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Molecular dynamics studies on the arginine kinase protein of *Aedes sollicitans*: Against the natural chemical compound, Gedunin

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Abstract

Plant extracts have, today, become an important additive in the food industry owing to the bioactive compounds present in them. They have antimicrobial and antioxidant activities which act as very good candidates to replace synthetic compounds. In this insilico study, we focus on Gedunin, a tetranortriterpenoid extracted from *Azadirachta indica*, the Indian neem tree, which inhibits the Arginine kinase present in *Aedes sollicitans*. The chemical structure of Gedunin was obtained from NCBI Pubchem chemical compound database for the purpose of drug docking. An automated drug docking server was used to analyse the binding interaction between Gedunin and arginine kinase protein. Gedunin directly interacts with the protein at various functional active sites. The results were analysed using molecular dynamic studies. In conclusion, we infer that natural products such as Gedunin can be effectively used to control havoc-causing species such as *Aedes sollicitans*. This has been proved using Insilico studies in our current research work.

Keywords: *Aedes sollicitans*, Gedunin, drug docking

1. Introduction

The Togaviridae family's alphavirus, known as the Eastern Equine Encephalitis Virus (EEEV), is a significant mosquito-borne pathogen that can cause severe encephalitis and case fatality rates of between 30% and 80% in people in North America and up to 95% in horses throughout the Americas. South American (SA) EEEV strains appear to be less virulent and/or contagious for humans in comparison to North American (NA) EEEV, which is associated with substantial morbidity and mortality. There are presently no approved human vaccinations for this virus despite the seriousness of the illness connected with symptomatic NAEDEV infection and its potential to be aerosolized and employed as a biological weapon. Despite the fact that North America has a low rate of natural attacks^[1], a reliable vaccination is required to routinely immunise laboratory staff and first-line epidemic responders in the event of a biologic attack. There are formalin-inactivated vaccines for veterinary use^[2], but they are not very immunogenic, need multiple doses, and may contain virulent, wild-type EEEV.

The imitation blood meals comprising the parent viruses (SINV, NAEDEV, and SADEV) as well as the chimaeras (SIN/NADEV and SIN/SADEV) were given to *Aedes sollicitans* and *Ae. taeniorhynchus*. 7–10 days after their emergence, cohorts of 50–100 female adult mosquitoes were put in 0.9-liter containers and sucrose-starved for a while before being given artificial blood meals. The artificial blood meals contained 10% (v/v) heat-inactivated foetal bovine serum (FBS) (Omega Scientific, Inc., Tarzana, CA), 0.25 M adenosine triphosphate, and 0.03 M sucrose as phagostimulants, together with 35% (v/v) packed defibrinated sheep erythrocytes (Colorado Serum Company, Denver, CO). *Virus suspension in minimal essential medium (MEM)* made up the remaining volume.

The imitated blood meal was placed on the nylon mesh fabric that covered the top of the carton housing the mosquitoes, wrapped in either an artificial membrane or sausage skin, and warmed to 37 °C in a Hemotek feeding equipment (Discovery Workshops, Accrington, United Kingdom). Fully gorged mosquitoes were withdrawn from the carton after an hour and incubated for 10 to 14 days under the same rearing conditions, which is longer than the

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extrinsic incubation period for the majority of alphaviruses, including EEEV that has been observed [3, 4]. To act as negative controls, a sample of each mosquito species was given an uncontaminated blood meal and observed under the identical circumstances.

According to the World Health Organisation (WHO), there are 20,000 different types of medicinal plants in 91 different nations [5]. Different kinds of these plants have been utilised throughout history by numerous cultures for their potential as medicines. Many species of plants in the Meliaceae family, which are employed in conventional medicine and pest management, fit that description. This family has more than 50 genera and around 1400 species, and it is found in tropical and subtropical areas [6].

Limonoids are the most significant of the several phytochemical components of this family that have been identified from diverse regions of plants. A variety of biological actions, including insecticidal, antifungal, antimalarial, antibacterial, antiviral, and anticancer properties, are attributed to limonoids [7]. It is essential to arrest the transfer of virus through mosquitoes. Controlling the spread of the virus through natural means would be of great benefit to human beings.

2. Methodology

Extraction of drug and target protein: The protein sequence of the target, Arginine kinase of *Aedes sollicitans* was retrieved from NCBI GenPept database [8] (<https://www.ncbi.nlm.nih.gov/protein/>) in FASTA format. Gedunin was retrieved from NCBI PubChem Compound database [9] (<https://www.ncbi.nlm.nih.gov/pccompound/>). The conversion of the structure of Gedunin (CID: 12004512) from 2D to 3D was performed using Discovery studio software for the purpose of drug docking studies.

Molecular Drug Docking: The selected mosquito protein sequence and the predicted 3D structure of Gedunin were subject to drug docking studies in order to find out the binding efficiency of Gedunin with arginine kinase of *Aedes sollicitans*. HDock server, a molecular drug docking server [10] (<http://hdock.phys.hust.edu.cn/>) was used for performing docking studies.

3. Results and Discussion

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>AUR38783.1 arginine kinase-like
protein, partial [Ochlerotatus
sollicitans]
PASNFGDVSDFANVDPTGEFVVSTRVRCGRSMQGYPF
NPCLTEAQYKEMEDKVSSTLSGLELEGELKGFYP
LTGMDKAVQQQLIDDHFLFKEGDRFLQAANACRFWPT
GRGIYHNDNKTFVLWCNEEDHLRIISMQMGDL
GQVYKRLVTAVNDIEKRIPFHHDRDLGFLTFCPTNLG
TTIRASVHIKVPKLAQDMAKLESADKYNLQVR
GTRGEHSEAEGGIYDISNKRRLMGLTEYD
```

Fig 1: FASTA sequence of mosquito protein retrieved from NCBI database

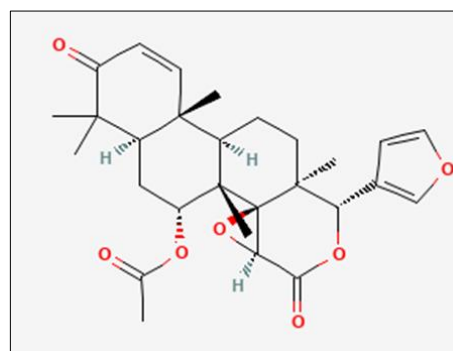


Fig 2: 2D structure of Gedunin with respective atoms retrieved from NCBI Pubchem compound database

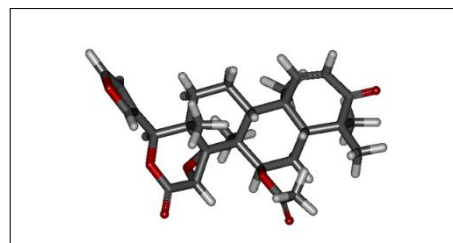


Fig 3: 3D structure of Gedunin with respective atoms in Ball and Stick model viewed using Discovery Studio software.

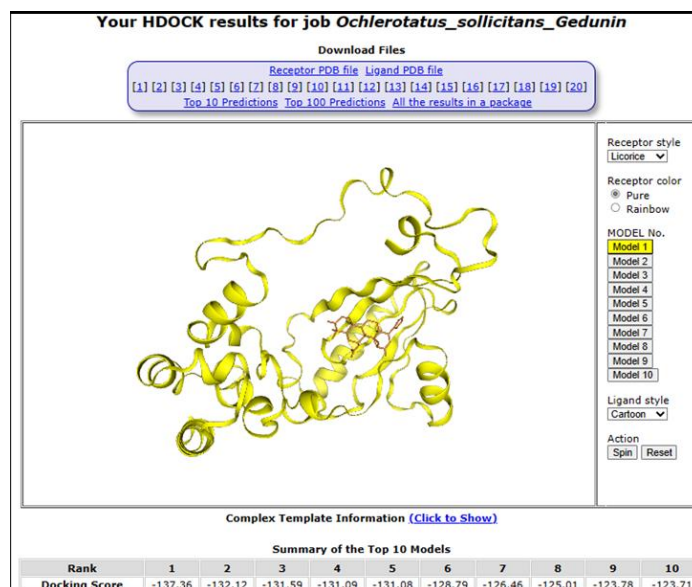


Fig 4: The above picture shows the docking results of Arginine kinase with Gedunin with the respective docking score of -137.36 Kcal/mol viewed using Discovery studio software

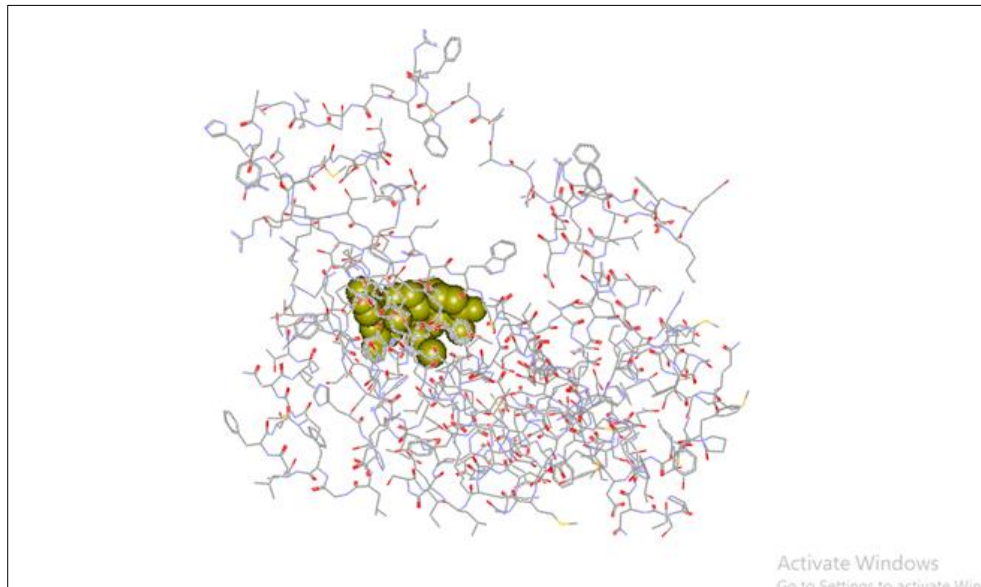


Fig 5: The above picture shows the H-bond interaction between Arginine kinase and Gedunin showing the amino acids present at the H-bond interacting sites viewed using Discovery studio software

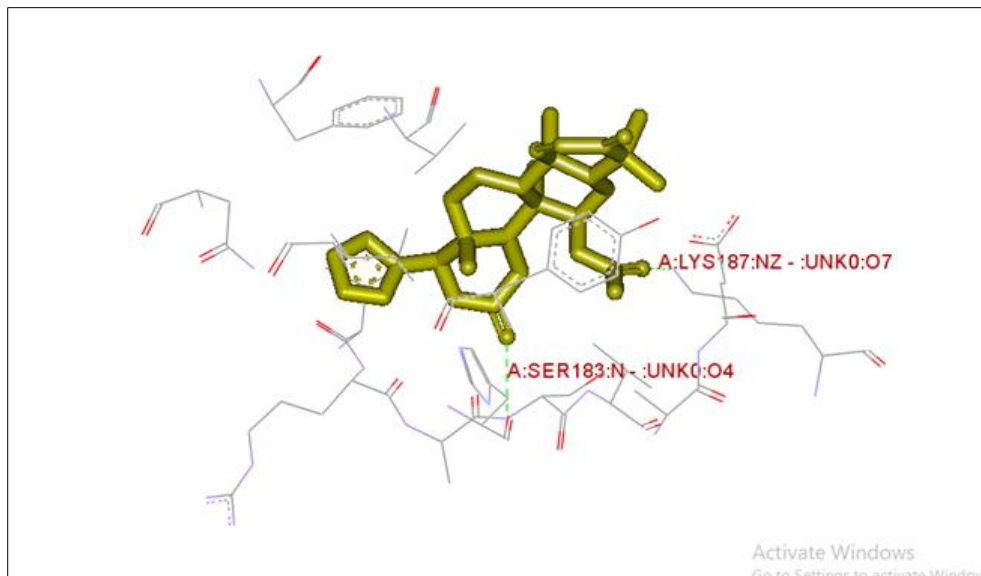


Fig 6: The above picture shows the Electrostatic interaction between Arginine kinase and Gedunin in surface model viewed using Discovery studio software

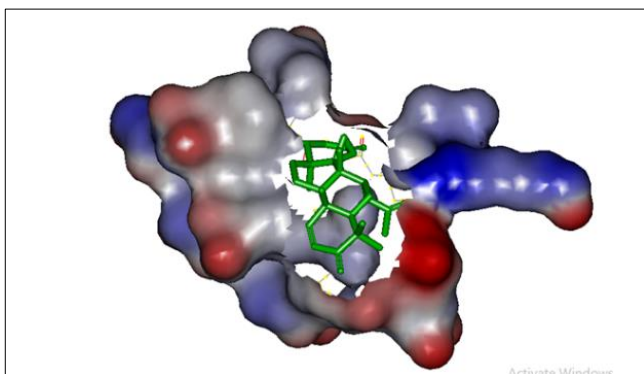


Fig 7: The above picture shows the electrostatic interaction between Arginine kinase and Gedunin in surface model viewed using Discovery studio software

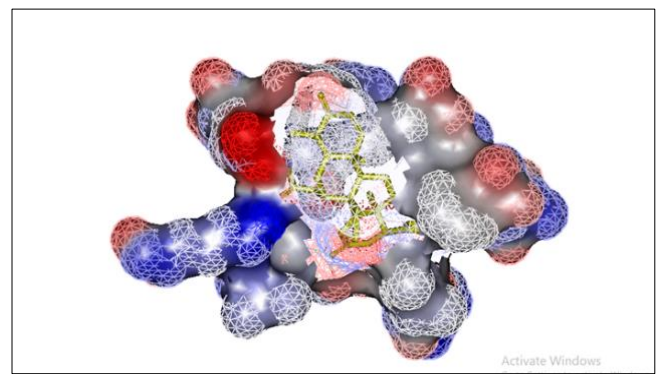


Fig 8: The above picture shows the hydrophobic interaction between Arginine kinase and Gedunin in surface model viewed using Discovery studio software

Table 1: Molecular Drug Interaction summary of drug and receptor with the binding score along with units (*Ochlerotatus sollicitans* - Gedunin)

Receptor	Drug
AUR38783.1 arginine kinase [<i>Ochlerotatus sollicitans</i>]	Gedunin (CID: 12004512) -137.36 kcal/mol

Our research focuses on the mosquito, *Aedes sollicitans* which has Arginine kinase (AK). Its nucleotide length is 714 bp (MG232415.1) and the length of its corresponding amino acid sequence is 238 aa (AUR38783.1) (Fig 1). Arginine kinase (AK) is an important enzyme which takes part in energy metabolism in invertebrates. It has been suggested as a target for controlling agricultural insect pests based on RNA interference (RNAi). One AK gene is present in *Aedes sollicitans*.^[11] Arginine kinase helps in the catalyzation of the reversible transfer of the γ -phosphoryl group (PO-4) of ATP into L-arginine^[12, 13]. AK is primarily identified in the tissues of muscle and heart. It is also found in the digestive glands, intestine, gills^[14] and eggs^[15].

AK is identified in insects like lepidopterans^[16, 17], beetles^[18], cockroaches^[19] hymenopterans^[20] and locusts^[21]. It is also identified in crustaceans^[22], echinoderms and mollusks^[23]. Since the sub-phylum Chelicerate has more than 75,000 species, arginine kinase from spider^[24] horseshoe crab^[25] and scorpion have been investigated.

Arginine kinase provides fast energy at the time of muscular contraction by means of back-phosphorylation of ADP by phospho-arginine^[26]. Besides maintaining energy, Arginine kinase also acts as an allergen. Of late, cross-reactivity has been identified in the AKs present in crustaceans and insects, perhaps owing to structural epitopes^[27].

Aedes sollicitans acts an important vector of dog heartworm, Venezuelan equine encephalitis and Eastern equine encephalitis. In this research study, it has been observed that Gedunin, a pentacyclic triterpenoid found in plants directly inhibits Arginine kinase of *Aedes sollicitans*. Our current research study focused on managing the diseases caused by mosquitoes such as *Aedes sollicitans* using plant derivatives such as Gedunin. This research work clearly demonstrates that Gedunin binds with Arginine kinase at its functional part, thereby, inhibiting it. Hence, Gedunin can be effectively used to control mosquitoes.

In our research work, HDOCK server was used to study the molecular dynamic interactions between Arginine kinase and Gedunin. HDOCK server (<http://hdock.phys.hust.edu.cn/>) is a well-integrated medium for template-based modelling, homology search, macromolecular docking, structure prediction, job management for quick and successful protein-protein docking and biological information incorporation. On providing input information for ligand and receptor molecules, the server accordingly speculates their interaction by means of a hybrid algorithm of docking based and not based on template. The HDOCK server is different from other docking servers as it is capable of supporting the input amino acid sequences and a hybrid docking strategy where experimental information on protein-protein binding site and small-angle X-ray scattering can be incorporated at the time of docking and after docking. Besides, HDOCK can also support protein-RNA/DNA docking with an intrinsic scoring function. The server provides template-based as well as

docking-based binding models of two molecules and permits interactive visualization and download.

Our docking results are clearly explained in Fig 2 which shows the 2D structure of Gedunin with coloured atom labels. The picture is extracted from NCBI PubChem compound database. Fig 3 shows the 3D structure of Gedunin in Ball and Stick model with coloured atoms labels. The 3D structure was viewed using an advanced molecular visualization software, named, Discovery Studio. Internal Electrostatic force exists between Gedunin and the arginine kinase of *Aedes sollicitans*. The Insilico H-Dock results coincide with the experimental data.^[28, 29, 30, 31, 32, 33, 34] The high negative binding value of -137.36 kcal/mol between Gedunin and arginine kinase shows that the binding is efficient. (Table: 1).

Fig.3, 4, 5,6,7,8 show the manner in which binding interaction occurs at the hydrogen bonds between arginine kinase and Gedunin. The results show the 3D interaction between the compounds with amino acid labels viewed using Discovery Studio Software. Fig 8. Distinctly shows the Hydrophobic interaction, between Gedunin and arginine kinase, and how Gedunin inhibits the functional part of arginine kinase. It was identified that the total length of arginine kinase was 714 bp aa. ATP:guanido phosphotransferase, C-terminal catalytic domain (IPR022414) Phosphagen (guanidino) kinases, (PS51510)^[35, 36, 37] are present. Gedunin binds at the functional part of arginine kinase (183 aa and 187 aa) thereby, down-regulating it.

4. Conclusion

Aedes sollicitans is primary vector of dog heartworm, Venezuelan equine encephalitis and Eastern equine encephalitis. In this research work, we clearly explain that arginine kinase of *Aedes sollicitans* effectively binds with Gedunin. Gedunin is a pentacyclic triterpenoid and a neem limonoid. The results revealed that the selected chemical compound, Gedunin can potentially bind the arginine kinase protein of *Aedes sollicitans*, thereby, inhibiting it. The molecular interactions clearly elucidated that arginine kinase is potentially down-regulated by Gedunin. Hence, Gedunin can be effectively used to control the havoc caused by *Aedes sollicitans*.

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