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# Efficacy of *Cassia fistula* against *Callosobruchus chinensis* (Linn.): An in-silico approach

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**Abstract**
*Cassia fistula* is a flowering plant belongs to family fabaceae and is widely distributed in tropical countries of the world. Various therapeutic and antibacterial activity of this plant has been studied so far. *Callosobruchus chinensis* is a major pest of leguminous stored seeds and cause huge amount of damage to seeds every year. The present study emphasizes on the in silico study of interactions of various chemical constituents of *C. fistula* against enzyme alpha amylase and glutathione S transferase of *C. chinensis*. The study also aims to find out the mortality rate of *C. chinensis* when treating them with methanolic crude extract of *C. fistula*. Among all the selected ligands, physcion shows minimum rerank score of -74.1435 against alpha amylase and phytol shows minimum rerank score of -73.452 against glutathione S transferase. The toxicity test shows that mortality of insects when treated with extract at 2, 4, 6, 8, 10 and 12 % concentration was 28, 35, 43, 53, 67 and 82% respectively and $LC_{50}$=5.671%. The minimum rerank scores of all selected ligands in in-silico study and the toxicity test results suggest that *C. fistula* can be potent controlling agent against *C. chinensis*.

**Keywords:** alpha amylase, controlling agent, glutathione s transferase, methanolic extract, phytochemicals, mortality

## Introduction
*Cassia fistula*, also known as Golden tree is a semi deciduous tree belongs to sub family Caesalpiniaceae of the leguminous family Fabeceae. It is native to India, Sri Lanka, the Amazon, Mauritius, South Africa, Mexico, China, West Indies, Thailand, Brazil etc. It has been documented in Indian literature for the use of liver disorders, rheumatism, skin infection, diabetes, leukoderma, intestinal disorders etc. (Sakulpanich & Gritsanapan, 2009) [19]. *C. fistula* can be used as a therapeutic agent in treatment of hypercholesterolemia as it contains fibers and mucilage (El-Saadany *et al*., 1991). This plant also has antibacterial activity against wide range of bacteria including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aerogenes* etc. (Perumal *et al*., 1998). Different chemical constituents of *C. fistula* include Aloeemodin, Camphor, Chromone, Chrysophanic, epicatechin etc. (Maqsood *et al*., 2020, Bahorun *et al*., 2005) [16, 2].
*Callosobruchus chinensis* is a major storage pest of pulses. The average damage of pulses by these pests may range from 5-10% in temperate and 20-30% in tropical countries (Kiradoo & Srivastava, 2010) [13]. Chemical control is the most effective way in controlling these pest (Jackai & Adalla, 1997) [10], but it leads to pest resistance and residual toxicity (Brent & Hollomon, 1998) [5]. Plant based pesticides are chiefly biodegradable, eco-friendly and sustainable alternatives to synthetic pesticides. Alpha amylase and Glutathione S Transferase are two important enzymes that controls physiological processes in *C. Chinensis*. Alpha amylase is a digestive enzyme act in polysaccharide digestion. Glutathione S Transferase catalyzes conjugation of Glutathione to various substrates and thus accelerates detoxification process by making the compound more soluble (Eaton and Bammler, 1999) [6]. Suppressing the activity of these two enzymes affects their normal physiological functioning and hence can be helpful in controlling this insect pest.
In silico pharmacology includes development of techniques that uses software to capture, analysis and integrate biological data from various sources. Thee software make simulations that helps to make predictions and ultimately provide advances in medicine and therapeutics (Ekins *et al*., 2007) [7]. This approach accelerates drug discovery process by efficiently predicting and modelling most relevant pharmacokinetics, metabolic and toxicity endpoints (Batool *et al*., 2019) [3].
The annual food grain storage losses in India caused by insects alone accounts for nearly Rupees Thirteen hundred crore (IGMRI report, 2015) [9]. *C. chinensis* is commonly encountered stored gain pest in India. The enzymes alpha amylase and glutathione S transferase play a vital role in their normal physiological functioning. Defect in the enzyme activity of any of these enzymes will affect their polysaccharide digestion and natural detoxification process ultimately leading them to physiologically compromised state. The present study emphasizes on the interaction of various chemical constituents of *C. fistula* with the enzymes alpha amylase & glutathione S transferase and hence finding the effective chemicals that suppresses the activity of these enzymes by blocking their active sites. The study also carried out to know the mortality of *C. chinensis* when treating them with various concentration of methanolic crude extract of *C. fistula*.

## Materials and Methods
Receptor and ligand preparation: The molecular structures of the receptors (enzymes) Alpha amylase (1VIW) and Glutathione S Transferase (1GNW) (Fig.1) were downloaded from PDB database (https://www.rcsb.org/).

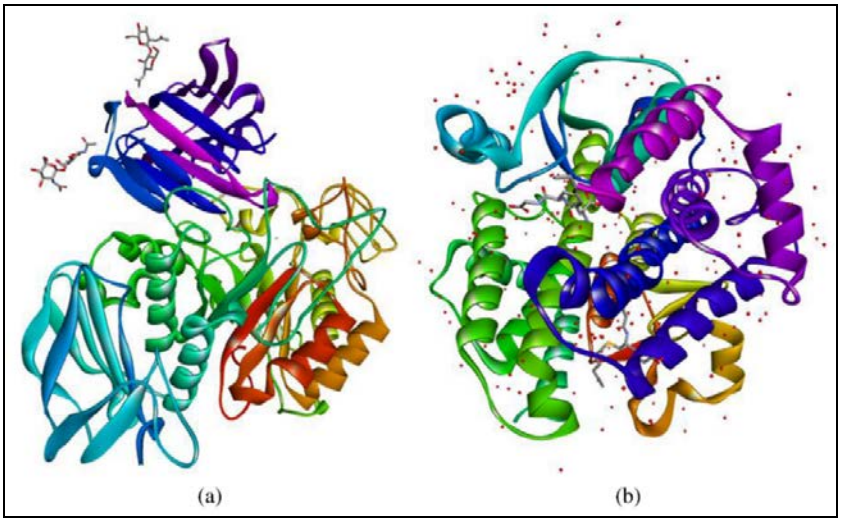


**Fig 1:** Structures of the receptors, (a) Alpha Amylase (1VIW) and (b) Glutathione S-Transferase (1PN9)

The structures of the ligands (phytochemicals) present in *C. fistula* (Fig. 2) were downloaded from Zinc database (https://zinc.docking.org/) which is a free database of commercially available compounds especially prepared for virtual screening. All the ligands were selected from previously published reviews.

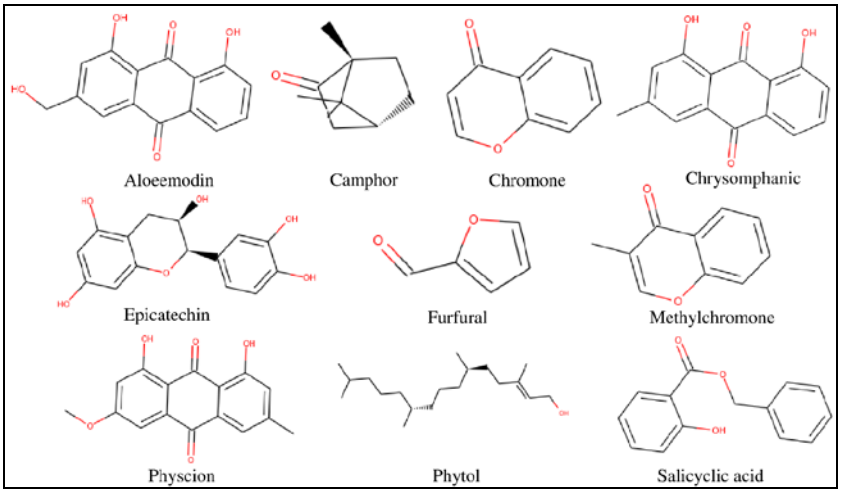


**Fig 2:** Structures of phytochemicals (ligands) present in *C. fistula*

Pharmacokinetics: The preliminary physicochemical properties and pharmacokinetics of ligands were analyzed using Swiss ADME web tool. The web tool is freely accessible (http://www.swissadme.ch) and provides computed parameters that would make a molecule affective as a drug.

Molecular docking: The possible molecular interactions between the receptors (enzymes) and the ligand molecules (phytochemicals) were predicted by using docking software, Molegro Virtual Docker (MVD 2010.4.0.0) for Windows. Rerank score was recorded for analyzing the receptor ligand interactions. The reranking coefficients uses the energy parameters such as E-Inter total, E-Inter (protein-ligand), Steric, Van der Waal's, H-Bond energy, E-Intra (tors, ligand atoms) etc.

Receptor-ligand interactions visualization: For visualization, BIOVIA Discovery Studio Visualizer was used. It is a free molecular modeling application used for viewing, sharing and analyzing protein and small molecule data developed by Dassault Systems BIOVIA 2021.

Collection and maintenance of stock culture of *C. chinensis*: The adult beetles were collected from Entomology Department, Assam Agricultural University, Jorhat (Assam) and are maintained on healthy seeds of *Vigna radiata* (green gram). Insect free healthy seeds were collected from Entomology Department, Assam Agricultural University, Jorhat (Assam) and various local farmers. The seeds were sundried or heated in oven at $60^0$C for 30-40 min to make them free from any hidden infestation and stored in a closed container in room temperature. The cultures were kept on plastic containers (12cm X 8cm) with their mouth tied with muslin cloth and sealed by rubber band and were kept in temperature ($30\pm2^0$C) and relative humidity (70±5%).

Collection of plants and extract preparation: The leaves of *C. fistula* were collected and identified using key book (Kanjilal *et al*., 1938) [12]. Collected sample was washed and dried in room temperature for 6 to 10 days and macerated using a grinder. The powdered material was subjected to methanol extraction using a Soxhlet apparatus for 15-18 hrs. Then the extract was dried using Rotary Evaporator and the dried residue is scratched out from the petri dish and kept in a clean airtight bottle for experimental use.

Direct toxicity test: The adult insects were chilled for a period of few minutes. Then the immobilized insects were kept in petri dish and 20 microliter solution of different concentrations (2, 4, 6, 8, 10, 12%) of extract were applied

on the dorsal side of each insect with the help of micropipette. After treatment the insects were transferred into a petri dish at 20 insects per petri dish and provided them with equal amount of food. The insects were examined daily and those did not move or respond to gentle touch were considered as dead. Insect mortalities were recorded after 24h up to 72h of treatment and the observed data was subjected to probit analysis (Finney, 1947).

## Results and Discussion

The efficacy of *C. fistula* against *C. chinensis* was studied using computer based molecular docking technique. Various phytochemicals present in *C. fistula* was identified by literature review. The receptors (enzymes) and ligands (phytochemicals) were downloaded in their compatible formats (.pdb &.mol2). The Swiss ADME web tool was used for analyzing physicochemical properties & pharmacokinetics of the ligands present in *C. fistula*. The studied ligands include aloeemodin, camphor, chromone, chrysophanic, epicatechin, furfural, methylchromone, physcion, phytol and salicyclic acid. All the studied ligands have high Gastro-Intestinal absorption property. Among the ligands, camphor, chromone, chrysophanic, furfural, methylchromone and salicyclic acid has blood-brain barrier permeant and hence can cross into extra cellular fluid of central nervous system. P-glycoprotein substrate is present in aloeemodin, epicatechin, physcion and phytol. Ligands such as chromone, chrysophanic, methylchromone, physcion and salicyclic acid possess inhibitors for cytochrome $P_{40}$ (CYP) superfamily of enzymes (Table 1&2).

**Table 1:** Physiochemical properties of selected ligands of *C. fistula*

| Sl. No | ZINC Id | Name of ligand | Formula | Molecular weight (gm/mol) | No. of heavy atoms | No. of arom. Heavy atoms | Fraction Csp3 | No. of rotatable bonds | No. of H-bond acceptors | No. of H-bond donors | Molar Refractivity | TPSA (A) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ZINC000004098644 | Aloeemodin | $C_{15}H_{10}O_5$ | 270.24 | 20 | 12 | 0.07 | 1 | 5 | 3 | 69.92 | 94.83 |
| 2 | ZINC000000967520 | Camphor | $C_{10}H_{16}O$ | 152.23 | 11 | 0 | 0.9 | 0 | 1 | 0 | 45.64 | 17.07 |
| 3 | ZINC000000057736 | Chromone | $C_9H_6O_2$ | 146.14 | 11 | 10 | 0 | 0 | 2 | 0 | 42.48 | 30.21 |
| 4 | ZINC000003861630 | Chrysophanic | $C_{15}H_{10}O_4$ | 254.24 | 19 | 12 | 0.07 | 0 | 4 | 2 | 68.76 | 74.6 |
| 5 | ZINC000000119988 | Epicatechin | $C_{15}H_{14}O_6$ | 290.27 | 21 | 12 | 0.2 | 1 | 6 | 5 | 74.33 | 110.38 |
| 6 | ZINC000003861345 | Furfural | $C_5H_4O_2$ | 96.08 | 7 | 5 | 0 | 1 | 2 | 0 | 24.1 | 30.21 |
| 7 | ZINC000000001707 | Methylchromone | $C_{10}H_8O_2$ | 160.17 | 12 | 10 | 0.1 | 0 | 2 | 0 | 47.45 | 30.21 |
| 8 | ZINC000003978794 | Physcion | $C_{16}H_{12}O_5$ | 284.26 | 21 | 12 | 0.12 | 1 | 5 | 2 | 75.25 | 83.83 |
| 9 | ZINC000003978631 | Phytol | $C_{19}H_{38}O$ | 282.5 | 20 | 0 | 0.89 | 12 | 1 | 1 | 94.13 | 20.23 |
| 10 | ZINC000000388079 | Salicyclic acid | $C_{14}H_{12}O_3$ | 228.24 | 17 | 12 | 0.07 | 4 | 3 | 1 | 64.23 | 46.53 |

**Table 2:** Pharmacokinetics of selected ligands of *C. fistula*

| Sl. No | ZINC Id | Name of ligand | GI absorption | BBB permeant | P-gp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | log Kp (skin permeation) (cm/s) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ZINC000004098644 | Aloeemodin | High | No | Yes | No | No | No | No | No | -6.66 |
| 2 | ZINC000000967520 | Camphor | High | Yes | No | No | No | No | No | No | -5.67 |
| 3 | ZINC000000057736 | Chromone | High | Yes | No | Yes | No | No | No | No | -6.21 |
| 4 | ZINC000003861630 | Chrysophanic | High | Yes | No | Yes | No | No | No | Yes | -5.34 |
| 5 | ZINC000000119988 | Epicatechin | High | No | Yes | No | No | No | No | No | -7.82 |
| 6 | ZINC000003861345 | Furfural | High | Yes | No | No | No | No | No | No | -6.6 |
| 7 | ZINC000000001707 | Methylchromone | High | Yes | No | Yes | No | No | No | No | -5.91 |
| 8 | ZINC000003978794 | Physcion | High | No | Yes | Yes | No | No | No | Yes | -5.88 |
| 9 | ZINC000003978631 | Phytol | High | No | Yes | No | No | No | No | No | -2.59 |
| 10 | ZINC000000388079 | Salicyclic acid | High | Yes | No | Yes | Yes | No | No | No | -5.43 |

Molecular docking shows the interactions of ligands with the receptor molecules. MolDock automatically identifies potential binding sites (cavities) using cavity detection algorithm. Rerank score was taking into account to study the receptor ligand interactions it gives better result than the docking score. All the selected ligands show low negative results with minimum of -74.1435 by physcion and maximum of -42.304 by camphor against alpha amylase (Fig. 1). Phytol shows minimum rerank score of -73.452 and aloeemodin shows maximum rerank score of -36.6025 against glutathione S transferase (Fig. 2). The rerank score is the weighted combination of MolDock score and Lennard-Jones approximations to steric energy and it indicates stable interactions between receptors and ligands (Thomson & Chistensen, 2006) [22]. The rerank score of selected ligand receptor interactions suggest that the *C. fistula* can be a potent extract for controlling *C. chinensis*. Earlier work by Shintu *et al*., (2020) [20] found that various isolated compounds from *Citrus sinensis* show good binding potential to agERG protein of *Anopheles gambiae* and hence can be a potential controlling agent. Singh *et al*., (2016) [21] reveals that flavanols show inhibition with ATP molecule at active site of heat shock protein 90. Another work by Mejia *et al*., (2018) [17] found that compounds isolated from *Calceolaria* show good binding potential to prophenoloxidase, acetylcholinesterase and ecdysone receptors of insects.

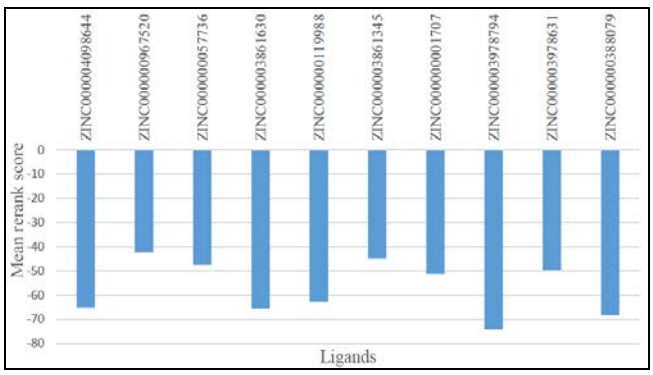


**Fig 3:** Graph showing rerank scores of ligands against Alpha amylase

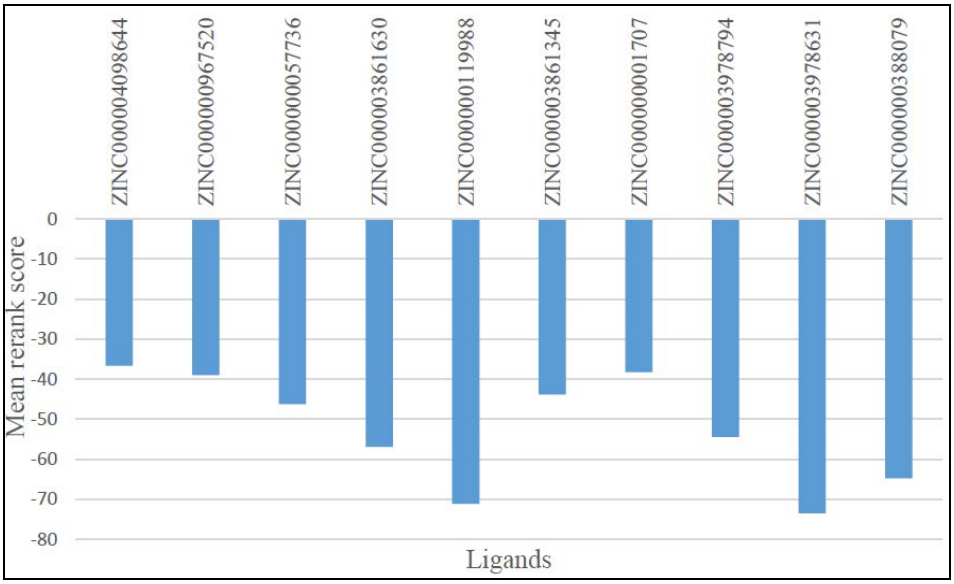


**Fig 4:** Graph showing rerank scores of ligands against Glutathione S Transferase

For visualizing the interactions of ligands with the receptors, Discovery Studio Visualizer was used and both 2D &3D images were analyzed. The molecular interactions include H-Bond, Van der Waals, C-H bond, covalent bond, alkyl bond, pi-alkyl bond, pi donor H-bond etc. The best 3 interactions of ligands with the receptors based on their rerank scores are shown below. (Fig 3&4).

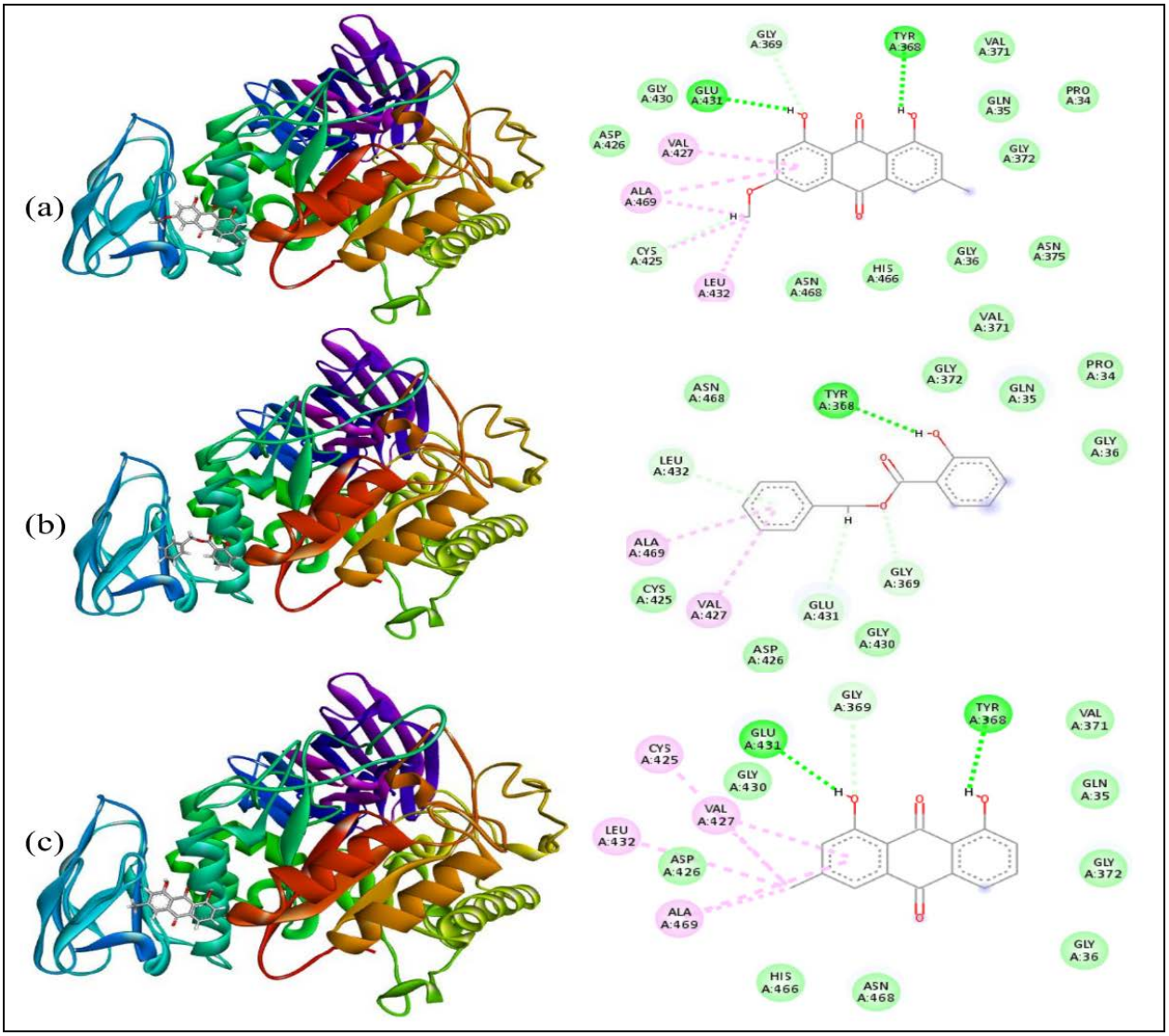


**Fig 5:** 2D & 3D visualization of ligands interaction with the alpha amylase at potential ligand binding cavity; (a) Physcion, (b) Salicyclic acid, (c) Chrysophanic

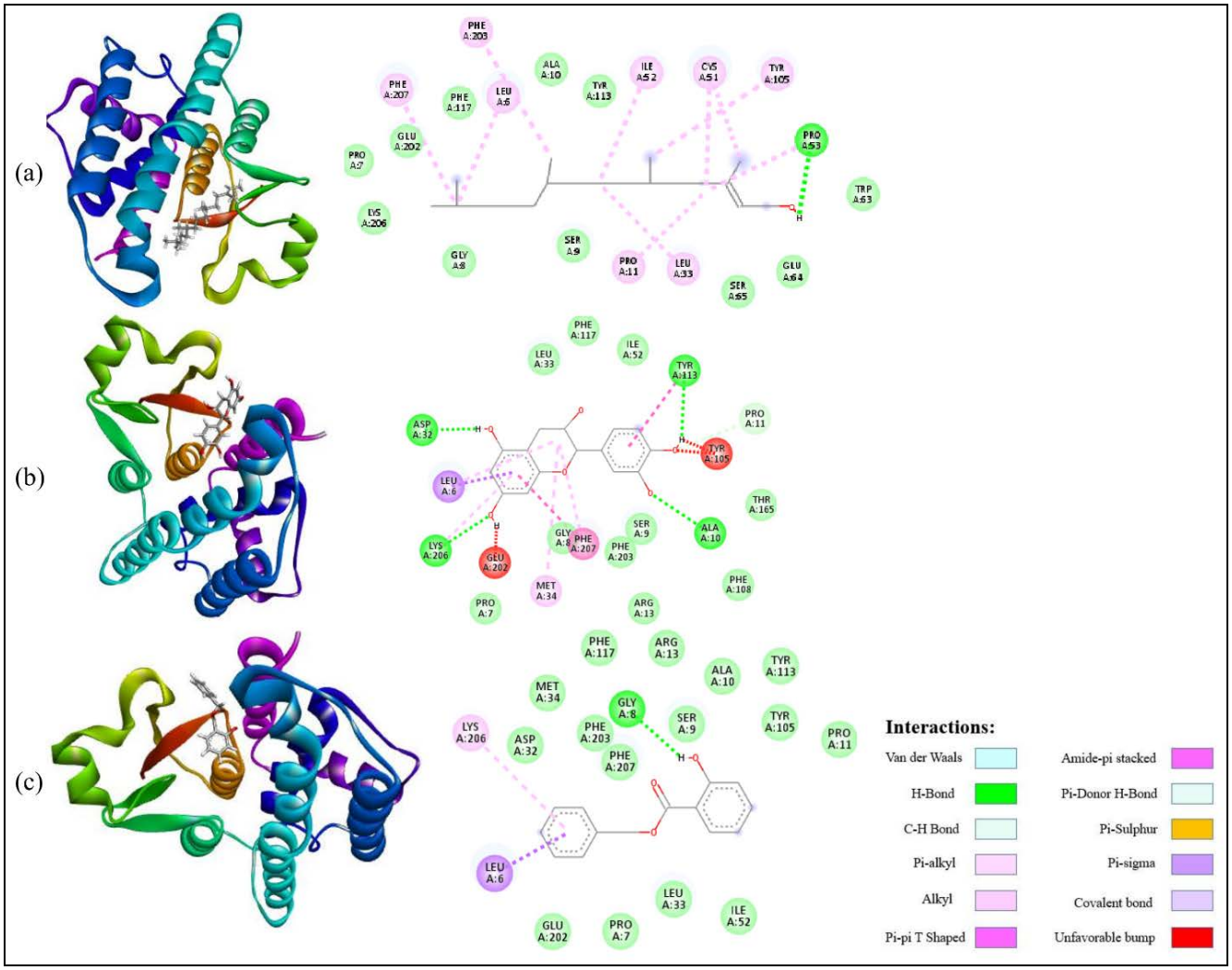


**Fig 6:** 2D & 3D visualization of ligands interaction with glutathione S transferase at potential ligand binding cavity; (a) Phytol, (b) Epicatechin, (c) Salicyclic acid

The toxic effect of methanolic crude extract of *C. fistula* from leaf were evaluated against *C. chinensis*. The number of dead insects were counted after 24h, 48h and 72h at different doses of the extract. The total percent mortality was observed after 72h and the corrected mortality was calculated by using Abbott's formula. The result of the toxicity test shows that % mortality of insects was dose dependent and the % mortality of insects when treated with extract at 2, 4, 6, 8, 10 and 12 % concentration was 28, 35, 43, 53, 67 and 82% respectively (Table 3). The probit analysis for the estimation of $LC_{50}$ shows that $LC_{50}$= 5.671% and the smaller significance F value for the regression suggest that the regression model is significant (Table 3). Earlier study by Jadhav and Pardeshi (2017) [11] found that fungal extract isolated from leaf of *Jatropha curcas* against *C. chinensis* show $LD_{50}$= 42.32mg/kg in methanol and 64.76mg/kg in ethyl acetate. Another study by Kumar *et al*., (2017) [14] found that neem oil @ 2.5ml/kg seeds was most effective against *C. chinensis*. Similar study by Bindu *et al*, (2015) [4] revealed that ethanolic extract of plant *Hydrocotyl asiatica* causes high mortality of *C. chinensis*. The aqueous extract of *Nicotiana tabacum* and *Azadirachta indica* produce maximum adult mortality (100%0 of *C. chinensis* followed by *Euclyptus globules* (86%) as studied by Rashid *et al*., (2018) [15]. The in silico and wet lab results of the leaf extract of *C. fistula* against *C. chinensis* suggest that it can also be a potent controlling agent. Assam is rich in its floral biodiversity and hence there is abundant number of *C. fistula* present in Assam. The commercial scale production of methanolic crude extract as well as specific physcion and phyol can be beneficial for farmers in controlling the damage caused by *C. chinensis*.

**Table 3:** % mortality and probit analysis of insects when treating them with various concentration of methanolic crude extract of *C. fistula*.

| Dose (% of Conc.) | Log % of Conc. | No. of Insects | Mean Mortality after 72h | Mean % Mortality (Rounded) | Corrected Mortality (%) | Probit Value | Regression Equation | Significance F value in regression | LC50 (% Conc.) |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.301029996 | 20 | 5.66 | 28 | 28 | 4.42 | y=1.734x+3.693 | 0.010327579 | 5.671 |
| 4 | 0.602059991 | 20 | 7 | 35 | 35 | 4.61 | | | |
| 6 | 0.77815125 | 20 | 8.6 | 43 | 43 | 4.82 | | | |
| 8 | 0.903089987 | 20 | 10.6 | 53 | 53 | 5.08 | | | |
| 10 | 1 | 20 | 13.3 | 67 | 67 | 5.44 | | | |
| 12 | 1.079181246 | 20 | 16.3 | 81 | 81 | 5.88 | | | |

## Conclusion

In conclusion, the present study reports the insecticidal effects of phytochemicals from leaves of *C. fistula* against *C. chinensis*. In-silico study shows low negative rerank scores suggesting a good interaction between ligands and the active sites of enzymes alpha amylase and glutathione S transferase. This interaction is helpful in controlling the insect pest as it hampers the normal activity of enzymes ultimately leading to the physiologically unfit state of the insect. Furthermore, toxicity test results also show that the methanolic crude extract of *C. fistula* is effective in controlling the insect and the mortality of insect is dose dependent with $LC_{50}$=5.671%. Thus the study suggest that *C. fistula* can be used as a potential controlling agent for the insect pest *C. chinensis*.

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