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Understanding the Molecular Mechanistic Anthelmintic Potential of *Trichosanthes dioica* Seed

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Abstract: ***Background:*** Herbal treatments have historically been used to cure a variety of human problems. To identify a single element that could be the basis for creating novel, therapeutically effective medicines, researchers examined herbal remedies. The genus Trichosanthes in the family Cucurbitaceae contains an annual or perennial herb that grows in tropical Asia and Australia. The pointed gourd, or parwal (Trichosanthes dioica Roxb), is mostly farmed as a vegetable. T. dioica leaf juice is employed in the treatment of edema, alopecia, subacute liver enlargement, as a febrifuge, and as a tonic. The Charaka Samhita lists fruit and leaf cures for jaundice and intoxication. The pharmacology of the various parts of T. dioica has been the subject of numerous scientific investigations, but some more traditionally important therapeutic benefits are still awaiting scientific confirmation. ***Aim:*** In the present study *in -vivo* and *in -silico* evaluation of anthelmintic potential of *Trichosanthes dioica* seed extracts was carried out. Further proposed mechanism of Anthelmintic efficacy of *Trichosanthes dioica* seed was determined by molecular docking. ***Method:*** In the current study, a molecular docking technique was used to try and identify β -tubulin of the helminth's protein inhibitors. A grid-based docking strategy was used to determine the binding using the Auto Dock software. Merck Molecular Force Field was used to build the 2D structures of compounds, convert them to 3D, and then energetically reduce them up to arms gradient of 0.01. (MMFF). ***Result:*** The ethyl acetate extract at 50 mg/mL was the most active against *Pheretimaposthuma*. The extract exhibited a paralytic effect at 3.0 min and caused actual death at 6.6 min. ThePEE, EAE & EE exhibited a dose dependent activity; however, the EAE effect at 50 mg/mL against *Pheretima posthuma* (earthworm) was not significantly different from that of albendazole, 10 mg/mL. The result of molecular docking was tabulated in table 7, showing binding energy -3.42 & -1.38 kcal/mol for betulin and karounidiol respectively. ***Conclusion:*** The anthelmintic potential of *T.dioica* seed was due to synergetic effect of Betulin and karounidiol *via* inhibition of the β -tubulin of the helminths.

Keywords: Anthelmintic efficacy, betulin and karounidiol, β-tubulin & molecular docking.

Research Paper

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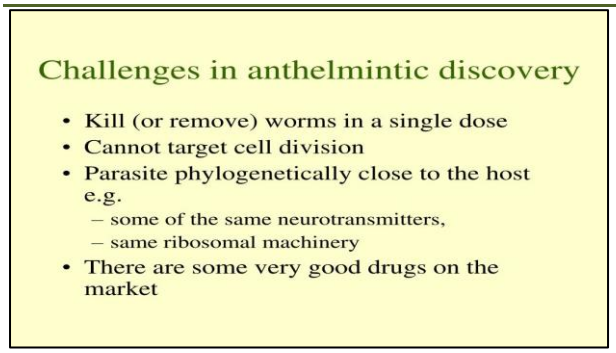
INTRODUCTION

Traditional herbal medicine practitioners have established that a number of native plants are therapeutically effective in treating a variety of illnesses. Natural components serve as the foundation for both synthetic and traditional herbal therapy [1-2]. Helminthiasis, an illness caused by helminths that prevents living things from growing normally, is a serious health issue. The most widespread infectious disease in the poor world is helminthiasis, which affects the human intestine. In addition to being extremely expensive, helminths are currently resistant to commercially available medications. Globally, parasitic illnesses continue to have a significant negative impact on the production of live animals. The parasitic worm Haemonchus contorn, which feeds on the blood of small ruminants, affects its host by causing anaemia, appetite loss, sluggish growth, and finally death. The production of healthy sheep and goats around the world is significantly hampered by H. contortus, a highly pathogenic parasite of small ruminants [3]. Researchers are trying to solve the issues by using anthelmintic drugs made from organic plant sources.



Trichosanthes is an annual or perennial herb that grows in Australia, Polynesia, and tropical Asia. It is a member of the Cucurbitaceae family. In several parts of India and Bangladesh, the pointed gourd (T. dioica), one of the important vegetables in the area, is referred to by the names parwal, palwal, parmal, patol, and potala [4]. The fruits and leaves of the plant are the only edible parts, and they can be prepared in a variety of ways on their own or in combination with other fruits, vegetables, and meats.

Trichosanthes

The distinctive quality of the seed peptides is their resistance to the action of silver nitrate, a sensitive dye frequently employed to stain proteins [5]. The chemical components of T. dioica include vitamins A, C, tannins, and saponin [6]. Analyses of the phytochemistry of aqueous and ethanolic extracts revealed the presence of tannins and saponins [7]. The most important component of the highly polar fraction of the nonsaponifiable lipid in the T. dioica seed extract is 7-oxidihydrokarounidol-3-benzoate.T. dioica has 24-ethylcholest-7-enol and 24-ethylcholest-7-enol as its two primary phytosterols [8]. Additionally, T. dioica seeds contain lectin, a protein that binds to carbohydrates (particularly galactose) and is related to type-II ribosomal inhibitory proteins (type-II RIP). A Gal/GalNAc-specific lectin from the seeds of T. dioica has been purified, physicochemically characterised, saccharide specificity determined, and chemically modified by Sultan and Kenoth (2004). Betulin and karounidiol was present in ethyl acetate extract as per literature survey [9]. Molecular docking of these active compounds with β-tubulin was carried out to illustrate the proposed mechanism of action. Molecular docking of anthelmintic drug with β-tubulin to study the activity by drug-tubulin interaction is already proven by Grace basumatary *et al.*, 2020 because inhibition of β -tubulin of the helminths can severely affect their vital cellular functions such as mitosis, motility, and transport. Therefore, in order to rationalize the anthelmintic activity of T. dioica seed the active compound (Betulin and karounidiol) was taken as lead molecule for elucidation of proposed activity and understand their possible interactions, molecular docking simulation of the compounds have been carried out against β -tubulin.

Experimental Works

***In-vivo* anthelmintic activity**

The anthelmintic activity was carried out using three doses (10, 25, 50 mg/mL) ofpetroleum ether, ethyl acetate extract, ethanolic extract and aqueous extract against the Indian earthworm (*Pheretima posthuma*) by adopting the standard procedures (Fried – Jaiyesimi *et al.*, 2011) (Figure 4). The earthworms of nearly the same size were used. Paralysis time (m) was noted when earthworms did not move except in the condition when the worms were robustly shaken.

The death time (m) was noted after observing that the worms did not move when shaken forcefully and when they were dipped in hot water (51°C) and followed by dullness of their original body colors. Albendazole (10 mg/mL) was used asthe reference drug [10].

Molecular docking studies

Ligand Preparation:

2D Structure of ligands like betulin and karaounidiol were drawn using Chem Sketch [11], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

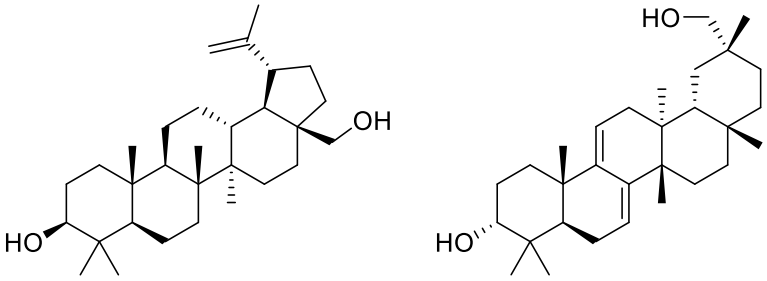


Figure 1: 2D structure of betulin and karaounidiol

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [11].

Table 1: Grid parameters used in current docking analysis of β-tubulin

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	β-tubulin	40	40	40	0.403	15.657	64.408	37.603

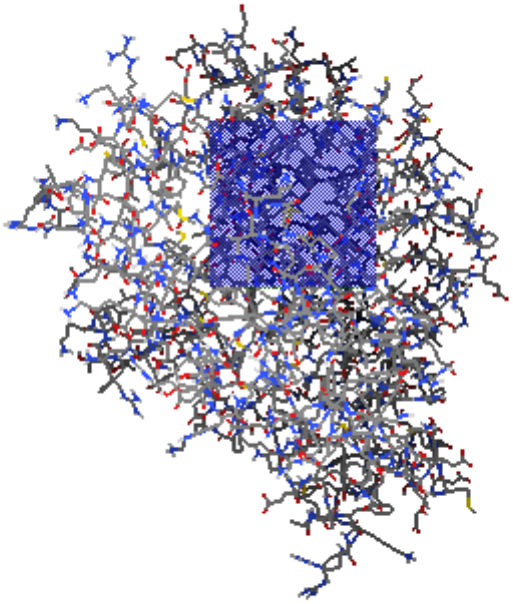


Figure 2: Grid box covering all active sites in β-tubulin receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [12].

Crystal structure

The crystal structure of the protein consisting of β-tubulin receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6fkj.pdb) registered in the Protein data bank was used [13]. The complex ligand was separated by using Chimera software.

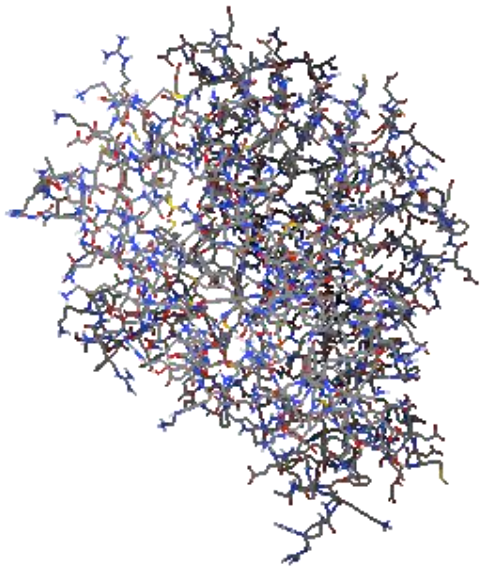


Figure 3: Crystal structure of β-tubulin receptor (PDB ID-6fkj)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain A was selected for experimental purpose and other chain B was removed from it. The bound ligand Heme was separated from the macromolecular complex by using software Chimera [14-15].

Molecular Docking Simulation Studies

Docking of ligands like butulin and karaounidiol against β-tubulin receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [16].

Toxicity & ADME-T Studies

The ligand molecules *viz.* betulin and karaounidiol were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [17-18].

RESULT AND DISCUSSION

Helminthiasis, an illness brought on by helminths that causes difficulty and stunted growth in living things, is a serious health issue. The most prevalent infectious illness in the poor world is helminthiasis, which affects the human gut. Helminths are now resistant to commercially available medications, and they are very expensive. Researchers are striving to filter the anthelmintic compounds from natural plant sources in an effort to solve the issues. The present focus is on finding and studying plants that have anthelmintic potential. Currently, the chemicals used to treat helminthiasis are costly, effective against just one type of parasite, and lose their potency after 20 min. The anthelmintic activity of *T.dioica* seeds may be attributed to the presence of these active components. The *in-vivo* anthelmintic activities determined in PEE, EAE & EE. In this study PEE, EAE & EE of *T.dioica* seed extract exhibited anthelmintic activities against *Pheretima posthuma* (Table 2 & fig.4).The result revealed that ethyl acetate seed extract of *T.dioica* showed maximum activity as compared with standard. The ethyl acetate extract at 50 mg/mL was the most active against *Pheretimaposthuma*. The extract exhibited a paralytic effect at 3.0 min and caused actual death at 6.6 min. The PEE, EAE & EE exhibited a dose dependent activity; however, the EAE effect at50 mg/mL against *Pheretima posthuma* (earthworm) was not significantly different from that of albendazole, 10 mg/mL.

As per literature survey Betulin and Karounidiol was present in ethyl acetate extract (Mahia Khandaker etal;2018). So these constituent was selected as lead molecule for the *in-silico* docking analysis. Molecular docking of anthelmintic drug with β-tubulin to study the activity by drug-tubulin interaction is already

proven by Grace basumatary etal;2020 because inhibition of β -tubulin of the helminths can severely affect their vital cellular functions such as mitosis, motility, and transport. Therefore, in order to rationalize the anthelmintic activity of the active compounds present in ethyl acetate seed extract of *T.dioica* and understand their possible interactions, molecular docking simulation of the compounds have been carried out against β -tubulin.

The plants *T.diocia* are traditionally utilized for the anthelmintic property from the immortal time. The exact mechanism of action for the anthelmintic response was still not revealed. With intent to propose the most probable mechanism of action the docking based computational analysis has been performed against the beta tubulin receptor. The result of molecular docking was tabulated in table 3, showing binding energy -3.42 & -1.38 kcal/mol for betulin and karounidiol respectively. The binding mode showed in fig.5-6 whereas 2D &3D binding interaction was shown in fig.7-12. Although betulin and karounidiol showed good interaction with selected ligand but highest binding interaction displayed by betulin with ***β -tubulin*** receptor having covalent bonding interaction with LEU 255, LEUA:252, LYS A:352, ALA A:354, LEU A:242 & ALA A:256 as well as conventional hydrogen binding at TYR A:202 with weak Vander wall's interaction at THR A:353, CYS A: 241, ALA A:317. The binding interaction of karounidiol on ***β -tubulin*** receptor displayed the conventional hydrogen bounding at ALA A:316, ILE A:378, CYS A :241, ILE A: 318,LEU A: 255, LEU A: 242,Val A:238, THY A:202 along with GLU A: 200,ASn A :167 (covalently),MET A: 259 (Pi-sigma) & VAL A:315,THR A:314,ASN A : 258, LEU A :252,THR A:239 (weak Vander wall's interaction).

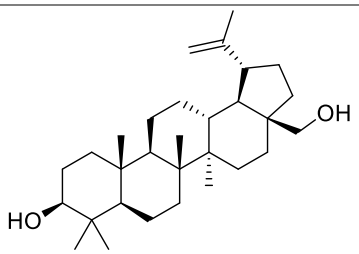
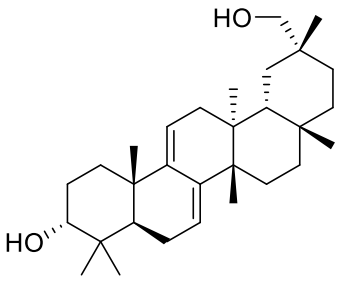
The pharmacokinetic profiling of the Betulin and Karounidiol ligand has revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like reproductive effects, irritant effect, and tumorogenic properties, but shows the presence of some mutagenicity. The pharmacokinetic and toxicity profiling results of flavonoids was shown in fig. 13-14.

Table 2: Effect of PEE, EAE & EE T.dioica seeds on paralytictime and death time of Pheretima posthuma

Extract	Dose (mg/mL)	Mean length of worms (cm)	Paralytic time(min) Mean±SEM	Death time (min) Mean±SEM
PEE	10	10	98.1±4.6*	406±28.5*
	25	9	81.8±20.5 *	312±15.7*
	50	11	24.0±67.1 *	215±15.0*
EAE	10	6	30.5±2.50*	22.2±2.30
	25	7	10.1±2.49	11.4±5.09
	50	6	3.0±1.35	6.06±0.98
EE	10	8	74.2±3.5*	512±30.3*
	25	7	22.9±21.6*	375±20.1*
	50	9	7.3±52.2*	213±16.1*
Albendazole	10	4	3.8±0.75	6.2±2.02

Fig. 4: *In-vivo* Anthelmintic Activity

Table 3: Results of docking of ligands like betulin and karaounidiol against β-tubulin receptor

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	Betulin		-3.42
2	Karaounidiol		-1.38

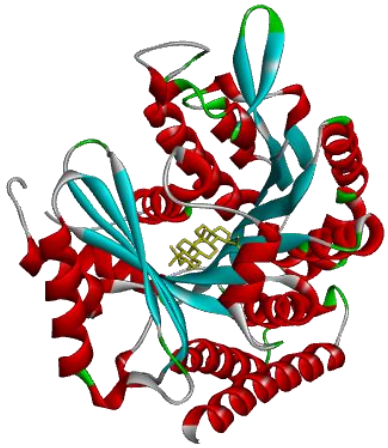


Figure 5: Binding mode of betulin within the active site of β-tubulin receptor

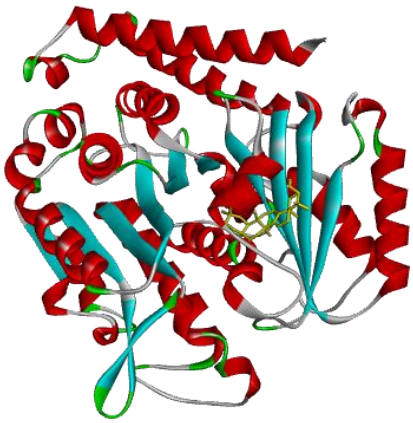


Figure 6: Binding mode of karaounidiol within the active site of β-tubulin receptor

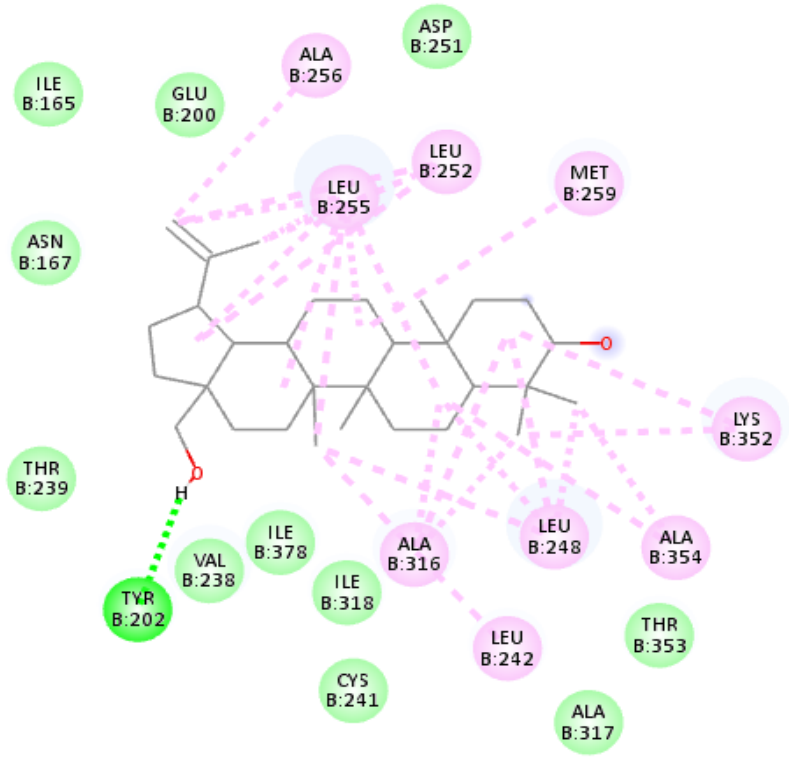


Figure 7: Two-dimensional binding mode of betulin within the active site of β-tubulin receptor

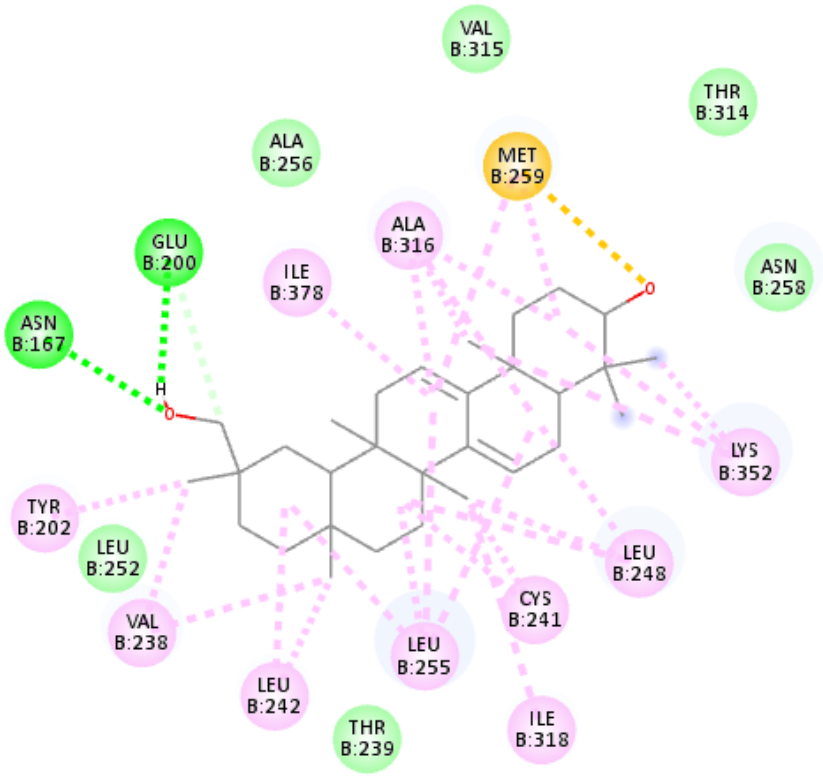


Figure 8: Two-dimensional binding mode of karaounidiol within the active site of β-tubulin receptor

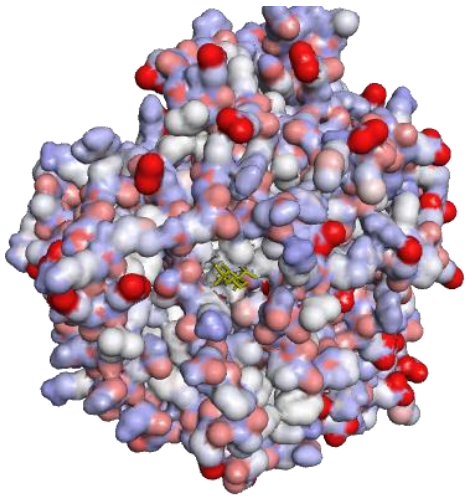


Figure 9: Three-dimensional binding conformation of betulin within the active site of β-tubulin receptor

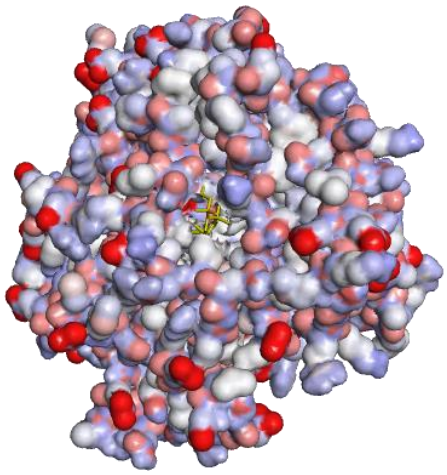


Figure 10: Three-dimensional binding conformation of karaounidiol within the active site of β-tubulin receptor

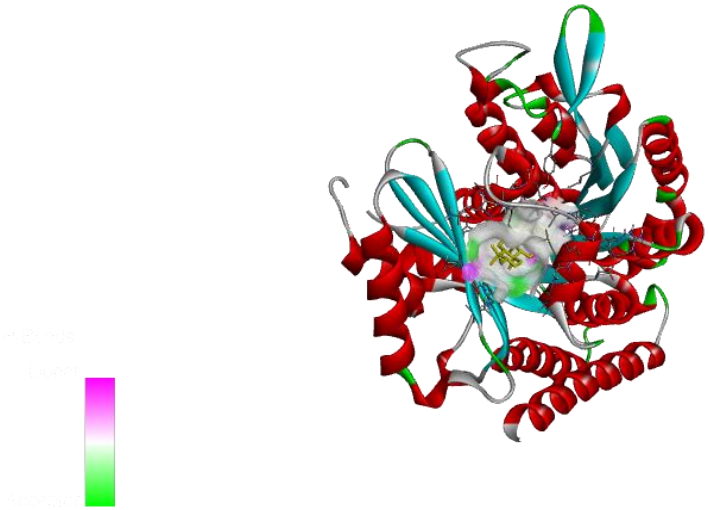


Figure 11: Three-dimensional binding mode of betulin within the active site of β-tubulin receptor

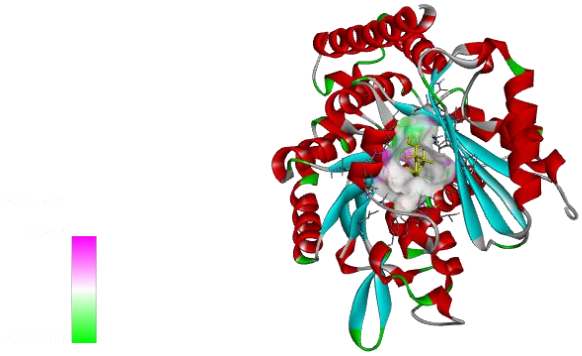


Figure 12: Three-dimensional binding mode of karaounidiol within the active site of β-tubulin receptor

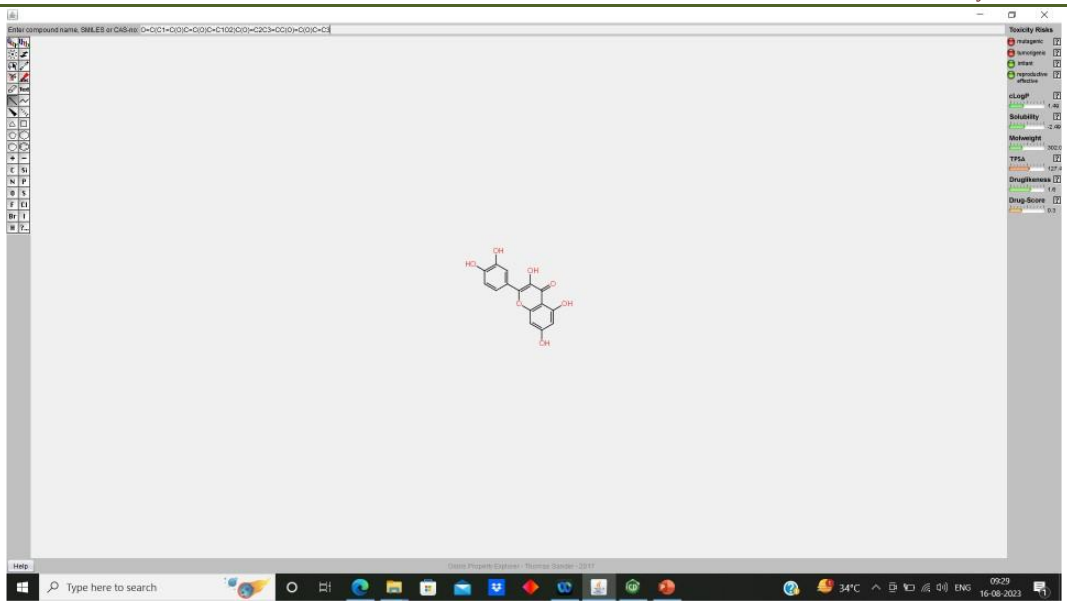


Figure 13: Pharmacokinetic and toxicity profiling of botulin

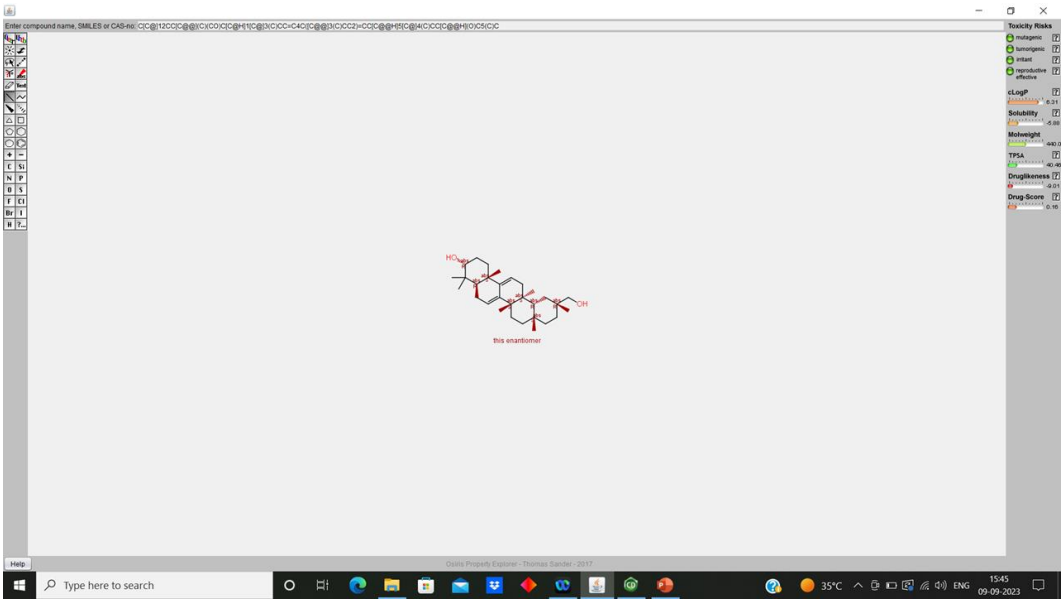
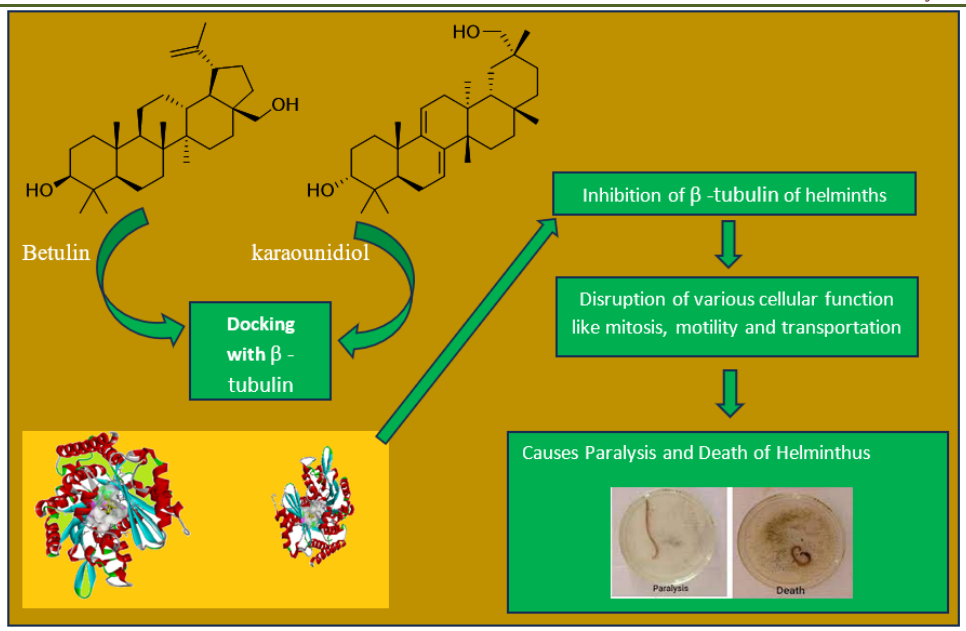


Figure 14: Pharmacokinetic and toxicity profiling of karaounidiol

Divulgence of Investigation

Helminth infections are among the most prevalent types of diseases seen in humans and impact a significant section of the population around the world. They are a significant contributor to the incidence of malnutrition, anaemia, eosinophilia, and pneumonia in underdeveloped nations, where they are a huge public health risk and a substantial threat to public safety. Anthelmintics are medications that either kill or expel helminths that are already infesting the body. The gastrointestinal tract is the home of the majority of helminths, although certain helminths also reside in other tissues, or their larvae move into other tissues. They do damage to the host by preventing him from consuming food, leading to blood loss, causing organ damage, obstructing the digestive or lymphatic tracts, and secreting poisons.

A variety of human conditions are treated with T. dioica by Ayurvedic practitioners in addition to those who practice traditional medicine in rural and tribal communities. It can be used by itself or in conjunction with other herbs as a treatment for hyperacidity in Ayurvedic medicine. Numerous usages as antiparasitic, antiperiodics, febrifuge, refrigerants, anticoagulants, blood purifiers, antiseptics, and tonics. They also said that it was used as a blood purifier. As per current investigation the proposed mechanism of action against Helminthes was diagrammatically shown as:



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