Anuppur, M.P., India. Dr. Arti Garg, Botanical Survey of India, Allahabad, identified the plant specimen (BSI/RGC/GOI/2022-23/283). The leaves and flowers were carefully rinsed with tap water to remove impurities. They were subsequently shade-dried, finely ground into a powder, and stored at 4°C for further use.

Plant extract preparation

Ten grams of dried plant powder were macerated in 100 ml of water. The suspension obtained was purified through a Whatman No. 1 filter paper. The aqueous extracts were then concentrated through lyophilization (LABCONCO) and labeled as LE (leaf extract) and FE (flower extract).

Phytochemical analysis

The Harborne method was employed to conduct phytochemical analysis with the LE and FE (Harborne, 1973). This screening aimed to identify the presence of flavonoids.

Assessment of total flavonoid content

The aluminum chloride technique was adopted to determine the total flavonoid content (Chang et al., 2020). The water was diluted for each extract until the concentration was 1mg/ml, and a calibration curve was created using quercetin (20-100µg/ml). The resulting mixture was formed by combining 2ml

of attenuated extracts and quercetin with 100µl of 10% AlCl₃ and 100µl of 0.1mM CH₃COOK. After 30 min. of incubation at 25°C, the absorbance was measured at 415 nm. To determine the overall flavonoid concentration, mg quercetin equivalent per gram (QE/g) dry weight of extracts was utilized.

Partial isolation of flavonoids

Thin-layer chromatography

The LE and FE were applied as spots using a capillary tube, and the plates were developed in a mobile phase composed of chloroform: methanol: acetonitrile (65:25:10, v/v). The developed plates were air-dried and examined under UV light (254nm) (Lina et al., 2013).

Column chromatography

Based on TLC results, the extract was subjected to column chromatography for further purification. In the stationary phase, a glass column (50 × 2.5 cm) was filled with silica gel (60-120 mesh). The column was equilibrated with chloroform, methanol, and acetonitrile, and the extract was dissolved in a minimal amount of DMSO and loaded onto the column. The column was eluted with a chloroform: methanol: acetonitrile (65:25:10 v/v/v) at a flow rate of 0.2 ml/min, and fractions of 2 ml each were collected. Further characterization was done to identify the presence of flavonoids in fractions.

Characterization of flavonoids

UV-Vis spectroscopy

The UV-Vis spectrophotometer (UV-1800, SHIMADZU) was used to measure the presence of phytocomponents, including flavonoids. The investigation, which focused on the electronic transitions connected to several functional groups, including O–H, N–H, C=O, C=C, C–C, C–H, and C–N, encompassed a wavelength range of 100–600 nm.

Liquid chromatography mass spectrometry (LC-MS)

The molecular weight and structure of the flavonoids were determined using an LC-MS SCIEX (QTRAP Enabled Triple Quad 5500+) with the following protocol. Column: C18 reverse-phase, Mobile Phase: Gradient elution with acetonitrile, Flow Rate: 0.3 ml/min, Injection Volume: 5 µL, Acquisition Method: Metabolome METHOD-60 Min)-22122023. Ionization Mode: Electrospray Ionization (ESI) in positive and negative modes, Capillary Voltage: 3.5 kV (positive mode) / 3.0 kV (negative mode), Source Temperature: 150°C, Desolvation Temperature: 400°C, Desolvation Gas Flow: 800 L/h, Algorithm Used: MQ4, the mass spectra were analyzed, and compounds were identified by comparing the obtained spectra with databases and literature.

***In silico* molecular docking analysis preparation of the targeted protein**

Acetylcholinesterase (AChE) was chosen as the targeted protein for computational docking because it is a crucial enzyme that regulates the nervous system of insects. The gene sequence was obtained from the National Center for Biotechnology Information (NCBI) (Fig.1) (<https://www.ncbi.nlm.nih.gov/>) and saved. The protein structure was predicted using the Google Alpha fold protein structure database for high-accuracy results (<https://alphafold.ebi.ac.uk/>), and the predicted protein was saved in the form of PDB and then into PDBQT. The resolution of the protein was 1.5 Å (Fig. 2).

Active site prediction

DogSiteScorer examined the active site of the targeted protein (<https://proteins.plus/help/dogsite>). The predicted active site pocket in Fig.3 was further used in molecular docking analysis. The grid box was adjusted within the AChE's adjusted active site with x (40), y (50), and z (38) dimensions and centers x, y, and z (0.816, -2.139, and -0.449) to ensure the docking configuration and algorithm.

Ligand preparations

After identifying the flavonoids (4'methoxypelargonindin, luteolinidin) from the leaves and flowers of *P. hysterothorus*, their potential insecticidal activity was considered. The synthetic insecticide N, N-diethyl-meta-toluamide (DEET) was considered a reference compound to compare the efficacy of the natural compounds. The molecular structure of these ligands was drawn in ChemDraw 16.0 and saved as a mol file. Further, the mol files of ligands were changed into the PDB files using the Avogadro molecule editor (<https://avogadro.cc/>) to enable docking studies.

In silico analysis

in silico molecular docking analysis was examined using AutoDock Vina 1.2.5 (<http://autodock.scripps.edu/>), a widely available software for docking analysis. All the targeted ligands, including the reference and the targeted protein, were converted into PDBQT from the PDB file. The docking procedure includes ligand conformation prediction and orientation inside the target protein's intended binding site. A docking comparison of the energies of each ligand against the target protein is used to determine the ligands having the most significant binding energy. Molecular docking was carried out utilizing Vina (<http://vina.scripps.edu>).

Results

Preliminary qualitative and quantitative analysis

The flavonoid concentration in the leaf and flower extracts of *P. hysterothorus* was assessed and quantified in mg QE/g dry weight. The leaf extract exhibited more flavonoid concentration (19.60±0.48mg QE/g dw) than the flower extract (18.68±0.72 mg QE/g dw) (Fig. 4). Three bands were observed in the leaf and flower samples, indicating the presence of conjugated double bonds or aromatic rings. No interaction or reactivity was detected between the silica gel stationary phase and the

mobile phase components due to its inertness and high surface area, ensuring the system's stability and the reliability of the separation process.

Column chromatography

The presence of flavonoids was confirmed in 5 out of 54 collected leaf fractions (L1, L2, L3, L4, L5) and 4 out of 54 collected flower fractions (F1, F2, F3, F4). L4 and F4 were selected for further characterization due to their more substantial positive results based on phytochemical investigation in the flavonoid assays.

UV-VIS spectroscopy

The UV-Vis spectral analysis of the flower and leaf fraction of *P. hysterothorus* was conducted to identify flavonoid compounds. The recorded spectra (Fig.5) exhibited significant absorbance peaks at 200-400nm, corresponding to flavonoids' typical UV absorption characteristics. The flavonoid structure determines these bands' particular positions and intensity, precisely the substitution pattern on its structural skeleton. Flavones and flavonols typically exhibit two major absorption bands: 240–280 nm, which corresponds to the benzoyl system, and 300–400 nm, which corresponds to the cinnamoyl system. hydroxyl groups can shift the absorption maxima and alter the intensity, especially in positions 3 and 5. (Fig. 5) Flavanones generally show a lower intensity band in the UV region (250–290 nm) and a less intense or absent (300-400) due to the lack of conjugation in the C-ring.

Liquid chromatography-mass spectroscopy

In the LC-MS analysis of *P. hysterothorus*,17 compounds were identified in the leaf fraction (L4) and 9 in the flower fraction (F4). In the flower fraction, flavonoid was identified with a 4'-Methoxypelargonidin retention time of 25.04 and an m/z 285.1207. However, in the leaf fraction, two flavonoids were identified: Luteolinidin, with a retention time of 19.54 and an m/z 271.0698, and 4'-Methoxypelargonidin, which appeared at a retention time of 22.19, with m/z 285.0555. (Fig.6 &7) (Table 1 & 2).

Table 1 LC-MS data of the leaf fraction of *P. hysterothorus*.

Found at mass(M/Z)	Precursor mass	Expected RT	Library score	Library hit
104.040	104.0494	1.13	60.4	N-Methyl-L-alanine
118.0597	118.080	1.22	99.1	3-Aminopentanoic acid
138.2484	138.240	1.32	59.4	3-Pyridylacetic acid
193.1662	193.164	1.34	91.8	(-)-Quinic acid
132.2359	132.240	1.37	76.0	Caldine
112.2040	112.200	1.46	92.5	Histamine
149.1137	149.160	9.21	85.6	Phthalic anhydride
78.9555	78.960	9.45	97.2	Dimethyl sulfoxide
400.1722	400.174	16.33	72.8	Palmitoylcarnitine
414.1769	414.175	17.91	100.0	Norbuprenorphine
153.1416	153.142	22.12	97.3	3-Methoxybenzoic acid
343.1596	343.159	23.55	53.5	2.alpha.-Mannobiose
285.1207	285.120	25.04	93.0	4'-Methoxypelargonidin cation
107.1539	107.160	37.05	97.9	Benzaldehyde
459.120	459.2036	55.17	60.1	His-Phe-Arg
601.656	601.6486	59.44	95.5	1-Oleoyl-2-linoleoyl-rac-glycerol
531.663	531.6647	59.55	84.2	Cyclo(Gln-Trp-Phe-Gly-Leu-Met)

Table 2 LC-MS data of the flower fraction of *P. hystrophorus*

Found at mass (m/z)	Precursor mass	Expected RT	Library score	Library hit
104.1179	104.040	1.07	76.8	N-Methyl-L-alanine
117.8297	117.840	1.11	99.5	3-Aminopentanoic acid
78.8453	78.840	1.72	96.2	Dimethyl sulfoxide
149.1049	149.107	3.30	96.9	Phthalic anhydride
271.0698	271.071	19.54	50.9	Luteolinidin cation
285.0555	285.055	22.19	92.8	4'-Methoxypelargonidin cation
107.1587	107.160	47.34	89.7	Benzaldehyde
601.5813	601.581	57.33	63.8	1-Oleoyl-2-linoleoyl-rac-glycerol
338.6378	338.640	57.80	55.1	Arg-Tyr

***In silico* molecular docking**

Protein and ligand PDBQT files were accessed for the molecular docking analysis. AutoDockVina was utilized to dock the protein with the ligands. The grid box was set along with the active site, which was predicted through DogSiteScorer. Molecular docking scores were generated by running the command. 4' methoxypelargonidin showed the best binding affinity at -9.3 Kcal/mol, followed by luteolinidin at -9.1 Kcal/mol, and the binding affinity of the reference compound (DEET) was -6.4 Kcal/mol, which was lower than both identified flavonoid compounds. The 2D and 3D visualization of interactions of both the identified flavonoids is shown in (Figs. 8 and 9). Table 3 displayed the conventional hydrogen bonding and Pi-Pi T-shaped bonding with amino acids TYR 258 (2.73, 3.51), LEU 420 (2.17), and TRP 417 (5.07), TYR 470 (5.04, 5.65). However, the molecular docking indicated a robust binding affinity between 4' methoxypelargonidin and amino acid residues with a docking score of -9.3 Kcal/mol. During the docking studies, an unfavorable donor interaction was also observed (LEU, 2.21). This is because a hydrogen bond donor in the ligand or the protein is oriented towards another hydrogen bond donor, creating repulsive interactions. The overall negative docking score indicates that the other favorable interactions likely compensate for this unfavorable interaction, contributing to the stability of the ligand-protein complex. On the other hand, luteolinidin (Table 4) conventional hydrogen bonding, Carbon hydrogen bonding Pi-anion bonding, Pi-donor hydrogen bonding, Pi-sigma, Pi-Pi stacked and Pi-Pi T shaped with amino acid residues of ASP 209 (3.12), SER (2.97), TYR 267 (1.95), ILE 207 (2.54), GLY 260 (3.38), ASP 209 (4.94), TYR 258 (3.38), ASP (3.85), TRP 221 (4.78, 4.67, 5.68, 4.64), and TYR (4.99).

Table 3 Interaction between AChE and 4'methoxypelargonidin

Amino Acid	Bond Name	Bond Distance
TYR 258	Conventional hydrogen	2.73
LEU 420	Conventional hydrogen	2.17
TYR 258	Carbon hydrogen	3.51
TRP 417	Pi-Pi T-shaped	5.07
TYR 470	Pi-Pi T-shaped	5.04
TYR 470	Pi-Pi T-shaped	5.65
LEU 420	Unfavorable donor	2.21

Table 4 Interaction between AChE and Luteolinidin

Amino acid	Bond Name	Bond Distance
ASP 209	Conventional hydrogen	3.12
SER 259	Conventional hydrogen	2.97
TYR 267	Conventional hydrogen	1.95
ILE 207	Conventional hydrogen	2.54
GLY 260	Carbon hydrogen	3.38
ASP 209	Pi-anion	4.94
TYR 258	Pi- donor hydrogen	3.38
ASP 209	Pi- sigma	3.85
TRP 221	Pi-Pi stacked	4.78
TRP 221	Pi-Pi stacked	4.67
TRP 221	Pi-Pi stacked	5.68
TRP 221	Pi-Pi stacked	4.64
TYR 258	Pi-Pi T shaped	4.99

Discussion

The extraction of crude and identifying flavonoids are essential in discovering insecticides derived from natural products. This method conforms to conventional phytochemical screening protocols and comprehensively evaluates bioactive chemicals. In an earlier investigation, the flower extract of *P. hystrophorus* exhibited more flavonoid concentration ($20.25 \pm 0.12 \mu\text{g QE/g dw}$) than the leaf extract ($2.64 \pm 0.20 \mu\text{g QE/g dw}$) (Pakkirisamy et al., 2017). Li et al. (2022) observed $14.23 \pm 0.10\text{mg QE/g dw}$ for

the ethanolic flower extract, which is less than the present finding (Li et al., 2022a). This disparity may be ascribed to the broader spectrum of flavonoids that aqueous solvents can solubilize, recognized for their robust antioxidant and insecticidal characteristics (Tapas et al., 2008). The higher flavonoid concentration in leaves relative to flowers can be ascribed to genetic variation and microenvironmental influences. Luteolinidin has also been isolated from *Sorghum bicolor* and *Zea mays*, contributed to the seed color, and exhibited antioxidants, anti-inflammatory, anticancer, and antimicrobial effects (Wu et al., 2023).

4'-Methoxypelargonidin, a methylated derivative of pelargonidin, has been reported in various fruits and vegetables, such as strawberries and red radishes, contributing to color and potential health benefits (Giusti & Wrolstad 2003). Methoxy pelargonidin has been recognized for reducing oxidative stress linked to chronic diseases like cardiovascular disease and cancer, and, in parallel, during in-silico analysis of flavonoids 4'-methoxypelargonidin and luteolinidin, identified from *P. hystrophorus*, demonstrated their strong binding affinity toward the target insect protein. These natural compounds exhibited conventional hydrogen bonding, hydrophobic interactions, and π - π stacking, contributing to their stability within the binding site. Moreover, their docking scores were comparable to or higher than those of N, N-diethyl-meta-toluamide (DEET), indicating their potential as effective insecticidal agents. These findings highlight the efficiency of computational approaches in predicting insecticidal activity, providing valuable insights for future experimental validation. The results suggest that these flavonoids could serve as promising natural alternatives to synthetic insecticides, offering a more sustainable and eco-friendly solution for pest management. Recent studies on flavonoids as natural insecticides are also gaining popularity nowadays. Malank et. al (2023) performed the molecular docking analysis of some identified flavonoids and screened their insecticidal activity against *Rhipicephalus microplus*.

Methylenebisphloridzin, thearubigin, fortunellin, quercetagenin-7-O-(6-O-caffeoyl- β -D-glucopyranoside), quercetagenin-7-O-(6-O-p-coumaroyl- β -glucopyranoside), rutin, and kaempferol 3-neohesperidoside showed the potential inhibitory effect against AChE. On the other hand, three flavonoids (quercetagenin-7-O-(6-O-caffeoyl- β -D-glucopyranoside, isorhamnetin, and liquiritin) were potent inhibitors against the triose phosphate isomerase (TIM) protein (Malak et al., 2023). Veronica Herrera-Mayorga et. al (2022) checked the in vitro and *in silico* larvicidal activity of the organic extracts of *Solidago graminifolia* with their flavonoid and polyphenol (Quercetin and chlorogenic acid) against *Spodoptera fugiperda*. Quercetin had an LC₅₀ of 0.157 mg/ml, and Chlorogenic acid did not show any insecticidal activity but also an antagonistic effect; the molecular docking score of quercetin was higher than that of the reference compound (Chlorpyrifos). (Verónica Herrera-Mayorga et al. 2022). Pathak et al. (2022) examined the molecular action of flavonoids against *Aedes aegypti* by targeting the enzyme Glutathione S-transferase (Pathak et al., 2022). Upasani et al. (2003). An *in-silico* study revealed that the inhibitory action of flavonoids suppressed the larval development of *Ae. aegypti*. demonstrated the potential insecticidal, ovicidal, and oviposition deterrent of foliage flavonoids from *Ricinus communis* against *Callosobruchus chinensis* (Upasani et al., 2003). Chellai muthu et al. (2013). Performed the ovicidal and oviposition deterrent activity of the flavonoid pectolinarigenin isolated from *Clerodendrum phlomidis* against *Eari vittela*. This displayed maximum ovicidal activity of 64.29% and oviposition deterrent activity of 100% at

100 ppm concentration against *E. vittella*. (Chellai Muthu et al. 2013). Nenaah (2014) isolated four flavonoids, i.e., isoglabratephrin, (+)-glabratephrin, tephroapollin-F, and lanceolatin-A, from *Tephrosia apollinea* L. and tested against *Sitophilus oryzae* (L), *Rhyzopertha dominica* (F), and *Tribolium castaneum* (Herbst) at concentrations of 0.875, 1.75, and 3.5 mg ml⁻¹. After parental exposure to flavonoids, the generation of F1 progeny in insects was impacted, with *S. oryzae* being the most vulnerable (Nenaah, 2014). This evidence supports our work regarding the *in silico* insecticidal activity of flavonoids from *P. hysterophorus*, and these can be purely isolated in the future and can be used as insecticidal agents. However, this study has a number of limitations. First, the study only used *in silico* docking, which forecasts binding affinity but cannot verify real enzyme inhibition or *in vivo* insecticidal effectiveness. Second, despite the fact that *P. hysterophorus* includes a diverse range of phytochemicals that may function antagonistically or synergistically, only two flavonoids were examined. These constraints emphasize the necessity of additional validation using larvicidal or insecticidal bioassays, as well as *in vitro* enzyme assays.

Conclusion

P. hysterophorus is a noxious weed, but the flavonoids identified from this plant may serve as promising candidates for insecticidal agents. This study reports, for the first time, the partial purification and identification of 4'-methoxypelargonidin and luteolinidin from *P. hysterophorus* and their predicted interaction with AChE of *P. papatasi* through *in silico* docking. While the previous studies have focused on its toxic and allelopathic properties, our findings indicate a potential role for its flavonoids as natural alternatives to synthetic insecticides. However, these results are based solely on computational predictions and partial purification, and their translation into practical applications requires further *in vitro* assay, toxicity evaluation, and *in vivo* insecticidal studies. In addition, flavonoids did not show any adverse effects on non-targeted organisms, but toxicity evaluation is still an essential prospect.

Declarations

Conflict of Interests

The authors declare no conflict of interest.

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Author Contribution

Heena Chaodhary and Parshant have written the Manuscript Ravindra Shukla and T. Srinivasan supervise and validate the manuscript Aman Saket and Shivani Singh assist during the making the draft

Data Availability

The experiment supports the findings of this study and is available in the supplementary material of this article.

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Figures

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>AFP20868.1 acetylcholinesterase [Phlebotomus papatasi]  
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Figure 1

Amino acid sequence of the targeted protein

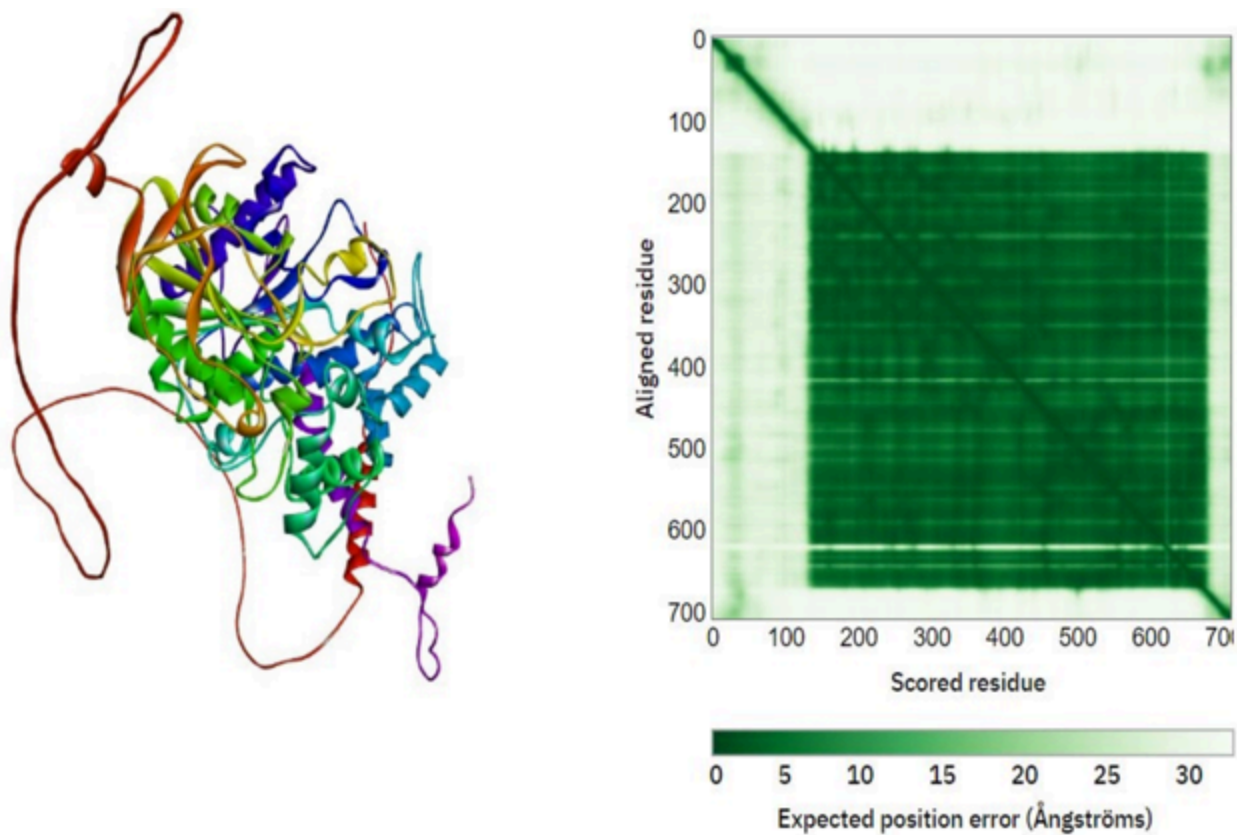


Figure 2

Preparation of the targeted protein

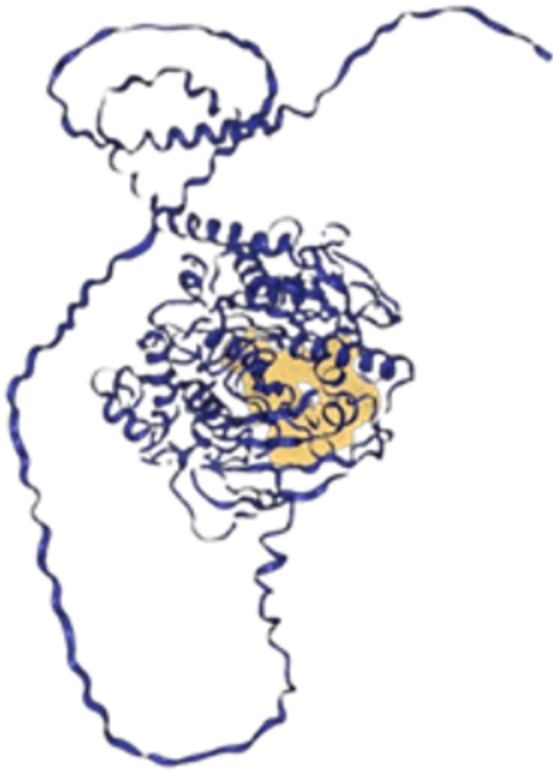


Figure 3

Active site of targeted protein

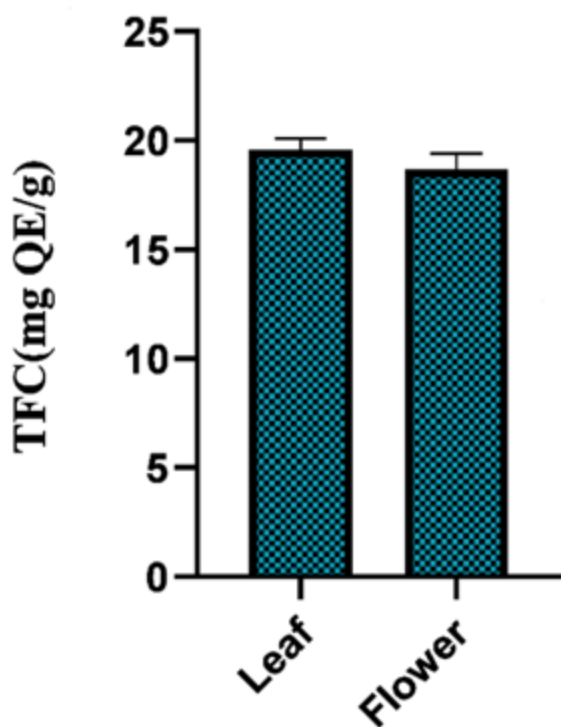


Figure 4

Total flavonoid content of LE and FE of *P. hysterophorus*

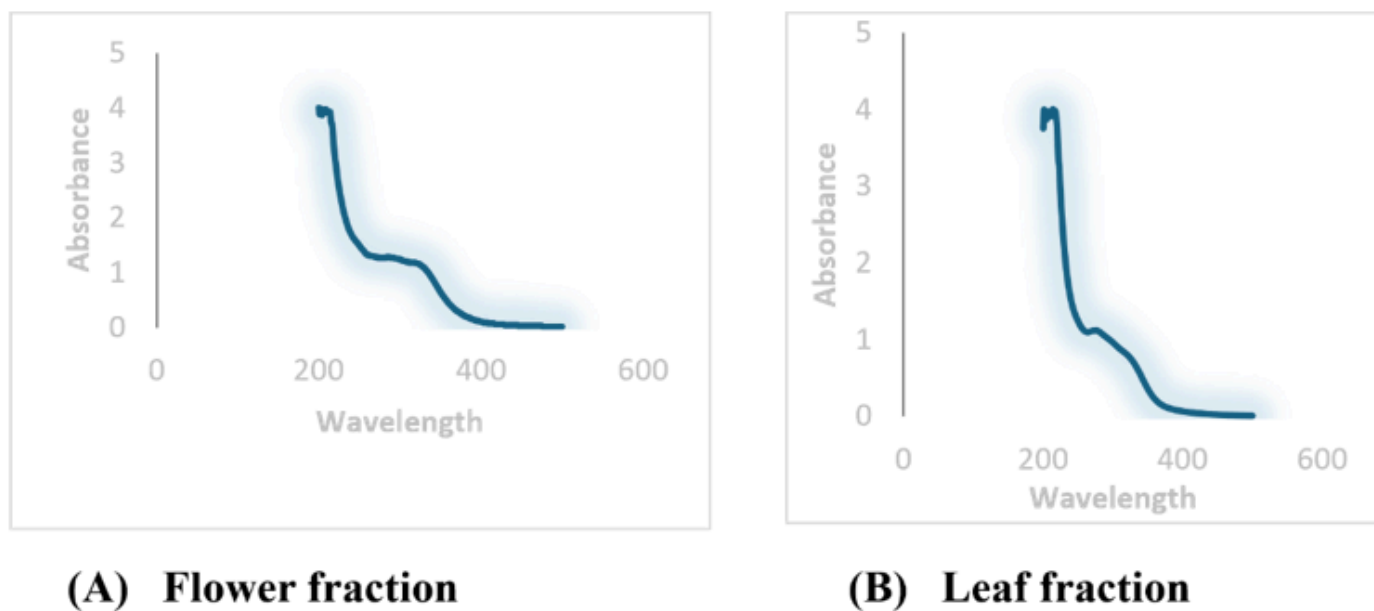


Figure 5

UV-VIS spectral analysis of flower (A) and leaf fraction (B) of *P. hysterophorus*

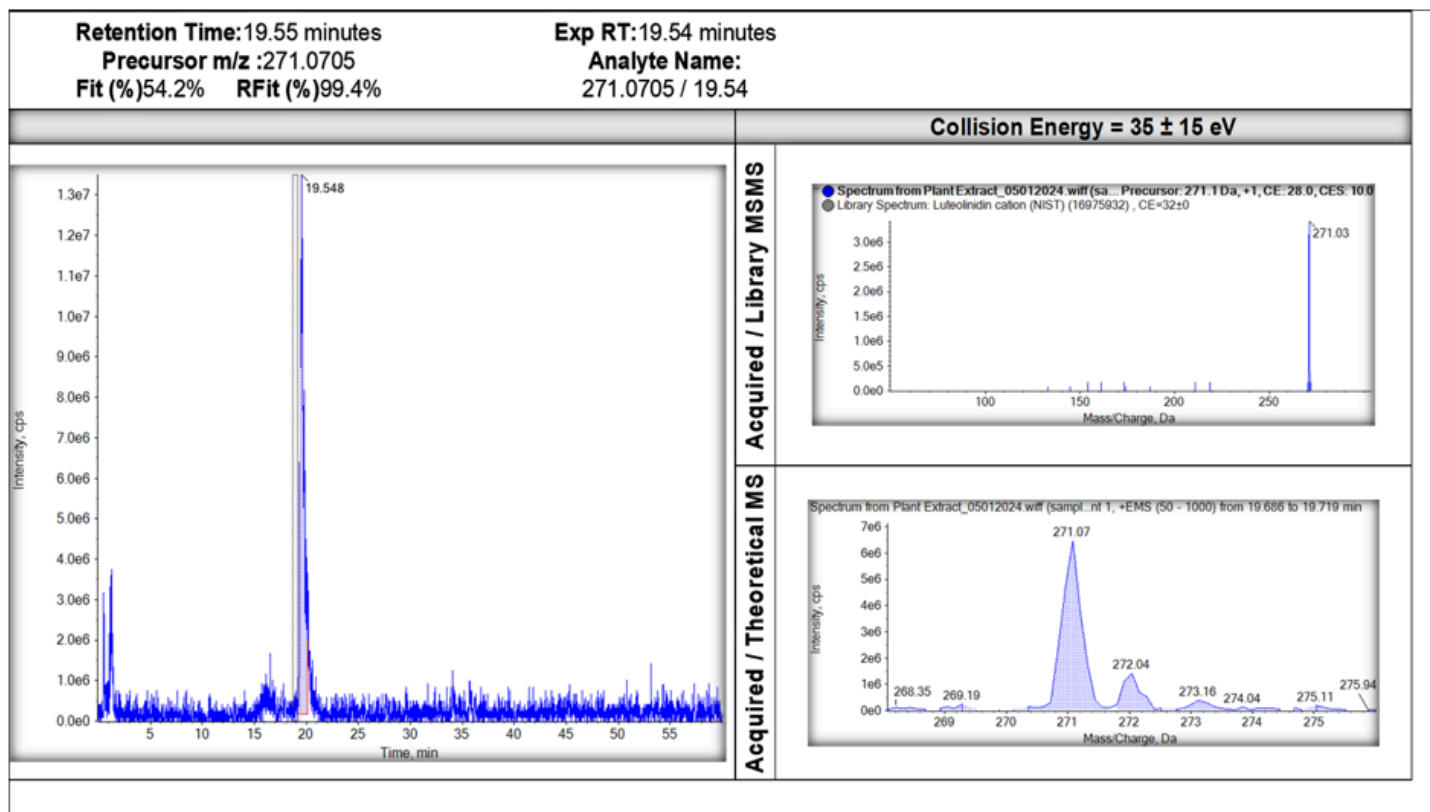


Figure 6

The LC-MS/MS spectrum displays the mass-to-charge ratio (m/z) 271.0705 corresponding to Luteolinidin in the Leaf fraction of *P. hysterophorus*

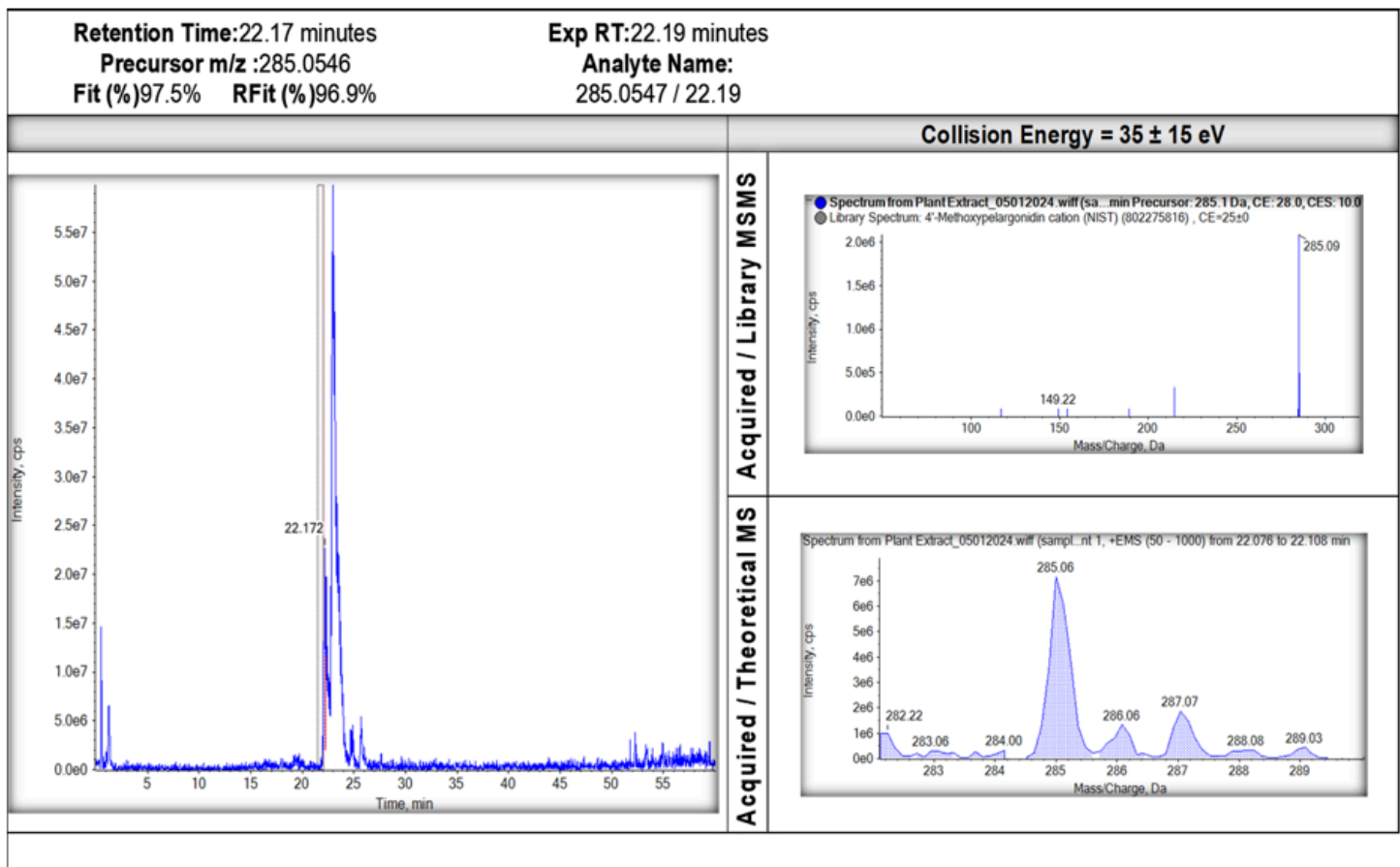


Figure 7

LC-MS/MS spectrum displays the mass-to-charge ratio (m/z) 285.0555 corresponding to 4'-Methoxypelargonidin in the *P. hysterophorus* leaf fraction.

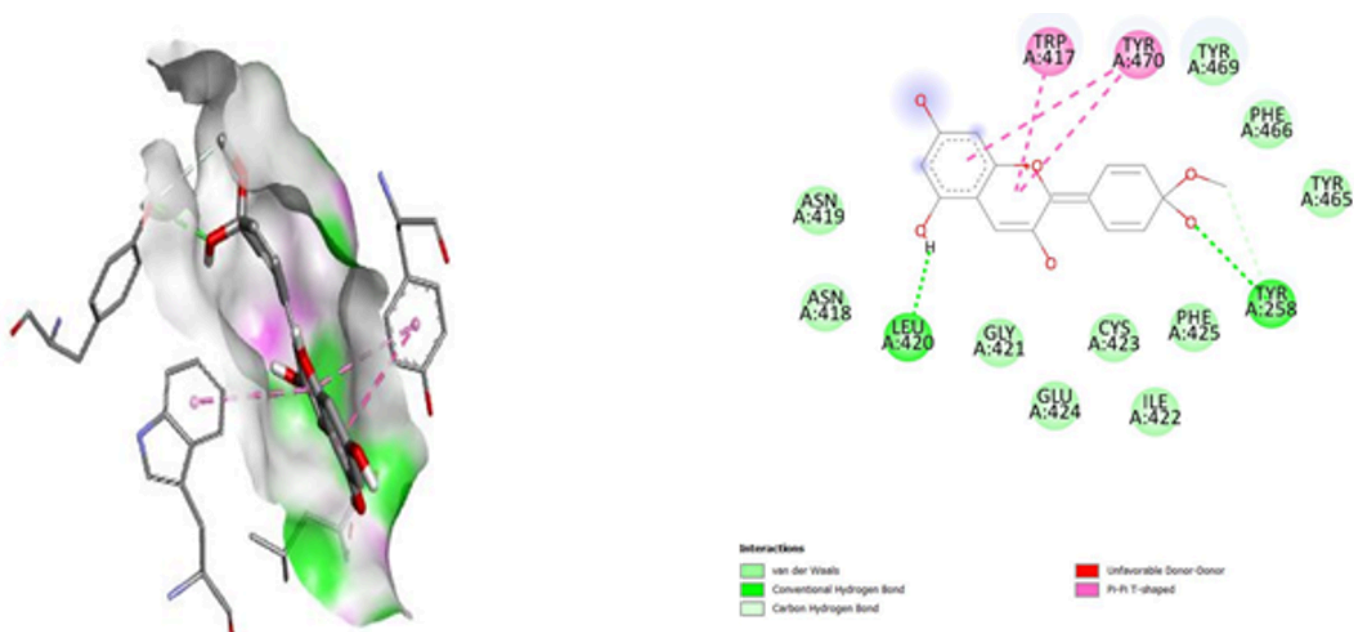


Figure 8

2D and 3D visualization of interaction between 4'Methoxypelargonidin and AChE

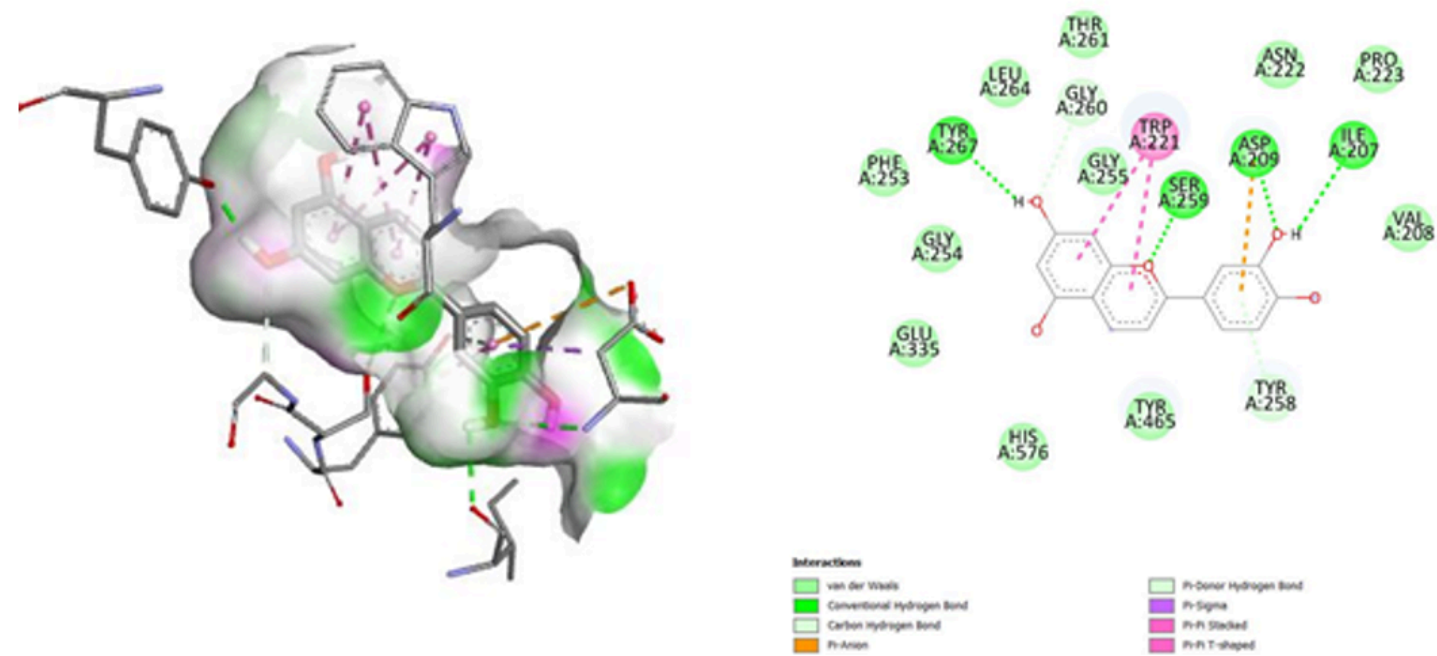


Figure 9

2D and 3D visualization of interaction between Luteolinidin and AChE

Supplementary Files

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