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Original Research Article

Deciphering of Anthelmintic Potential of *Annona squamosa* Leaf: Molecular Mechanistic

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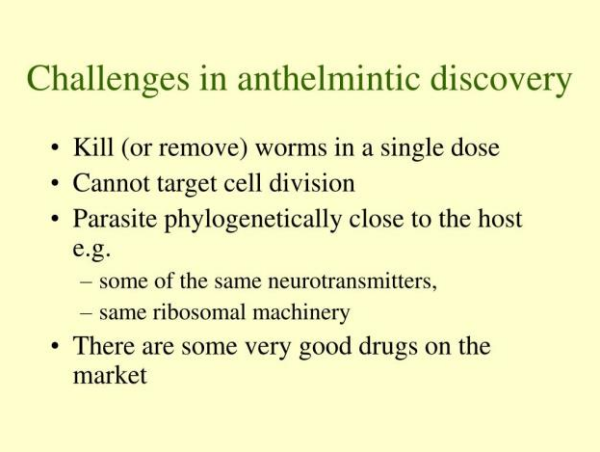
Abstract: Herbal treatments have historically been utilized to treat various human diseases. To find a single ingredient that could be the basis for creating new therapeutically effective products, researchers studied herbal medicines. *Annona squamosa* Linn has anti-inflammatory, hepatoprotective, anti-diabetic, cytotoxic, gene-toxic, and anticancer properties. The primary active components of *A. squamosa* leaves, flavonoids, have been utilised to treat a variety of human ailments. Quercetin and rutin, two flavonoids, have been shown to have anticancer, antiviral, anti-inflammatory, and heart disease preventive properties. In the present study *in -vivo* and *in -silico* evaluation of anthelmintic potential of *Annona squamosa* leaf methanolic extract was carried out. Further proposed mechanism of Anthelmintic efficacy of *Annona squamosa* leaf was determined by molecular docking.

Keywords: *Annona squamosa*, Quercetin, docking score, molecular docking & β-tubulin.

INTRODUCTION

Several native plants' therapeutic efficacy in treating a range of ailments has been documented by traditional herbal medicine practitioners. Both synthetic and conventional herbal medicine are built on natural ingredients [1, 2]. A severe health problem is helminthiasis, an infection brought on by helminths that impairs living things' ability to grow normally. Helminthiasis, which affects the human intestine, is the most common infectious disease in the developing world. Helminths are exceedingly expensive and are now resistant to commercially available medicines. The production of live animals continues to be severely hampered by parasite diseases on a global scale. Haemonchus contorn, a parasitic worm that feeds on the blood of small ruminants, causes anaemia, loss of appetite, sluggish growth, and ultimately death in its host. H. contortus, a highly pathogenic parasite of small ruminants, is a significant impediment to the global production of healthy sheep and goats [3]. Using anthelmintic medicines derived from natural plant sources, researchers are working to find answers to the problems.



The therapeutic efficacy of many indigenous plants, for diverse diseases has been defined with the aid of conventional herbal medicinal practitioners [4]. *Annona squamosa* (A. squamosa) L. (Family: Annonaceae), generally known as custard apple [5].

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Annona squamosa

The main flavonoids found the leaves of *A.squamosa* are quercetin-3-O robinobioside, rutin, quercetin-3-O-β-D-glucoside, kaempferol-3-O-robinobioside, and kaempferol-3-O-rutinoside [6]. As per literature survey rutin and quercetin was present in methanolic leaf extract. 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 *Annona squamosa* leaf the active compound (quercetin) 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 [7].

Experimental Works

***In-vivo* anthelmintic activity**

The anthelmintic activity was carried out using three doses (10, 25, 50 mg/mL) of methanolic leaf extract against the Indian earthworm (*Pheretima posthuma*) by adopting the standard procedures. 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.

Earthworms used for Anthelmintic Activity

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 colours. Albendazole (10 mg/mL) was used as the reference drug [8].

Ligand Preparation:

2D Structure of ligand (quercetin) was drawn using ChemSketch [9], the two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structure of ligand (quercetin) is given below:

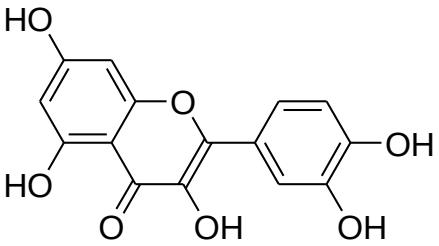


Figure 1: 2D structure of quercetin

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 between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.392 Å and No. of points considered are 40, 40 and 40 points in the x, y, and z dimensions and 16.741, 11.602 and 27.751 as x, y, z centers [10].

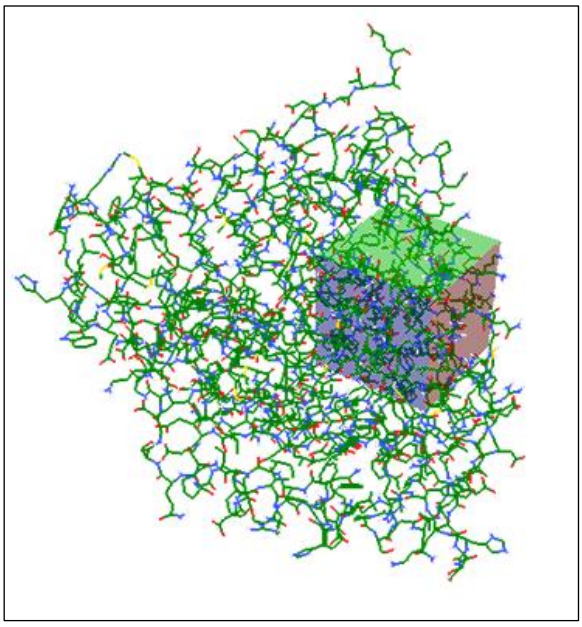


Figure 2: Grid box covering all active sites in receptor

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 [10].

Docking of beta-tubulin with Quercetin

Crystal structure

The crystal structure of the protein consisting of receptor associated with bound ligand mebendazole is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (7odn.pdb) registered in the Protein data bank was used. The bound ligand serotonin is found within the receptor [11].

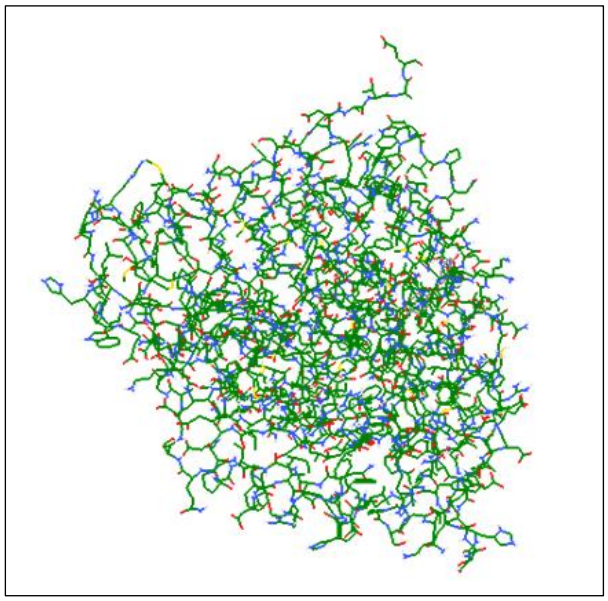


Figure 3: Crystal structure of beta-tubulin receptor with bound mebendazole (PDB ID-7odn)

Processing of Protein

The downloaded receptor protein is having two chains, i.e. chain A, and B. Out of these two chains, chain B was selected for experimental purpose and other chains were removed from it. The bound ligand mebendazole was separated from the macromolecular complex by using software Chimera [12].

Molecular Docking Simulation Studies

Docking of quercetin ligand on beta-tubulin was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [13].

Toxicity & ADME-T Studies

The modified lead molecules are 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 [14].

RESULTS AND DISCUSSION

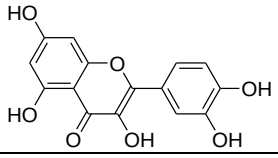
In-vivo anthelmintic potential of methanolic leaves extract of *A.squamosa* with different concentration i.e. 10mg/ml, 25mg/ml & 50mg/ml was carried out on *Pheretima posthuman*. The outcome of the study revealed that the 25mg/ml of extract showed the significant result having paralytic time (min) 11.8 ± 18.5* as compared with standard drug albendazole (Table 1).

As per literature survey rutin and quercetin was present in methanolic leaf extract. 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 *Annona squamosa* leaf the active compound (quercetin) 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. The molecular docking of quercetin with beta tubulin receptor revealed that (Table 2), the it has exhibited the chemical interaction with the amino acids in the active pockets which is showed in Figure.4. Theoretically, the ligand molecule has shown encouraging docking score. The docking result of quercetin revealed that their docking scores was -7.13 kcal mol^{-1}, and it can be predicted as good inhibitor of beta tubulin receptor. The pharmacokinetic profile of quercetin reveals that it is having good pharmacokinetic profile without presence of any major toxic effects. The pharmacokinetic and toxicity profiling results of quercetin were shown in Figure 5.

Table 1: Effect of methanolic leaf extract of *A.squamosa* 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
Methanolic leaf extract of *A.squamosa*	10	10	94.1+14.6*	398+28.5*
	25	9	11.8+18.5 *	105+15.7*
	50	11	32.0+77.1 *	311+15.0*
Albendazole	10	4	3.9+0.65	7.2+2.02

Table 2: Molecular docking result

S. No	Compound Name	Structure	Binding Energy (Kcal/mole)	Ki (μM)
1.	Quercetin		-7.13	5.90

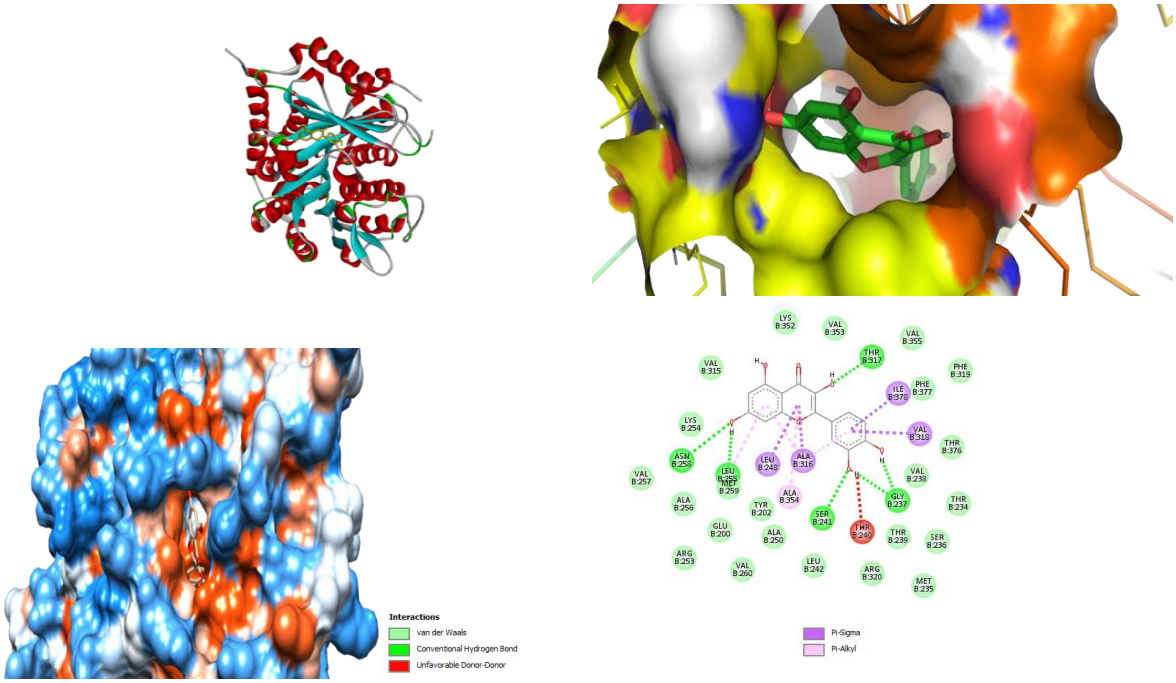


Figure 4: Binding mode of quercetin within the active site of beta-tubulin receptor

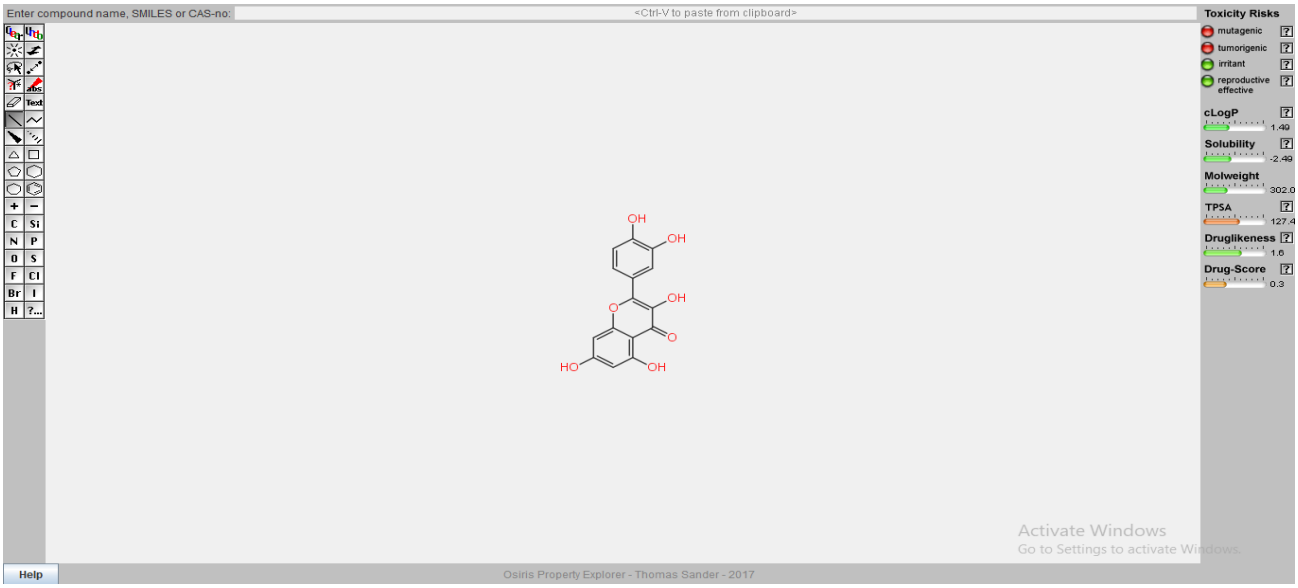
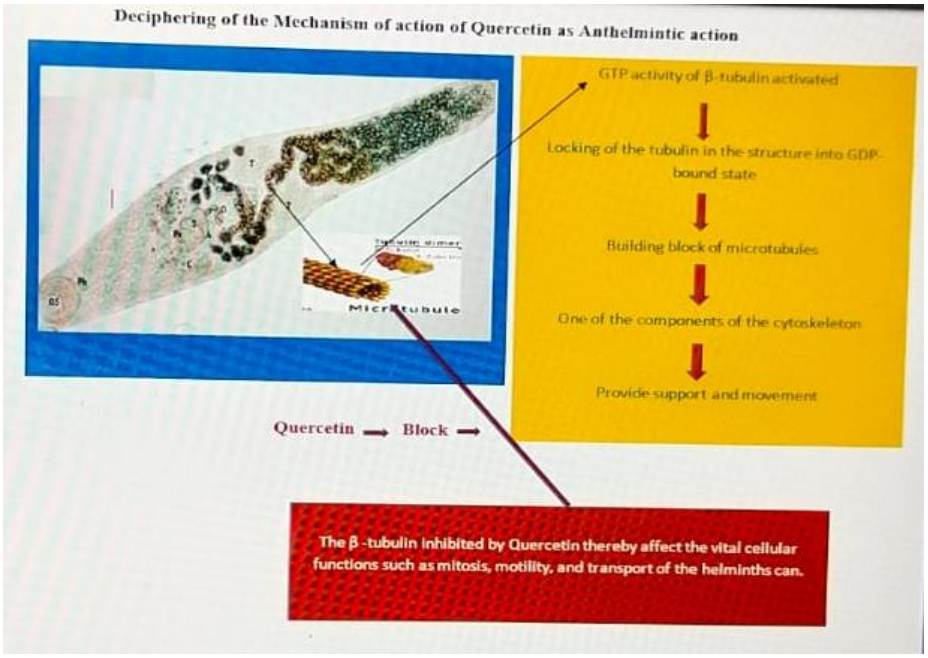


Figure 5: Pharmacokinetic and toxicity profiling of quercetin

CONCLUSION

In this study, the 25 mg/mL of *A.squamosa* Linn leaf exhibited relevant anthelmintic activity against *Pheretima posthuma*. Quercetin was taken as lead molecule for *in-silico* docking study. The docking result of quercetin revealed that their docking scores was -7.13 kcal mol^{-1}, and it can be predicted as good inhibitor of beta tubulin receptor. From the work we conclude that traditional usage of *Annona squamosa* leaf for treating worm infestations has been validated.



REFERENCE

1. Himesh, S., Singhai, A. K., & Sarvesh, S. (2012). Quantification of ascorbic acid in leaves of Annona squamosa. *Int. J. pharm. pharm. sci*, *4*, 144-147.
2. Himesh, S., Mishra, K., Sarvesh, S., & Singhai, A. K. (2012). Characterization of Azadirachtin from ethanolic extract of leaves of Azadirachta indica. *Journal of Pharmacy Research*, *5*(1), 199-201.
3. Waller, P. J., & Thamsborg, S. M. (2004). Nematode control in 'green'ruminant production systems. *Trends in parasitology*, *20*(10), 493-497.
4. Himesh, S., Sharan, P. S., Mishra, K., Govind, N., & Singhai, A. K. (2011). Qualitative and quantitative profile of curcumin from ethanolic extract of Curcuma longa. *Int Res J Pharm*, *2*(4), 180-184.
5. Himesh, S., Singhai, A. K., & Sarvesh, S. (2012). Quantification of ascorbic acid in leaves of Annona squamosa. *Int. J. pharm. pharm. sci*, *4*, 144-147.
6. Zhu, H., Chen, L., Yu, J., Cui, L., Ali, I., Song, X., ... & Wang, X. (2020). Flavonoid epimers from custard apple leaves, a rapid screening and separation by HSCCC and their antioxidant and hypoglycaemic activities evaluation. *Scientific reports*, *10*(1), 8819.
7. Soni, H., & Malik, J. (2022). Isolation, characterization and radical scavenging activities of rutin isolated from leaves of Annona squamosa. *Journal of Pharmacognosy and Phytochemistry*, *11*(2), 222-228.
8. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
9. Malik, J. K., Soni, H., Sharma, S., & Sarankar, S. (2020). Hydroxychloroquine as potent inhibitor of COVID-19 main protease: Grid based docking approach. *Eurasian Journal of Medicine and Oncology*, *4*(3), 219-226.
10. Soni, H., Gautam, D., Sharma, S., & Malik, J. (2020). Rifampicin as potent inhibitor of COVID-19 main protease: In-silico docking approach. *Saudi Journal of Medical and Pharmaceutical Sciences*, *6*(9), 588-593.
11. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
12. Soni, S., Malik, J. K., Sarankar, S. K., & Soni, H. (2019). Rutin as a potent inhibitor of dihydrofolate reductase: A computational design and docking. *EAS J. Pharm. Pharmacol*, *1*, 130-134.
13. Soni, N. U. P. U. R., Pardasani, K. R., & Mujwar, S. O. M. D. U. T. T. (2015). In silico analysis of dietary agents as anticancer inhibitors of insulin like growth factor 1 receptor (IGF1R). *J Pharm Pharm Sci*, *7*(9), 191-196.
14. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.

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