ANTIFUNGAL ACTIVITY OF A SECONDARY METABOLITE OF AZADIRACHTA INDICA AND ITS DERIVATIVES – AN IN SILICO STUDY

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ABSTRACT

Objective: This study was aimed to inhibit the 1, 3 β-glucan synthase with azadirachtin or with the derivatives by docking method.

Methods: The homology model of the protein 1, 3 β-glucan synthase was prepared with “easy modellar” using query sequence and template and it was validated with procheck of Ramachandran plot. The ligand was selected from the PubChem database, and the .sdf file was downloaded which was converted to another file format with open babel. The .pdb files of protein and ligand were uploaded for rough docking with iGEMDOCK, and finally, the accurate docking was made with autodock vina. The docked poses were visualized with PYMOL then saved. The derivatives of the ligand were validated with procheck of Ramachandran plot.

Results: The results obtained from iGEMDOCK and Autodock Vina were tabulated. It was found out that the Azadirachtin and the derivatives are effective in binding 1, 3 β Glucan synthase and thereby inhibiting the formation and integrity of fungal cell wall.

Conclusion: In this study, the secondary metabolite Azadirachtin and the derivatives are showing inhibitory action against the model protein 1, 3 β-glucan synthase and it was suggested that the external application of the ligand and its derivatives can be used because of their poor oral bioavailability.

Keywords: Azadirachtin, 1, 3-β-Glucan synthase, Dermatophytes, Open babel, SWISS ADME, iGEMDOCK, Autodock vina.

INTRODUCTION

Azadirachta indica was commonly known as Neem plant, (synonym melia azadirachta) is an evergreen, fast-growing tree commonly found in arid areas of India, Africa, and America. The neem tree has been described as A. indica as early as 1830 by De Jussieu [1], and it belongs to a Family Meliaceae. Every part of the tree has been used in traditional medicine for various human ailments [2-6]. Myriad of secondary metabolites [7] from different parts of the tree have been found to be effective on a wide spectrum of diseases, including dermatophytosis. In the period of Harappa culture around 4500 years back neem was used in medical treatment [8]. A. indica is a small deciduous with a rounded crown with a height of 5–15 m and a width of 5–7 m [9]. Due to its more efficacy, better tolerability and null adverse effects, Azadirachta, a chemical compound belongs to Limoind group, a tetranorriterpenoid obtained from the neem [10]. Fungal cells are composed of a rigid cell wall, mostly made up of chitin and glucan. 1, 3 β-gelan is a major constituent of the fungal cell wall constitutes of about 30–80%. 1, 3 β-gelan attached to the core polymer by 1, 6 β branches and forms a branched polymer [11-13]. 1, 3 β-glucan helix is a coiled spring-like structure provides a degree of elasticity and tensile strength to the cell wall [13]. It is the building block for fungal cell wall and is synthesized by 1, 3 β-glucan synthase, a well-characterized plasma membrane-associated enzyme with multiple transmembrane domains [11-15]. The enzyme utilizes cytoplasmic UDP-glucose as a substrate and ads up glucose molecules to the growing linear glucan polymer [16]. Whenever required to strengthen the cell wall, the fungi produce 1, 3 β Glucan by the activation of glucan synthase. Caspofungin, Micafungin, and Anidulafungin belong to Echinocandin family used in the treatment of various fungal infections [17]. They act by inhibiting 1, 3 β-glucan synthase resulting in cell swelling and cell death of the fungi. The Echinocandins are currently being used for the treatment of life-threatening infections caused by aspergillosis and candidiasis organisms. The novel method of drug discovery is in silico method which helps to identify drug targets with the help of computer-aided bioinformatics software. The software is helpful in analyzing the protein, the target for drug action with possible predicted active site, generate ligands as lead molecule, check for druglikeness, dock the proteins or target with ligand or molecule, hierarchized them based on binding affinities and generating the structure-activity relative (SAR) molecules with physicochemical, druglikeness, and medicinal properties.

METHODS

Preparation of protein

1, 3 β-glucan synthase plays a vital role in the synthesis of fungal cell wall. The 3D structure of this protein is not available in PubChem database. The homology modeling of this macromolecule was generated.

Homology modeling

Homology modeling was developed with the help of software “easy modellar”. The query sequence and the template were retrieved from National Center for Biotechnology Information (NCBI), the query sequence was aligned with the template sequence and the model was generated. The generated 3D structure of the macromolecule or model protein was validated by Ramachandran Plot.

Preparation of ligand

A. indica is known for many secondary metabolites, and they are used in many clinical conditions. From the literature, azadirachtin was selected as the secondary metabolite of A. indica. The ligand was directly obtained from PubChem database which is a free database available for compounds for virtual screening. From the PubChem database, the structure was downloaded in .sdf file format. Then .sdf file was converted...
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into.pdb file and/or .mol file format with software OPENBABEL. The 105 derivatives (SAR molecules) of the selected secondary metabolite were prepared with the help of SWISS ADME online tool. The same SWISS ADME software was used to generate the physicochemical, pharmacokinetics, medicinal, and druglikeness properties of the secondary metabolite, Azadirachtin and the derivatives. The best suited 10 derivatives were selected from the 105 SAR molecules based on the binding affinity and other chemical properties. Rough docking was performed with iGEMDOCK 2.0 software with a population size of 150 and 70 generations set as default. Lipinski’s rule also called as the rule of five (RO5) is a rule of thumb to evaluate the druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that may likely active per orally in human beings.

Components of the rule
For compounds that have better oral bioavailability, should not violate more than one of the following criteria in Lipinski’s rule [18,19]

- No more than five hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass <500 Daltons
- An octanol-water partition coefficient [20]
- Log $P$ not >5.

Protein-ligand docking
The protein-ligand docking was performed by autodock vina, an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and ligands and were presented hierarchically based on binding affinities.

RESULTS

Protein-ligand preparation
The homology model of target protein, 1, 3 β glucan synthase was docked with the small molecule called azadirachtin and also with the 105 derivatives or SAR compounds. The homology model of macromolecule or protein or drug target was validated with the Ramachandran plot was shown in Fig. 1. The 10 derivatives were selected based on the

![Ramachandran plot](image)

Fig. 1: Ramachandran plot shows the amino acids that are favored, allowed and disallowed in model protein 1, 3 β glucan synthase

| Protein with ligand | Total energy (Kcal/mol) | VDW (Kcal/mol) | H bond (Kcal/mol) | Electrostatic (Kcal/mol) | Aver con pair (Kcal/mol) |
|---------------------|-------------------------|----------------|------------------|-------------------------|-------------------------|
| 1,3 β glucan synthase- azadirachtin | −219.317 | −197.687 | −21.6302 | 0 | 26.5686 |
| 1,3 β glucan synthase- azadirachtin D 03 (SAR1) | −152.989 | −139.841 | −13.1479 | 0 | 17.8235 |
| 1,3 β glucan synthase- azadirachtin D 02 (SAR2) | −29.777 | −25.809 | −13.9683 | 0 | 28.6667 |
| 1,3 β glucan synthase- azadirachtin D 19 (SAR3) | −173.747 | −170.247 | −3.5 | 0 | 23.9216 |
| 1,3 β glucan synthase- azadirachtin D 22 (SAR4) | −241 | −232.437 | −8.56344 | 0 | 29.7451 |
| 1,3 β glucan synthase- azadirachtin D 43 (SAR5) | −186.469 | −184.088 | −5.68824 | 0 | 24.75 |
| 1,3 β glucan synthase- azadirachtin D 56 (SAR6) | −143.1 | −119.845 | −2.38066 | 0 | 24.75 |
| 1,3 β glucan synthase- azadirachtin D 58 (SAR7) | −173.873 | −168.185 | −5.68824 | 0 | 24.6863 |
| 1,3 β glucan synthase- azadirachtin D 65 (SAR8) | −177.247 | −176.63 | −0.61666 | 0 | 25.56 |
| 1,3 β glucan synthase- azadirachtin D 70 (SAR9) | −221.85 | −221.85 | 0 | 0 | 27.6275 |
| 1,3 β glucan synthase-azadirachtin D 81 (SAR10) | −243.297 | −236.12 | −7.17628 | 0 | 29.6923 |

VDW: Van der Waals force, H Bond: Hydrogen bond
binding affinity and the physicochemical, medicinal, and druglikeness properties. The docked poses of the Azadiractin and the derivatives were shown in Fig. 2. The energy values, Van der Waals force, H-bond were derived by rough docking with iGEMDOCK of the Azadiractin

![Docking poses of secondary metabolite of Azadiracta indica, azadiractin and the derivatives](image)

**Table 2:** The results showing the binding affinity of 1,3 β Glucan Synthase with Azadiractin and the derivatives

| Name of the protein and ligand                  | Binding affinity | RMSD | Upper bound | Lower bound |
|-----------------------------------------------|------------------|------|-------------|-------------|
| 1,3 β glucan synthase-azadiractin             | −13.3            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D03 (SAR1) | −13.9            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D02 (SAR2) | −19.0            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D19 (SAR3) | −13.9            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D22 (SAR4) | −18.4            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D43 (SAR5) | −13.2            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D56 (SAR6) | −14.0            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D58 (SAR7) | −14.9            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D65 (SAR8) | −14.9            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D70 (SAR9) | −18.5            |      | 0           | 0           |
| 1,3 β glucan synthase-azadiractin D81 (SAR10)| −19.1            |      | 0           | 0           |

RMSD: Root mean square deviation
Table 3: The general properties of a secondary metabolite of *A. indica*, azadirachtin and the derivatives

| Name the of ligand | Chemical formula | Structure | Isomeric SMILES | IUPAC |
|--------------------|------------------|-----------|-----------------|-------|
| Azadirachtin       | C₃₅H₄₄O₁₆        | ![Structure](image1) | C/C[C][C][=O] 1[C][C@@H][1OC(=O)] 4[C@@O][C][C@@H][[(C@@H)3OC2]]O [C@@@][120][C@@@][2][C][C@@H][2][C@@@H] 10[C][H]1[C@@@][2][O]C=C01)[1OC(=O)O]OC(C(=O)[OC][C] | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[(1S,2S,6S,8S,9R,11S)-2-hydroxy-11-methyl-5,7,10-trioxatetracyclo[6.3.1.0²,₆.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[2E]-2-methylbut-2-enoyl]oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D3 (SAR 1) | C₃₅H₄₅NO₁₅ | ![Structure](image2) | C/C[C][C][=O] 1[C][C@@H][1OC(=O)] 4[C@@O][C][C@@H][[(C@@H)3OC2]]O [C@@@][120][C@@@][2][C][C@@H][2][C@@@H] 10[C][H]1[C@@@][2][O]C=C01)[1OC(=O)O]OC(C(=O)[OC][C] | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[(1S,2S,6S,8S,9R,11S)-2-hydroxy-11-methyl-7,10-dioxa-5-azatetracyclo[6.3.1.0²,₆.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[2E]-2-methylbut-2-enoyl]oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D2 (SAR 2) | C₃₆H₄₆O₁₅ | ![Structure](image3) | C/C[C][C][=O] 1[C][C@@H][1OC(=O)] 4[C@@O][C][C@@H][[(C@@H)3OC2]]O [C@@@][120][C@@@][2][C][C@@H][2][C@@@H] 10[C][H]1[C@@@][2][O]C=C01)[1OC(=O)O]OC(C(=O)[OC][C] | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[(1S,2S,6R,8S,9R,11S)-2-hydroxy-11-methyl-7,10-dioxa-5-azatetracyclo[6.3.1.0²,₆.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[2E]-2-methylbut-2-enoyl]oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D19 (SAR 3) | C₃₅H₄₅NO₁₅ | ![Structure](image4) | C/C[C][C][=O] 1[C][C@@H][1OC(=O)] 4[C@@O][C][C@@H][[(C@@H)3OC2]]O [C@@@][120][C@@@][2][C][C@@H][2][C@@@H] 10[C][H]1[C@@@][2][O]C=C01)[1OC(=O)O]OC(C(=O)[OC][C] | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[(1S,2S,6R,8S,9R,11S)-2-hydroxy-11-methyl-5,10-dioxa-7-azatetracyclo[6.3.1.0²,₆.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[2E]-2-methylbut-2-enoyl]oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Name of ligand | Chemical formula | Structure | Isomer SMILES | IUPAC |
|---------------|------------------|-----------|---------------|-------|
| Azadirachtin D22 (SAR 4) | C_{35}H_{45}O_{15}P | ![Structure](image) | C/C=C/C(=O)OC@H] C(=O)OC@H] [C[@H] C(=O)OC@H] [C[@H] C(=O)OC@H] | 4,11-diethyl (1S,4S,5R,6S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[[1S,2S,5,8S,9S,11S)-2-hydroxy-11-methyl-10-dioxa-7-phosphatetraacyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[[2E]-2-methylbut-2-enoyl] oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D43 (SAR 5) | C_{35}H_{45}NO_{16} | ![Structure](image) | NCC(=O)[C[@H] C(=O)OC@H] C(=O)OC@H] C(=O)OC@H] [C[@H] C(=O)OC@H] | 4,11-diethyl (1S,4S,5R,6S,8R,11S,12R,14S,15R)-12-[[2-aminoacetyl] oxy]-4,7-dihydroxy-6-[[1S,2S,5,8S,9R,11S)-2-hydroxy-11-methyl-5,7,10-trioxtetraacyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[[2E]-2-methylbut-2-enoyl] oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D56 (SAR 6) | C_{35}H_{45}O_{15} | ![Structure](image) | CO[C[@H] C(=O)OC@H] C(=O)OC@H] C(=O)OC@H] | 4,11-diethyl (1S,4S,5R,6S,8R,11S,12R,14S,15R)-12-ethoxy-4,7-dihydroxy-6-[[1S,2S,5,8S,9R,11S)-2-hydroxy-11-methyl-5,7,10-trioxtetraacyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[[2E]-2-methylbut-2-enoyl] oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D58 (SAR 7) | C_{35}H_{45}NO_{15} | ![Structure](image) | C/C=C/C(=O)OC@H] C(=O)OC@H] [C[@H] C(=O)OC@H] | 4,11-diethyl (1S,4S,5R,6S,8R,11S,12R,14S,15R)-12-[ethanimidoxyloxy]-4,7-dihydroxy-6-[[1S,2S,5,8S,9R,11S)-2-hydroxy-11-methyl-5,7,10-trioxtetraacyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[[[2E]-2-methylbut-2-enoyl] oxy]-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Name the of ligand | Chemical formula | Structure | Smiles | IUPAC |
|--------------------|------------------|-----------|--------|-------|
| Azadirachtin D65 (SAR 8) | C_{35}H_{45}O_{15} | ![Structure](image1) | COC(=O)C@@I(O)OC[C@]23[C@@H]3[C@H]1[C@H]3[C@@H]120[C@]2[C@H]2 | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[[15,25,6S,8S,9R,11S]-2-hydroxy-11-methyl-5,7,10-trioxatetracyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[(2E)-2-methylbut-2-en-1-yl]oxy)-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D70 (SAR 9) | C_{35}H_{45}O_{15}P | ![Structure](image2) | COC(=O)C@@I(O)OC[C@]23[C@@H]3[C@H]1[C@H]3[C@@H]120[C@]2[C@H]2 | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-4,7-dihydroxy-6-[[15,25,6S,8S,9R,11S]-2-hydroxy-11-methyl-5,7,10-trioxatetracyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-14-[(2E)-2-methyl-1-phosphanylidenebut-2-en-1-yl]oxy)-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |
| Azadirachtin D81 (SAR 10) | C_{35}H_{43}BrO_{16} | ![Structure](image3) | BrC=C(C)=C(OC(=O)C@)1C[=O]1C@H]3[C@H]1[C@H]3[C@@H]120[C@]2[C@H]2 | 4,11-dimethyl (1S,4S,5R,6S,7S,8R,11S,12R,14S,15R)-12-(acetyloxy)-14-[[2Z]-2-(bromomethyl)but-2-enoyl]oxy)-4,7-dihydroxy-6-[[15,25,6S,8S,9R,11S]-2-hydroxy-11-methyl-5,7,10-trioxatetracyclo[6.3.1.0²,⁶.0⁹,¹¹]dodec-3-en-9-yl]-6-methyl-3,9-dioxatetracyclo[6.6.1.0¹,⁵.0¹¹,¹⁵]pentadecane-4,11-dicarboxylate |

**Table 3: (Continued)**

SMILES: Simplified molecular input line entry specification, IUPAC: International union of pure and applied chemistry. A. indica: Azadirachta indica
Table 4: The physicochemical properties of a secondary metabolite of *A. indica*, azadirachtin and the derivatives

| Name of the ligand | Molecular weight (g/mol) | Number heavy atoms | Number arom. heavy atoms | Fraction CSP3 | Number rotatable bonds | Number H-bond acceptors | Number H-bond donors | Molar refractivity | TPSA (Å²) |
|--------------------|--------------------------|-------------------|-------------------------|---------------|-----------------------|------------------------|-----------------|------------------|----------|
| Azadirachtin       | 720.71                   | 51                | 0                       | 0.77          | 10                    | 16                     | 3               | 165.92           | 215.34   |
| Azadirachtin D3 (SAR 1) | 719.73                 | 51                | 0                       | 0.77          | 10                    | 15                     | 4               | 171.55           | 218.14   |
| Azadirachtin D2 (SAR 2) | 718.74                 | 51                | 0                       | 0.78          | 10                    | 15                     | 3               | 169.64           | 206.11   |
| Azadirachtin D19 (SAR 3) | 719.73                 | 51                | 0                       | 0.77          | 10                    | 15                     | 4               | 171.55           | 218.14   |
| Azadirachtin D22 (SAR 4) | 736.7                  | 51                | 0                       | 0.77          | 10                    | 11                     | 3               | 173.15           | 219.7    |
| Azadirachtin D43 (SAR 5) | 735.73                 | 52                | 0                       | 0.77          | 11                    | 17                     | 4               | 168.63           | 241.36   |
| Azadirachtin D56 (SAR 6) | 705.72                 | 50                | 0                       | 0.77          | 10                    | 15                     | 3               | 166.98           | 198.27   |
| Azadirachtin D58 (SAR 7) | 719.73                 | 51                | 0                       | 0.77          | 10                    | 16                     | 4               | 169.31           | 222.12   |
| Azadirachtin D65 (SAR 8) | 705.72                 | 50                | 0                       | 0.77          | 10                    | 15                     | 3               | 166.98           | 198.27   |
| Azadirachtin D70 (SAR 9) | 736.7                  | 51                | 0                       | 0.77          | 10                    | 15                     | 3               | 174.24           | 232.41   |
| Azadirachtin D81 (SAR 10) | 799.61                 | 52                | 0                       | 0.77          | 11                    | 16                     | 3               | 173.79           | 215.34   |

TPSA: Topological polar surface area, H-bond: Hydrogen bond, *A. indica*: Azadirachta indica

Table 5: The lipophilicity of a secondary metabolite of *A. indica*, Azadirachtin and the derivatives

| Name of the ligand | Log $P_{o/w}$ (LOGP) | Log $P_{o/w}$ (XLOGP) | Log $P_{o/w}$ (WLOGP) | Log $P_{o/w}$ (MLOGP) | Log $P_{o/w}$ (SILICOS-IT) | Consensus log $P_{o/w}$ |
|--------------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------------|--------------------------|
| Azadirachtin       | 2.90                 | 1.09                  | -0.20                 | -0.47                 | 1.07                        | 0.88                     |
| Azadirachtin D3 (SAR 1) | 3.51                  | 1.1                   | -1.01                 | -0.47                 | 0.76                        | 0.78                     |
| Azadirachtin D2 (SAR 2) | 4.44                  | 1.57                  | 0.26                  | 0.04                  | 1.72                        | 1.61                     |
| Azadirachtin D19 (SAR 3) | 3.24                  | 0.81                  | -1.01                 | -0.47                 | 0.76                        | 0.66                     |
| Azadirachtin D22 (SAR 4) | 3.67                  | 0.93                  | 0.46                  | -0.14                 | 1.1                         | 1.2                      |
| Azadirachtin D43 (SAR 5) | 4.31                  | 0.18                  | -1.26                 | -1.19                 | 0.24                        | 0.45                     |
| Azadirachtin D56 (SAR 6) | 3.76                  | 1.42                  | 0.06                  | -0.55                 | 1.51                        | 1.24                     |
| Azadirachtin D58 (SAR 7) | 3.99                  | 1.18                  | 0.25                  | -0.47                 | 1.2                         | 1.23                     |
| Azadirachtin D65 (SAR 8) | 3.53                  | 1.36                  | 0.06                  | -0.55                 | 1.51                        | 1.18                     |
| Azadirachtin D70 (SAR 9) | 3.53                  | 0.34                  | 0.54                  | -0.55                 | 0.93                        | 0.96                     |
| Azadirachtin D81 (SAR 10) | 4.55                  | 1.28                  | 0.17                  | -0.2                  | 1.64                        | 1.49                     |

o/w: Octanol/water, *A. indica*: Azadirachta indica

Table 6: The hydrophilicity of a secondary metabolite of *A. indica*, Azadirachtin and the derivatives

| Name of the ligand | Solubility | Class | Log S (ESOL) | Class | Log S (Ali) | Solubility | Class | Log S (SILICOS-IT) | Solubility | Class |
|--------------------|------------|-------|--------------|-------|-------------|------------|-------|-------------------|------------|-------|
| Azadirachtin       | 3.33E–02 mg/ml; | Moderately | -5.20 | 4.50E–03 mg/ml; | Moderately | -1.40 | 2.86E+01 mg/ml; | Soluble | 3.97E–02 mol/l |
| Azadirachtin D3 (SAR 1) | 4.62E–05 mol/l | Soluble | 6.25E–06 mol/l | Soluble | 3.83E–05 mg/ml; | Moderately | -1.71 | 1.39E+01 mg/ml; | Soluble | 1.94E+02 mol/l |
| Azadirachtin D2 (SAR 2) | 4.62E–05 mg/ml; | Moderately | -5.51 | 2.23E–03 mg/ml; | Moderately | -1.94 | 8.21E+00 mg/ml; | Soluble | 1.14E+02 mol/l |
| Azadirachtin D19 (SAR 3) | 2.37E–05 mol/l | Soluble | 3.10E–06 mol/l | Soluble | 7.66E–03 mg/ml; | Moderately | -1.71 | 1.39E+01 mg/ml; | Soluble | 1.94E+02 mol/l |
| Azadirachtin D22 (SAR 4) | 5.06E–02 mg/ml; | Moderately | -4.97 | 7.66E–03 mg/ml; | Moderately | -1.71 | 1.39E+01 mg/ml; | Soluble | 1.94E+02 mol/l |
| Azadirachtin D43 (SAR 5) | 1.29E–01 mg/ml; | Soluble | 1.15E–02 mg/ml; | Soluble | 1.56E–05 mol/l | Moderately | -1.04 | 9.20E–02 mol/l | Soluble | 9.35E–02 mol/l |
| Azadirachtin D56 (SAR 6) | 1.63E–04 mol/l | Soluble | 1.56E–05 mol/l | Soluble | 4.57E–03 mg/ml; | Moderately | -1.88 | 9.35E+00 mg/ml; | Soluble | 1.32E–00 mol/l |
| Azadirachtin D58 (SAR 7) | 2.96E–02 mg/ml; | Moderately | -5.13 | 2.61E–03 mg/ml; | Moderately | -1.55 | 2.04E+01 mg/ml; | Soluble | 2.84E–02 mol/l |
| Azadirachtin D65 (SAR 8) | 4.11E–05 mol/l | Soluble | 3.63E–06 mol/l | Soluble | 2.61E–03 mg/ml; | Moderately | -1.55 | 2.04E+01 mg/ml; | Soluble | 2.84E–02 mol/l |
| Azadirachtin D70 (SAR 9) | 2.37E–02 mg/ml; | Moderately | -4.81 | 1.15E–02 mg/ml; | Soluble | 1.15E–02 mg/ml; | Moderately | -1.88 | 9.35E+00 mg/ml; | Soluble | 1.32E–00 mol/l |
| Azadirachtin D81 (SAR 10) | 1.09E–04 mol/l | Soluble | 1.64E–05 mol/l | Soluble | 7.14E–03 mg/ml; | Moderately | -2.15 | 5.71E+00 mg/ml; | Soluble | 2.64E–02 mol/l |

A. indica: Azadirachta indica
Table 7: The pharmacokinetics properties of a secondary metabolite of *A. indica*, azadirachtin and the derivatives

| Name of ligand | Gl absorption | BBB permeability | P-gp substrate | CYP 1A2 inhibitor | CYP 2C19 inhibitor | CYP 2C9 inhibitor | CYP 2D6 inhibitor | CYP 3A4 inhibitor | Log Kp (skin permeation) cm/s |
|----------------|----------------|------------------|----------------|------------------|-------------------|-------------------|-------------------|-------------------|-----------------------------|
| Azadirachtin | Low | No | Yes | No | No | No | No | No | -9.92 |
| Azadirachtin D3 (SAR 1) | Low | No | Yes | No | No | No | No | No | -9.91 |
| Azadirachtin D2 (SAR 2) | Low | No | Yes | No | No | No | No | No | -9.57 |
| Azadirachtin D9 (SAR 3) | Low | No | Yes | No | No | No | No | No | -10.12 |
| Azadirachtin D22 (SAR 4) | Low | No | Yes | No | No | No | No | No | -10.13 |
| Azadirachtin D43 (SAR 5) | Low | No | Yes | No | No | No | No | No | -10.66 |
| Azadirachtin D56 (SAR 6) | Low | No | Yes | No | No | No | No | No | -9.6 |
| Azadirachtin D58 (SAR 7) | Low | No | Yes | No | No | No | No | No | -9.85 |
| Azadirachtin D65 (SAR 8) | Low | No | Yes | No | No | No | No | No | -9.64 |
| Azadirachtin D70 (SAR 9) | Low | No | Yes | No | No | No | No | No | -10.55 |
| Azadirachtin D81 (SAR 10) | Low | No | Yes | No | No | No | No | No | -10.27 |

Gl absorption: Gastrointestinal absorption, BBB: Blood brain barrier, CYP: Cytochrome P. *A. indica*: Azadirachta indica

Table 8: The druglikeness of a secondary metabolite of *A. indica*, azadirachtin and the derivatives

| Name of the ligand | Lipinski | Ghose | Veber | Egan | Muegge | Bioavailability score |
|-------------------|----------|-------|-------|------|--------|-----------------------|
| Azadirachtin | 2 violations | 3 violations | 1 violation | 1 violation | 4 violations | 0.17 |
| Azadirachtin D3 (SAR 1) | 2 violations | 4 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D2 (SAR 2) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D19 (SAR 3) | 2 violations | 4 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D22 (SAR 4) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D43 (SAR 5) | 2 violations | 4 violations | 2 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D56 (SAR 6) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D58 (SAR 7) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D65 (SAR 8) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D70 (SAR 9) | 2 violations | 3 violations | 1 violations | 1 violations | 4 violations | 0.17 |
| Azadirachtin D81 (SAR 10) | 2 violations | 3 violations | 2 violations | 1 violations | 4 violations | 0.17 |

Table 9: The toxicity of a secondary metabolite of *A. indica*, azadirachtin and the derivatives

| Name of the ligand | hERG inhibition | AMES toxicity | Carcinogens | Acute oral toxicity | Rat acute toxicity (LD 50 mg/) |
|-------------------|------------------|---------------|-------------|---------------------|-------------------------------|
| Azadirachtin | 0.9919 | 0.7563 | 0.9455 | 0.6952 | 4.3477 |
| Azadirachtin D3 (SAR 1) | 0.9969 | 0.5609 | 0.9550 | 0.4926 | 3.0765 |
| Azadirachtin D2 (SAR 2) | 0.9919 | 0.7563 | 0.9455 | 0.6952 | 4.3477 |
| Azadirachtin D19 (SAR 3) | 0.9972 | 0.5171 | 0.9455 | 0.4294 | 3.1422 |
| Azadirachtin D22 (SAR 4) | 0.9917 | 0.7483 | 0.9503 | 0.5852 | 3.8622 |
| Azadirachtin D43 (SAR 5) | 0.9805 | 0.6369 | 0.9330 | 0.3630 | 3.6150 |
| Azadirachtin D56 (SAR 6) | 0.9887 | 0.6849 | 0.9393 | 0.6161 | 4.2870 |
| Azadirachtin D58 (SAR 7) | 0.9988 | 0.6087 | 0.9133 | 0.3976 | 3.3092 |
| Azadirachtin D65 (SAR 8) | 0.9880 | 0.7287 | 0.9470 | 0.7958 | 4.7577 |
| Azadirachtin D70 (SAR 9) | 0.9908 | 0.7306 | 0.9449 | 0.6260 | 4.1677 |
| Azadirachtin D81 (SAR 10) | 0.9097 | 0.6846 | 0.9346 | 0.6098 | 4.2446 |

hERG: Human ether-a-go-go-related gene, *A. indica*: Azadirachta indica

(TPSA) were presented in Table 4. The lipophilicity and hydrophilicity of azadirachtin and the SAR compounds were shown in Tables 5 and 6, respectively. The pharmacokinetic properties of azadirachtin and the SAR compounds were presented in Table 7. The druglikeness of the azadirachtin and the SAR compounds were shown in Table 8.

**Ramachandran plot**

The Ramachandran plot is the way to visualize the dihedral angles $\psi$ (phi) and $\varphi$ (psi) of a protein backbone [21]. Due to steric hindrances that occur between adjacent atoms within a protein structure, the $\psi$ (phi) and $\varphi$ (psi) values are usually constrained within specific areas of the plot, particularly for ordered structures such as helices and sheets. The dihedral angles or torsion angles for loop regions in a given protein do not often occupy particular regions in the plot unlike secondary structure elements such as $\alpha$-helices or $\beta$-sheets, but they may occupy any regions that are sterically permitted. The 1, 3 β glucan synthase protein structure was validated using procheck and from the Ramachandran plot, it was inferred that the modeled protein contains 87.5% of amino acid residues in the favored region, 6.9% in allowed region, and 5.6% in amino acid residues in disallowed region.

In Table 1, the secondary metabolite, azadirachtin shows energy values as $-219.317$ and $-197.687$ between protein and ligand. The SAR 10 was showing more than the secondary metabolite as energy values $-243.297$ and $-236.12$. In Table 2 summarizes that the binding affinity between protein and ligand for azadirachtin was $-13.3$ and the SAR 10 molecule was $-19.1$. The more energy value, Van der Waals force, and binding affinity between protein and ligand show more likely to be a new drug entity.

In Table 3 summarizes the general properties such as molecular formula, chemical structure, simplified molecular input line entry specification (SMILES), and IUPAC name of a secondary metabolite of *A. indica*, azadirachtin and the derivatives.

In Table 4 showing molecular weight, number of atoms, fraction CSP3, number of rotatable bonds, molar refractivity, and TPSA, where it shows that the molecular weight is more than 500, number of atoms are in the permissible range of 20-70, molar refractivity is more than 130, polar...
surface area is also more than 140 angstroms squared in Azadirachtin and also the derivatives implies that it is a poor oral bioavailability.

Table 5 is showing the log p octanol-water partition coefficient values of the azadirachtin, and the derivatives are in the range of permissible –0.4–5.6 range that implies a good lipophilic compounds. The consensus log p_{ow} means an average of all five predictions is also in the permissible range.

The Table 6 is showing the hydrophilicity property of the azadirachtin and the derivatives which are mostly moderately soluble.

Table 7 is showing the pharmacokinetic property of azadirachtin, and the derivatives implies the oral bioavailability is poor, and drug penetration to skin is high.

From the Table 8 summarizes that the azadirachtin and the derivatives do not obey the Lipinski’s rule of 5 and other filters for a new drug molecule, and the bioavailability score is also very low. This implies that the oral bioavailability of these compounds is poor.

Table 9 summarizes the toxicity of azadirachtin and the derivatives, which these compounds are non-toxic in hERG, AMES, carcinogenicity, acute oral toxicity, and LD50 in rats.

DISCUSSION

Fungi are ubiquitous constitute a very diverse group of organisms. They evolved and adapted to live in a wide variety of environmental and ecological niches. Most of the fungal infections in human beings are superficial and relatively innocuous, but some can cause devastating diseases such as invasive aspergillosis and systemic candidiasis. The currently available drugs for fungal diseases are amphotericin B, nystatin, griseofulvin, fluconazole, clotrimazole, miconazole, ketoconazole, fluconazole, terbinafine, tolnaftate, salicylic acid, and benzoic acid [23]. Medicinal plants are very widely used in modern days as these are safe and devoid of untoward events. A. indica is one of the plants having a myriad of medicinal properties. The whole plant is used against human ailments, especially for infections caused by bacteria, fungi, and viral organisms. New drug development is a tedious process which takes 15–20 years to develop a successful new drug entity. In silico method of drug discovery is helping us to discover newer ligands or molecules or drug substances which can reduce the pre-clinical study period. 1, 3 β glucan synthase, a model protein selected from literature as a drug target whose 3D structure was not available in NCBI. The homology was generated with easy modellar and it was validated with Procheck of Ramachandran plot. Azadirachtin, the secondary metabolite was selected as ligand. The protein and ligand were docked with iGEMDOCK and Autodock Vina [24], the results were retrieved on the basis of energy values, and also the derivatives implies that it is a poor oral bioavailability.

CONCLUSION

In this study, the secondary metabolite azadirachtin and the derivatives are showing inhibitory action against the model protein 1, 3 β glucan synthase. It was suggested that the protein-ligand interaction for a new drug entity between the 1, 3 β glucan synthase and azadirachtin or with SARs were more reliable as external application being they are having poor oral bioavailability.

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CONFLICT OF INTEREST

Declared none

AUTHORS CONTRIBUTION

Simhadri.V.S.D.N.A.Nagesh – Principle Investigator
DR. M. Munippamm– Guide
DR. I. Kannan – Co-Guide
DR. S. Viswanathan – Provided Required Software for Study

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