Structure-based in-silico analysis of phytocompounds from Cleistanthus collinus for the development of effective insecticide against Tribolium castaneum

Soma Prasad Sahoo
Odisha University of Technology and Research
Bighneswar Baliyarsingh
bighnesh.singh@gmail.com
Odisha University of Technology and Research

Research Article

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Abstract

Effective pest control requires the use of chemicals that are highly specific, safe, and eco-friendly. The richness of phytochemicals in the *Cleistanthus collinus*, a medicinal plant, indicates its plausibility role in controlling insects. This study aimed to identify potential insecticidal compounds from the leaves of *Cleistanthus collinus* against four different types of insect receptors of *Tribolium castaneum* through structure-based virtual screening. Homology modeling was used to generate 3D structures for each receptor, and the compound with the highest binding affinity was identified using Discovery Biovia Studio-2021. The screened phytocompound's insecticidal likeness was further assessed using the Tice rule that exhibit any deviations. Cedrene was found to be the best ligand for the acetylcholinesterase receptor, cytochrome p450 receptor, and GABA receptor, while cedrene and 1,2,3-benzenetriol were found to be the best ligands for the endoglucanase gene receptor. Molecular docking analysis revealed that the selected compounds interacted with the active sites of their respective receptors and, the linear relationship of inhibition constants with binding energy corroborates the insecticidal potential of phytocompounds. This study suggests that phytochemicals from *C. collinus* have the potential to act as insecticides by interacting with multiple receptors, offering insights for the development of novel and eco-friendly insecticides for pest control.

Keywords: Phytocompounds, Molecular docking, Phytopesticide, Insect receptor, Modeller, Tice Rule.

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1. Introduction

The goals of food security are not only being challenged by limited arable land, growing population, or weather variability but also crop damage due to infestations of pests. The problem of food loss during the pre-and post-harvest period mostly affects tropical and subtropical parts of the world. Among the different losses in post-harvest operations, storage loss can reach up to 7.5% of total production [1]. The yield and quality of crops are drastically reduced by insect pests, rendering them unsuitable for sowing, as well as unfit for food or feed. The economic impact of stored grain insect infestations is larger since they result in weight losses of 30 - 40% of the grains [2]. In particular, the Coleopteran insect, *Tribolium castaneum* (Herbst) is a polyphagous pest of cereal grains and many other foods. It severely affects food in flour or starch form among which wheat and corn flours are more suitable feed for *T. castaneum* [3]. On the other hand, synthetic pesticides, although effective in controlling pests, pose vivid detrimental consequences ranging from environmental pollution, and soil contamination to induction of health risks in humans and non-target species. Additionally, pests can develop resistance to these chemicals, necessitating stronger, potentially more harmful pesticides. Addressing these issues calls for the development of bio-insecticides to minimize the adverse effects of pesticides as well as achieve protection from insect pests.

Phyto-products and botanicals are viable substitutes to synthetic pesticides for the preservation of stored grains since they are environmentally safe. Insecticides, anti-feedants, repellents, larvicides, ovicides, and development-regulators of insect are just a few of the many uses for plant-derived compounds [4, 5]. Moreover, one of the critical advantage of phytopesticides is its ability to degrade and recycle the elements easily, therefore less likely to induce the pesticide resistance in insects. The utility of plant products against stored grain insects has been studied and important active components of plant products exhibit its potential as a grain protectant [6, 7]. However, only a small percentage of botanicals, such as neem plant derived products, have been widely used as commercial goods in insect pest management measures [8, 9].

The plant *Cleistanthus collinus* (Roxb.) belongs to the family Phyllanthaceae with geoavaliability in India, Sri Lanka, and other parts of South Asia and is also well known for its pharmacological usage. Preliminary studies have indicated the insecticidal potential of *C. collinus* against leafhoppers, whiteflies [10], and stored-grain pest [11]. The insecticidal activity was attributed to the presence of diverse secondary metabolites, such as triterpenoids, alkaloids, and flavonoids, and some plant-specific compounds like collinusin and cleistanthins [12]. Although, several mass-spectrometry (LC/GC – MS) data on the identification of phytocompounds in extracts of plants were reported, their role as an insecticide was rarely attributed. Additionally, the in-vitro analysis of identifying and screening phytocompounds for their insecticidal role will be cumbersome. However, the in-silico studies of molecular docking and interaction are useful in screening and identifying the lead phytocompounds in the development of natural insecticides.

The goal of the current computational research was to identify potential insecticidal phytocompounds derived from the *C. collinus* against the pest, *T. castaneum*. This study focused on analyzing the optimal receptor-ligand binding energies and molecular interactions between phyto-ligands and insect receptors/critical proteins using a molecular docking approach. Furthermore, the inhibition constants were evaluated as well as Tice rule was employed to assess the insecticidal likeness of the screened phytocompounds.

2. Experimental procedures

2.1. *Rational of selecting protein-targets of the pest*

One of the likely approaches of addressing the issue of pest control is to target critical proteins in the insects that are either part of the insect nervous or muscular system or actively participate in the growth and development of the pest or proteins likely involved in the development of insect resistance. One of the important neurotransmitters ACh, through AChE, plays a critical role in the regulation of locomotion activity as well as feeding behavior. In addition, the nervous system of *T. castaneum* expresses a high amount of AChE

(acetylcholinesterase) thereby regulating physiological and developmental processes [13]. In addition to its role in insecticide resistance, CYP450 enzymes are also involved in the regulation of ecdysteroid hormone levels, which are critical for regulating growth, and metamorphosis in insects [14]. Endoglucanase enzymes are produced by the salivary glands and the midgut of *T. castaneum* larvae and adults enabling the digestion of plant material and also playing a role in other physiological processes such as molting and development [15]. One of the major neurotransmitters GABA through its receptor has been involved in a variety of physiological and behavioral processes in beetles, including locomotion, feeding, and reproduction [16]. Understanding the interaction of these selected proteins/receptors of *T. castaneum* with phyto-compounds can provide important insights into the development of new insecticidal lead compound for pest control.

2.2. Homology modelling and evaluations

Comparative modelling was performed to predict the three-dimensional structures of target proteins/ receptors, namely acetylcholinesterase (AchE), cytochrome p450 (CYP450), endoglucanase (EdGl), and gamma-aminobutyric acid-gated (GABA) ion channel receptor. The protein sequences were obtained from the UniProt Knowledgebase in FASTA format, with accession codes D6WU45, A0A0F7R7X8, A0A139WGK8, and A8DMU2, respectively. The chosen proteins' amino acid sequences were 'BLASTp' searched against the Protein Data Bank (PDB) for appropriate template structures [17]. Then the MODELLER 10v2 [18] was used to predict the 3D structures of the chosen proteins. In order to select the three-dimensional (3D) structures of the chosen proteins, the top-ranked structures generated through homology modelling were chosen as ideal templates. Different assessment tools like ERRAT which examines the statistics of non-bonded interactions between various types of atoms, PROCHECK which assesses the 3D location of each atom and residues of protein structures and Verify3D

74 which helps in evaluating an atomic model's (3D) compliance with its own (1D) amino acid
75 sequence [19, 20] were used to evaluate the effectiveness of the predicted protein models.
76

2.3. Selection and retrieval of phytocompounds

77 *Cleistanthus collinus* (Roxb.) (Benth.) has a richness of various phytocompounds and
78 toxic constituents. From several studies pertaining GC-MS analysis of the leaf extracts of *C.*
79 *collinus* [21], a pool of phytocompounds representing a significant proportion in the constitution
80 were selected by analyzing the area-percentage of peaks. Then a total of 10 phytocompounds were
81 selected after corroborating with scientific literature for their bioactivity or toxicity which were (i)
82 thiophene, (ii) cedrane, (iii) n-hexadecanoic acid, (iv) 1,2,3-benzene-triol, (v) oleic acid, (vi)
83 bumetrizole, (vii) phytol, (viii) 9,12,15-octadecatrienoic acid, (ix) 3-O-methyl-d glucose, (x)
84 quinazoline. The 'sdf' files of the phyto-compounds were retrieved from the 'PubChem' database.
85 Along with the compound IDs, the 'Smiles' scores were compiled.
86

2.4. Molecular docking

87 The structures of target proteins/receptors were prepared using 'Discovery Biovia Studio'-
88 2021 prior to docking. During this process, the water molecules and heteroatoms were deleted
89 along with any additional chains that were observed and then polar hydrogens were added. The
90 docking was carried out by a virtual screening method through PyRx software (Virtual Screening
91 Tool, Ver 0.8). All the phyto-compounds and target proteins/receptors have been converted to
92 PDBQT format and the energy minimization was done using the 'Open Babel' tool. The
93 molecular docking was performed individually between the screened target proteins/receptors of
94 *T. castaneum* against the selected phytocompounds of the *C. collinus* under the 'AutoDock Vina'.
95 A genetic algorithm (GA) for protein-protein docking was used as the primary search method, and
96 the docking site on this target protein was expressed by forming a grid box. The best 'AutoDock'
97 outputs of molecular docking were selected based on their binding energy. The interactions

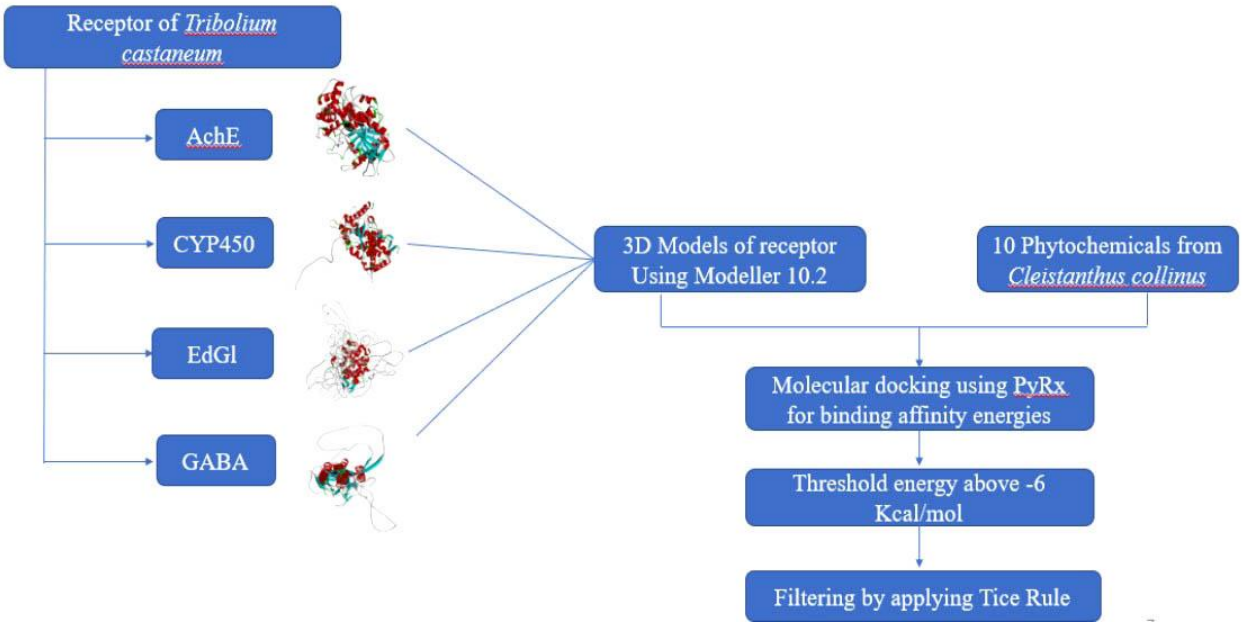
considered to be significant when the energy value is less than -1 kcal/mol [22]. Accordingly all the screened 10 phytocompounds were analyzed and those phytocompounds exhibiting docking energy values less than −6 kcal/mol against any of the selected insect receptors/ target proteins were considered. These are subsequently evaluated for insecticidal potential as the more negative the binding energy between the ligand and receptor the better is the binding between them (Fig 1).

2.5. Evaluation of Tice rule for insecticidal potency

The creation of new insecticides is dependent on the bioavailability and bioactivity of compounds. The Tice rule is a useful tool for predicting insecticidal potency based on molecular properties, and the computed properties of the best docked compounds were evaluated accordingly. According to this rule, potential insecticidal compounds should have (i) a molecular weight of less than or equal to 500 g/mol, (ii) number of hydrogen-bond donors should have less than or equal to 3, (iii) number of hydrogen-bond acceptors should be less than or equal to 12, (iv) the partition coefficient (log P) should be less than or equal to 5, and (v) no. of rotatable bonds should be less than or equal to 12 [23]. The molecular properties of investigated compounds were accessed using 'smile' scores, obtained from the 'PubChem' database and online 'Molinspiration cheminformatics' software (Fig 1).

2.6. Inhibition constant (IC50)

IC50 is the concentration where 50% of insecticidal activity was achieved. The examined molecule should have a lower binding energy value (higher negative value) to be the more potent insecticide, the reason being more free energy is released when the receptor and ligand interact more easily. The inhibition constants were estimated using the formula $Ki = \exp^{(\nabla G/RT)}$ [24], where '∇G' is the binding energy in kcal/mol, 'R' is the universal gas constant, and 'T' is the temperature.

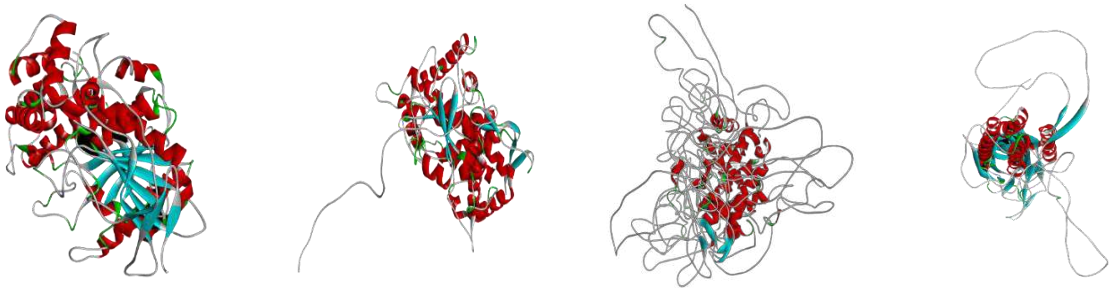


(Fig 1: Schematic representation of experimental procedure followed)

3. Results

3.1. Structures of receptors/ target proteins

Using Homology modelling, five 3D structures were generated for each of the selected target proteins (i.e. acetylcholinesterase, cytochrome p450, endoglucanase, and GABA receptor). Optimal models (Fig 2) of AChE (- 58534.91), CYP450 (- 55186.59), EdGl (- 81653.79), and GABA receptor (- 45426.15) were screened based on their lowest DOPE score.



(a) (b) (c) (d)

[Fig 2: In-silico representation of Optimal 3D structure of target proteins such as (a) AChE (b) CYP450 (c) EdGl (d) GABA receptor]

3.2. In-silico molecular docking analysis

130 *In-silico* docking experiments were performed to evaluate the binding potential of 10
131 phytocompounds from *C. collinus* with the optimized models of target proteins. The energy scores
132 were estimated individually using 'AutoDock' for each phytocompounds (Table 1).
133

Table 1: Binding energy (BE) of phytocompounds from *C. collinus* with the chosen target proteins from *T. casataneum.*

Ligands	**Targets** with their binding energy (BE) in kcal/mol			
	AChE	**CYP450**	**EdGl**	**GABA**
1,2,3-benzenetriol	-5.3	-5.2	-6.0	-5.1
3-O-methyl-d-glucose	-4.9	-5.1	-4.9	-4.8
9,12,15-Octadecatrienoic acid	-5.7	-6.4	-6.8	-5.4
Bumetrizole	-8.8	-7.5	-8.2	-8.3
Cedrene	-6.9	-6.4	-7.3	-7.3
N-hexadecenoic acid	-5.6	-4.9	-5.8	-4.2
Oleic acid	-5.3	-5.4	-5.1	-5.3
Phytol	-5.9	-5.5	-5.7	-5.2
Quinazoline	-6.3	-5.5	-5.6	-5.1
Thiophene	-3.1	-3.4	-3.8	-2.9

[BE- Binding energy in kcal/mol, AChE- acetylcholinesterase, CYP450- cytochrome p450, EdGl- endoglucanase, GABA- GABA receptor, Ligands- phytocompounds]

134

135 A ligand's binding energy is a measure of how strongly it interacts with its target protein.
136 The energy value less than -6.0 kcal/mol was considered in this study as the threshold for
137 significant interactions. Out of 10 screened phytocompounds only four phytocompounds namely
138 1,2,3-benzenetriol, 9,12,15-octadecatrienoic acid, bumetrizole, and cedrene showed strong

interaction with the endoglucanase (EdGl) while three phytocompounds *viz.* bumetrizole, cedrene, and quinazoline against acetylcholinesterase receptor (AChE) and the phytocompounds 9,12,15-octadecatrienoic acid, bumetrizole, and cedrene against cytochrome p450 (CYP450) have showed strong binding. However, only two phyto-compounds, bumetrizole and cedrene showed strong interaction with GABA receptors. Among all examined phytocompounds, bumetrizole exhibited the highest binding towards each of the receptors *viz.* AChE (-8.8 kcal/mol), CYP450 (-7.5 kcal/mol), EdGl (-8.2 kcal/mol), GABA (-8.3 kcal/mol) and strongest interaction being with acetylcholinesterase receptor. It can be inferred that bumetrizole can be a plausible inhibitor of AChE. Moreover, most of the phytocompounds exhibited stronger interaction with EdGl and AChE compared to CYP450 and GABA receptors. On the other hand, the binding energy of phytocompounds like thiophene, 3-o-methyl-d-glucose, oleic acid, and phytol was unable to surpass the chosen threshold value with any of the target proteins/receptors, thereby indicating weak interaction. Lowest binding was observed between thiophene and the GABA receptor (-2.9 kcal/mol) suggesting thiophene may not be a potent ligand for the GABA receptor and would not be a potent inhibitor. Furthermore, 'Molinspiration' was used to assess the molecular characteristics (Table 2) of the screened phyto-compounds to fit them according to the 'Tice Rule', a crucial method for predicting the insecticidal potential.

Table 2: Chemical characteristics top-scored phytocompounds of *C. collinus*.

Phytocompounds	**miLogP**	**MW**	**nON**	**nOHNH**	**nViolation**	**nrotb**
Bumetrizole	5.50	315.80	4	1	1	2
Cedrene	4.76	204.36	0	0	0	0
Quinazoline	1.54	130.15	2	0	0	0
9,12,15 Octadecatrienoic acid	5.84	278.44	2	1	2	13
1,2,3-benzenetriol	0.73	126.11	3	3	0	0

['Molinspiration' characterized phytocompounds. miLogP- molinspiration logP value, MW-

molecular weight, nON- number of hydrogen bond acceptors, nOHNH- number of hydrogen bond donors, nviolations- number of rule of five violations, and nrotb- number of rotatable bonds]

157

158 The chemical characteristics of the phytocompounds were revealed by these attributes
159 (Table-2), which help us to understand their biological actions as well as their potential
160 bioavailability. The molecular weight of bumetrizole is the highest among all the examined
161 phytocompounds, even though it is comparably similar to that of octadecatrienoic. Quinazoline
162 and 1,2,3-benzenetriol, on the other hand, have comparable molecular weights but are
163 significantly lesser than bumetrizole and octadecatrienoic. Quinazoline and 1,2,3-benzenetriol
164 have hydrophilic properties, as evidenced by their lower miLogP values, while bumetrizole and
165 octadecatrienoic acid exhibited greater miLogP values, showing their hydrophobic nature. The
166 miLogP value of cedrene indicates moderate hydrophobicity. Bumetrizole demonstrated the
167 highest number of hydrogen bond acceptors (4) among the tested phytocompounds followed by
168 1,2,3-benzene-triol (3) and quinazoline (2) and octadecatrienoic acid (2) indicating possession of
169 potential interaction sites with other molecules. However, cedrene was analyzed of not having any
170 hydrogen bond acceptors, suggesting its limitation in quick or hydrogen-bonding favoured
171 interactions. Apart from that, among five screened phytocompounds such as 1,2,3-benzene-triol
172 (3), bumetrizole (1) and octadecatrienoic acid (1) possess hydrogen bond donors while cedrene
173 and quinazoline don't have any hydrogen bond donors which possibly limit their strong
174 interaction with target molecules. The flexibility of octadecatrienoic acid was significantly higher
175 from all other phytocompounds with 13 numbers of rotatable bonds. While cedrene, quinazoline,
176 and 1,2,3-benzene-triol were evaluated as rigid structures.

177 Quinazoline and cedrene had the highest binding affinities to the acetylcholinesterase
178 protein and did not deviate from the Tice rule, making them the top two phytocompounds with
179 insecticidal potential. Cedrene has one of the highest cytochrome-450 binding affinity energies

and exhibited zero 'Tice' rule deviations. In terms of 'Tice' rule deviations, cedrene and 1,2,3-benzenetriol ranked first and second phytocompounds with the highest binding affinity towards the endoglucanase. Similarly, cedrene exhibited highest binding affinities towards the GABA receptor. Thus, cedrene emerged as the most promising candidate among the phytocompounds examined attributed to its high binding affinities for a range of receptors, including acetylcholinesterase, cytochrome-450, endoglucanase, and GABA recptor. Additionally, the acetylcholinesterase and endoglucanase revealed substantial binding affinity energies towards quinazoline and 1,2,3-benzenetriol, respectively. These simulated testing of phytochemicals from *C. collinus* against *T. castaneum* receptors produced promising outcomes.

3.3. Binding analysis of phytocompounds and target proteins

All visualization and analysis of the 3D and 2D interactions between the target proteins and phytocompounds were performed using the 'Discovery Biovia Studio'-2021. The optimal docking pose (Fig 3) with screened phytocompounds were also determined to reveal the highest number of interacting residues in the target protein's active side, and the inhibition constants were calculated. Among them, cedrane and quinazoline showed excellent bioactivity characteristics and insecticide-likeness (Table 3).

Table 3: Insecticide likeness and binding characteristics of phytocompounds of *C. collinus* with the target proteins of *T. casataneum.*

Target proteins	**Ligands**	**Binding energy**	**Inhibition constant**	**Nature of interaction**	**Contact residue**
AChE	Cedrene	-6.9	8.3	Alkyl interaction	Met315,Cys422
	Quinazoline	-6.3	23	Pi-anion interaction	Glu320
				Pi-sulfur interaction	Met311
				Pi-sigma interaction	Ile416

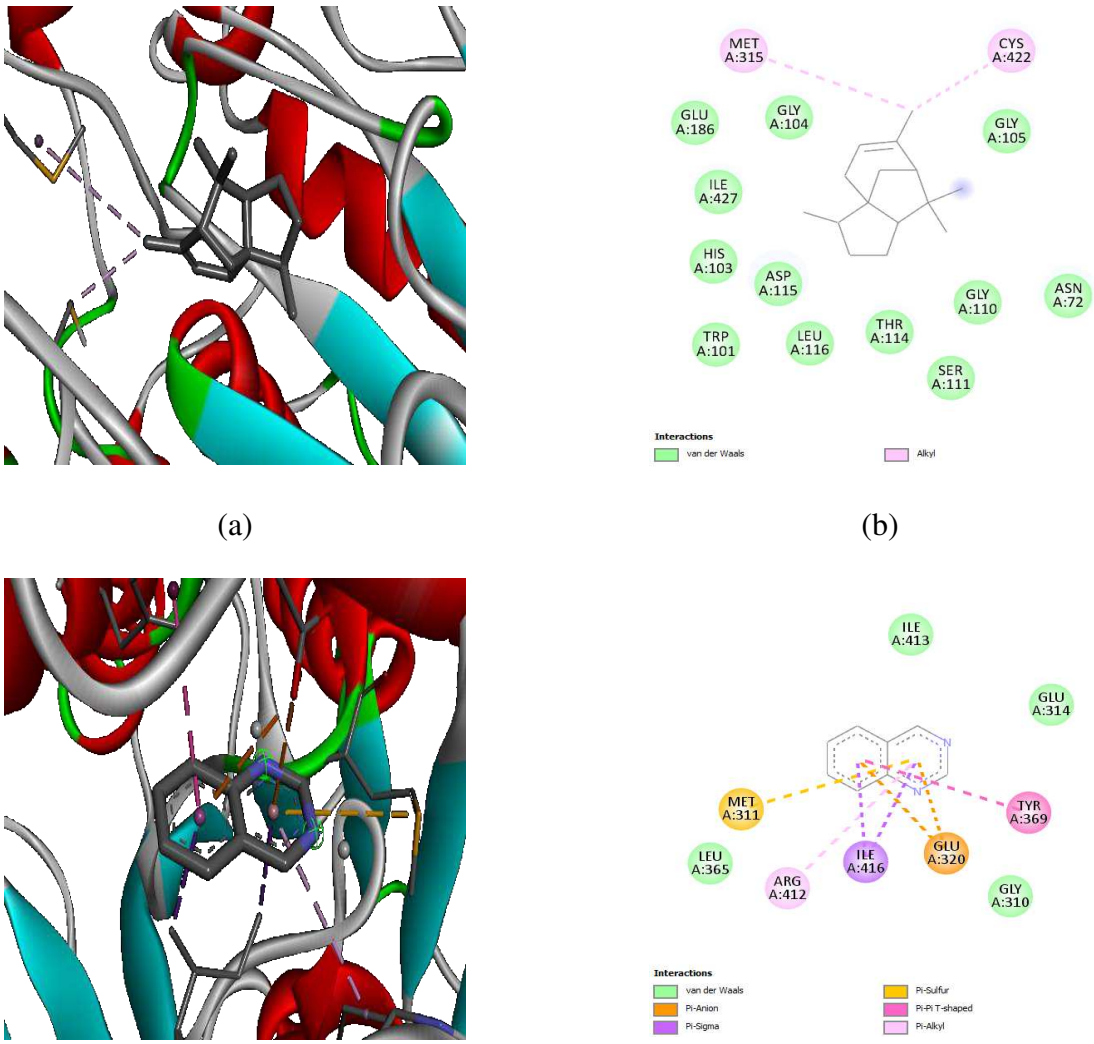
				Pi-alkyl interaction	Arg412
				Pi-pi T shaped	Tyr369
CYP-450	Cedrene	-6.4	19	Alkyl interaction	Leu223, Leu220 Pro221, Arg73
EdGl	Cedrene	-6.0	38	Alkyl interaction	Glu421, Asp189, Asn426
	1,2,3-benzenetriol	-7.3	4.2	Alkyl interaction	Leu483, Val362, Phe486
				Pi-Alkyl interaction	Ala346
GABA receptor	Cedrene	-7.3	4.2	Alkyl interaction	Ile469, Val476, Phe477

Binding energy in kcal/mol, inhibition constant in μM, amino acids at contact site.

196

197 Cedrene demonstrated strong binding with all the tested target proteins/receptors with
198 binding energy ranging from -6.0 kcal/mol (against Edgl) to -7.3 kcal/mol (GABA receptor) and
199 inhibition constant ranging between 4.2 μM to 38 μM. Although the interacting amino acids differ
200 with regard to the target proteins, alkyl interaction is the primary mechanism through which they
201 interact and potentially disrupt their normal functioning. As insect neurological systems are
202 heavily dependent on GABA receptors, modulating GABA receptors can cause brain activity to
203 be hyperexcited or inhibited. In line with this, the experimental result of strong binding (-7.3
204 kcal/mol) and inhibitory activity of cedrene with GABA receptor suggest its insecticide likeness.
205 Similarly, the results with other target proteins suggest that cedrene is one of the lead insecticide
206 molecules as it exhibited strong interactions with multiple receptors involved in insect biology.
207 With a binding energy of -6.3 kcal/mol and an inhibition constant of 23 M, the quinazoline
208 likewise demonstrated substantial interaction with AChE. Quinazoline also forms diverse types of
209 pi-interaction with target proteins such as pi-anion interaction with Glu320, pi-sulfur interaction

with Met311, pi-sigma interaction with Ile416, pi-alkyl interaction with Arg412, and a pi-pi T-shaped interaction with Tyr369. Similarly, another phytocompound, 1,2,3-benzenetriol, exhibited stronger interaction with endoglucanase through alkyl interaction and pi-alkyl interaction at the contacting side with a binding energy of -7.3 kcal/mol and an inhibition constant of 4.2 µM. This depicts it primarily affects the endoglucanase and is less likely with other receptors. Thus present in-silico observations depicted high affinity interactions between the phytocompounds and the target receptors/proteins and also the ability to interfere with these receptors' usual function, yielding an insecticidal impact.



(a) (b)

(c) (d)

[Fig 3: Schematic representation of the interactions between the screened phytocompounds and

acetylcholinesterase and displaying the pose view and their active pockets. (a) 3D view and (b) 2D view of Cedrene interaction with acetylcholinesterase. Similarly (c) 3D view and (d) 2D view of Quinazoline interaction with acetylcholinesterase]

4. Discussions

In-vitro studies have demonstrated that phytochemicals or botanicals exhibit multiple bioactivity role(s) such as insecticidal, larvicidal, antifungal, and antimicrobial properties and traditionally have been in practice as grain protectants against insects [5, 25]. Preliminary in-vitro studies have demonstrated that the phytochemicals of *C. collinus* have insecticidal potential against diverse insects such as *Sitophilus oryza, T. castaneum* and *Spodoptera litura* etc. [11, 26, 27]. Even though several phytocompounds have been discovered and identified, little research has been done on their efficacy as insecticides. However, the present in-silico study focuses on finding a lead insecticidal phytocompound against the red flour beetle, *T. castaneum* derived from *C. collinus*. This study has employed homology modeling to generate 3D structures for key target proteins *viz.* acetylcholinesterase (AChE), cytochrome P450 (CYP450), endoglucanase (EdGl), and the GABA receptor, and molecular docking analysis of ten phytocompounds of *C. collinus* against these targets which is then followed by the evaluation with Tice rule for best-docked phytocompounds to identify insecticide-like compound. To gain insight into the molecular characteristics of the screened phytocompounds, various properties such as molecular weight, hydrophobicity (miLogP), hydrogen bond donors, hydrogen bond acceptors, and flexibility (rotatable bonds) were assessed simultaneously. Among the characteristics assessed, bumetrizole stood out with the highest molecular weight and the most hydrogen bond acceptors, indicating its potential for versatile interactions with other molecules. The flexibility of octadecatrienoic acid was significantly higher than other phytocompounds, suggesting its potential to adapt to various binding orientations. Stable interaction between ligands and target proteins not only depends on hydrogen bonds but also non-bond interactions and non-hydrogen interactions. Usually, the non-

bonded and hydrogen bond interactions contribute to the stable conformation of the protein-ligand complex for optimal activity [28] which supports the present study that certain phytocompounds even with lesser availability of hydrogen bonding sites exhibited stronger interaction during molecular docking analysis.

The best-docked phyto-molecules were selected based on the binding energy and good interaction with the active site's residues. The results indicated that screened phytocompounds of *C. collinus* exhibit strong binding interactions with the selected target proteins, with binding energies well below the threshold of -6.0 Kcal/mol, signifying robust interactions [29]. One of the central findings from the study of the assessment of binding energy is that the phytocompounds, 1,2,3-benzenetriol, 9,12,15-octadecatrienoic acid, bumetrizole, and cedrene exhibited strong interactions with EdGl, while bumetrizole, cedrene, and quinazoline displaying strong binding to AChE. Notably, bumetrizole displayed the highest binding affinity for all four target proteins, with the strongest interaction observed with AChE, suggesting its potential as a potent inhibitor of AChE. Moreover, most of the phytocompounds exhibited stronger interactions with EdGl and AChE compared to CYP450 and the GABA receptor. This differentiation in binding patterns across proteins highlights the specificity of these phytocompounds in interacting with their respective targets.

In terms of the 'Tice' rule, which is important for predicting insecticidal lead compounds, cedrene and quinazoline were the top performers, showing excellent bioactivity characteristics and adhering to the rule. Cedrene, in particular, exhibited high binding affinities towards the chosen receptors such as AChE, CYP450, EdGl, and the GABA receptor. Cedrene demonstrated strong binding interactions with all the tested target proteins/receptors, with binding energies ranging from -6.0 kcal/mol to -7.3 kcal/mol. In line with this, similar kind of binding energy was estimated between the interactions of curcumin and its derivatives with AChE1 protein of *C. pipiens* and *A. gambiae* [30]. Even if, cedrene lacked hydrogen bond acceptors which may limit its ability to form hydrogen-bond dominated interactions, the alkyl interactions primarily involved

and favored the strong interactions with inhibition constants ranging from 4.2 μM to 38 μM. In addition to this, cedrene also exhibited stronger docking confirmation towards CYP450 and GABA receptors with a binding energy of -6.4 kcal/mol and -7.3 kcal/mol, respectively. Similarly, Quinazoline also exhibited significant interaction with AChE, with a binding energy of -6.3 kcal/mol and an inhibition constant of 23 M which is in accordance with studies of Uddin et al., (2020) where they demonstrated that phenolic groups exhibit strong interaction with binding energy less than -4 kcal/mol. Unlike cedrene, quinazoline interacts with chosen target proteins through pi-anion, pi-sulfur, pi-sigma, pi-alkyl, and pi-pi T-shaped interactions. In accordance with present studies, the in-silico docking analysis of Khanna and Ranganathan, (2011) revealed that the phytomolecules like benzimdazole and piperazine have insecticidal ability by targeting tubulin-1 chain receptor and acetylcholine receptor in worms. They suggested that binding to the receptor may commonly include piperazine-like substructures containing a nitrogen atom in the piperazine ring. Furthermore, the linear relationship between binding energy and inhibition constant observed in this study is consistent with previous research on the inhibition of acetylcholinesterase by phytochemicals. For example, a study by Yadav et al., (2015) reported a linear correlation between the inhibition constant and binding energy of non-azadirachtin limonoids from *Azadirachta indica* towards acetylcholinesterase. Similarly, 1,2,3-benzenetriol displayed strong interaction with endoglucanase, with a binding energy of -7.3 kcal/mol and an inhibition constant of 4.2 μM. The 1,2,3-benzenetriol forms a stable ligand-receptor complex with the help of alkyl and pi-alkyl interactions. Overall, the results of this study suggest that *C. collinus* phytochemicals, particularly cedrene, quinazoline, and 1,2,3-benzenetriol have the potential to act as insecticide by interacting with multiple receptors. These findings could have important implications for the development of novel and eco-friendly insecticides for storage pest control.

5. Conclusion

This study provides valuable insights into the binding interactions between selected phytocompounds and target proteins relevant to the biocontrol of insects. It can be concluded that phytocompounds of *Cleistanthus collinus* are potent inhibitors of enzymes and neuro-sensory proteins. Cedrene, in particular, emerged as a promising candidate due to its strong binding affinities across multiple receptors. The study also suggests that phytocompounds, especially quinazoline, and 1,2,3-benzenetriol exhibit the least binding energy with the target proteins of *T. castaneum* and do not violate the 'Tice' rule and can be developed into insecticide, offering new avenues for pest control strategies. Future research in this area could focus on in-vivo studies to validate the insecticidal potential of these phytocompounds and investigate their safety and environmental impact. Additionally, the molecular characteristics identified here could guide further modification and optimization of phytocompounds for enhanced insecticidal activity.

6. List of Abbreviations

IC50- Inhibition constant

SMILES- Simplified Molecular Input Line Entry System

pdbqt- Protein data bank, Partial charge (Q), Atom type (P)

sdf - Structural data file

miLogP- Octanol/water partition coefficient

MW- Molecular Weight

nON- No. of Hydrogen bond acceptor

nOHNA- No. of Hydrogen bond donor

nViolations- No. of Violations

nrotb- No. of rotatable bonds

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8. Declarations

a. Funding

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b. Conflicts of interest/Competing interests

The authors declare that they have no competing interests

c. Ethics approval and consent to participate

Not applicable

d. Availability of data and materials

All data generated or analyzed during this study are included in this published article

e. Code availability

Not applicable

f. Authors' consent

All authors read and approved the final manuscript.

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