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Computational Analysis and Molecular Modelling of Tryptophan Synthatase as Novel Antifungal Drug Target Against Bioactive Compounds of *Punica Granatum*.

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KEYWORDS

Computational Analysis, Molecular Modelling, Tryptophan Synthatase, Bioactive Compounds

ABSTRACT:

Fungal infections are a major worldwide health concern due to the rise in immunocompromised people with weakened immune systems. Invasive aspergillosis, an infection caused by fungi of the Aspergillus species, is the leading cause of invasive aspergillosis. Even with the development of new, potent antifungal medications, invasive aspergillosis still has a death rate above 40%. To address this, researchers have turned to herbal substances, mostly phytochemicals, in an effort to produce safe and commercially feasible antifungals. Pomegranates have long been recognized for their therapeutic benefits, and their chemical components have raised research interest in recent years. The goal is to find a phytochemical present in pomegranate which acts as inhibitor of novel drug target tryptophan synthase of Aspergillus fumigatus.

INTRODUCTION

Every year, fungal infections cause over a million fatalities, making them a serious worldwide health concern. The primary cause of this is the rise in immunocompromised people with weakened immune systems, such as those with primary immunological deficiencies, cancer chemotherapy, HIV/AIDS, hematologic and solid organ transplants. Invasive aspergillosis is an infection caused by fungi of the Aspergillus species, is a major threat which occurs mainly in immunosuppressed patients[1].Among the airborne saprophytic fungus, *Aspergillus fumigatus* is currently the most important airborne fungal pathogen and the leading cause of invasive aspergillosis (~85% of cases). Many of this fungus's conidia are continuously inhaled by humans and animals. Even with the development of new, potent antifungal medications, invasive aspergillosis still has a death rate above 40%.Moreover, the development of drug resistance complicates the course of treatment further. New antifungal medications are therefore desperately needed [2,3].Antifungal drugs that are now available for treating Aspergillosis work on these three molecular targets: β-1,3-glucan synthase for Echinocandin, ergosterol for Polyene, and 14 alpha demethylase for Azoles. These antifungal medications exhibit high levels of toxicity and resistance. Therefore, it is crucial to develop novel medications that have lower toxicity and address the issue of drug resistance [4].The rise of antifungal resistance has compelled researchers to turn to herbal substances, mostly phytochemicals, in an effort to produce safe and commercially feasible antifungals. This is because, despite the introduction of new and novel antifungal medications, their production and impact are gradual [5].

Nine amino acids Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine are classified as essential amino acids because they cannot be synthesized by human **[6].** Fungal enzymes that are involved in the manufacture of critical amino acids for humans could be a valuable source of new molecular targets for antifungal chemotherapy. However, since human blood contains an oligopeptide pool and amino acids that may be obtained externally, it may be debatable whether inhibiting the manufacture of human essential amino acids would be an effective antifungal treatment. Since human blood levels of these amino acids are

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particularly low to prevent the rescue of amino acid concentrations induced by blockage of its synthesis routes in human pathogen cells, the tryptophan and methionine pathways appear particularly promising from this perspective **[7].** Srivastava et al. in his article lists the potential therapeutic targets present in the several metabolic pathways found in the Aspergillus genome and goes into comprehensive detail on how they function biologically. They came up with a list of 33 unique therapeutic targets found in the Aspergillus genome, which could help in the antifungal drug discovery. Since human tryptophan biosynthesis pathway genes are lacking, tryptophan synthase represents a great target for the development of novel antifungal medicines **[4].**

Pomegranates (Punica granatum) have garnered significant attention for their pharmacological activities due to their rich composition of bioactive compounds such as anthocyanins, rutin, polyphenols, punicalagin, pedunculagin, ellagic acid, and quercetin. These compounds contribute to numerous health benefits across various bodily systems. For instance, pomegranates have been shown to support cardiovascular health by reducing blood pressure, improving lipid profiles, and reducing arterial plaque [8].They also offer neuroprotective effects, potentially safeguarding against neurodegenerative diseases such as Alzheimer's [9]. Moreover, pomegranates possess anti-ulcerogenic properties, aiding in the prevention and healing of ulcers [10]. They also exhibit hepatoprotective effects, promoting liver health and protecting against liver damage[11]. The antibacterial and antifungal activities of pomegranates help in fighting off various infections [12].In addition to these benefits, pomegranate compounds have shown anti-angiogenic properties, which can inhibit the formation of new blood vessels, an important factor in cancer treatment [13]. They also demonstrate anti-tumor activity by preventing the growth and spread of tumors [14] and cytoprotection by protecting cells from damage [15].Pomegranates also play a role in diabetes prevention by regulating blood sugar levels and improving insulin sensitivity [16]. They can protect the skin from UV-induced damage [17] and may enhance male fertility by improving sperm quality [18].

In this paper, my aim is to screen out best fit phytochemical of pomegrante which act as antifungal medication against novel drug target tryptophan synthase of *Aspergillus fumigatus*.

MATERIALS AND METHOD

Homology modeling

Homology modeling, also known as comparative modeling, is a method used to predict the three-dimensional structure of a protein based on its amino acid sequence and the known structures of homologous proteins (templates). This technique leverages the evolutionary conservation of protein structures to generate accurate models for proteins whose structures have not been experimentally determined.

Homology modeling involves several key steps. First, template identification is performed by using protein BLAST to find homologous proteins with known structures, selecting templates with high sequence similarity and structural coverage. Next, sequence alignment aligns the target protein sequence with the chosen templates using multiple sequence alignment tools. Model building then constructs the core structure based on conserved regions, and models variable regions (loops) and side chains of the target protein. Model optimization refines the initial model using energy minimization and molecular dynamics simulations to resolve steric clashes and improve geometry. Finally, model validation assesses the quality of the generated model which is done by [19,20]

Modeller software was used for model generation of tryptophan synthase. The accuracy of the protein model was determined by the PROCHECK tool (https://saves.mbi.ucla.edu/).

Ligand selection

In the present study, different phytochemicals of punica granatum was selected. These selected phytochemicals are Catechin, Cyanidin3,5-diglucoside, Ellagic acid, Punicic acid, Ellagitannin, Gallotannin, Kaempferol, Luteolin, Punicalagin, Quercitin, Rutin, Cyanidin-3-diglucoside, Gallic acid, Genistein. The three-dimensional structures of phytochemicals in simple data format (SDF) were obtained from the PubChem server

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(https://pubchem.ncbi. nlm.nih.gov/compound). Open babel is used to convert the different phytochemicals compound in different format like pdb format [21].

Lipinski filter

The Lipinski Rule of Five is a set of guidelines used to evaluate the drug-likeness of a compound, predicting its potential as an orally active drug in humans. Nevertheless, the rule provides a useful framework for medicinal chemists to assess the pharmacokinetic profile of new chemical entities during drug development[22]. This Lipinski filter is available at http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp is used to screen out the 10 phytochemical from 15 phytochemicals. These ten phytochemicals are Catechin, Ellagic acid, Punicic acid, Kaempferol, Luteolin, Quercitin, Cyanidin-3-diglucoside, Gallic acid, Linoleic acid, Genestein and Epigallocatechin Gallate..

Molecular docking

Molecular docking is a computational tool used to examine how ligands (compounds) and protein targets interact. The binding affinity of the ligand to the protein target is thought to be a determinant of the docking score or binding energy obtained from the docking[23]. Molecular docking of different phytochemicals of punica granatum is performed against novel drug target Tryptophan synthase with the help of software AutoDockTool Version 1,5.7.

ADME prediction

ADME prediction of all filtered phytochemicals is done by using different online adme prediction tool like SWISS ADME Tool (http://www.swissadme.ch/index.php), pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) and Pro Tox (https://tox.charite.de/protox3/index.php?site=home) .

RESULT AND DISCUSSION

Molecular modeling

These are the templates obtained from protein blast against pdb database with its query cover and percentage indentity [Table-1].These templates are used in modeller software to get five models with dope score. The lower the DOPE score, the better the model is. Model 1 is selected as a best model of tryptophan synthase[Table-2].Model 1 is then validated by using Ramachandran plot[24]. The ramachandran plot obtained show 92% residue in most favoured region [Figure-].

Table-1: Templates used in homology modeling with its query cover and identity taken from RCSB PDB

S.no	Templates	Query cover(%)	Percentage Identity(%)
1	5OCW	78	43.43
2	5KIN	79	43.35
3	4NEG	79	43.09
4	8EGY	79	41.91

Table-2: DOPE score obtained using MODELLER software

Model	Dope Score (Discrete Optimized Protein Energy)
Model 1	-45560.382813
Model 2	-45414.890625
Model 3	-45133.089844
Model 4	-44723.488281
Model 5	-45031.039063

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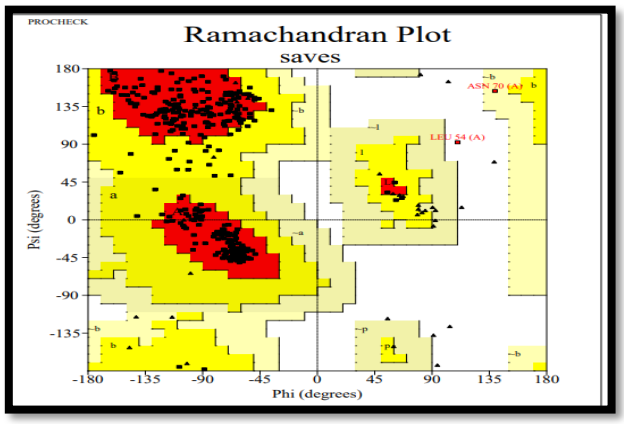

Figure 1-Ramachandran plot of model 1

Lipinski filter

Lipinski filter is used to filter out the phytochemicals which do not follow the rules [Table-3].

Table-3: Physicochemical properties of different phytochemicals of Punica granatum

	Phytochemicals	**Lipinski filter**				
S.no		**Molecular mass**	**logP**	**Hydrogen bond donor**	**Hydrogen bond acceptor**	**Molecular refractivity**
1	Catechin	290	1.09	5	6	68
2	Cyanidin 3,5-diglucoside	709	2.30	9	19	150
3	Ellagic acid	302	1.24	4	8	68.45
4	Punicic acid	278	4.47	1	2	89.54
5	Ellagitannin	992	0.62	9	27	194.81
6	Gallotannin	1700	2.82	23	46	338
7	Kaempferol	286	0.64	4	6	62.82
8	Luteolin	286	0.83	4	6	63.43
9	Punicalagin	1084	-1.54	10	30	206.91
10	Quercitin	302	0.52	5	7	64.36
11	Rutin	610	1.83	10	16	132.33
12	Cyanidin-3-diglucoside	484.50	1.15	11	8	101.84
13	Gallic acid	170	0.50	4	5	38.39
14	Genistein	270	0.94	3	5	61.94
15	Epigallocatechin Gallate	458	2.23	8	11	108.92

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Molecular docking

The filtered phytochemicals are then docked with protein tryptophan synthase and binding energy is thus obtained. [**Table-4**]

Table 4- Essential phytochemical with its binding energy

S.no	Phytochemicals	Binding energy
1	Kaempferol	-5.61
2	Ellagic acid	-5.16
3	Luteolin	-4.07
4	Gallic acid	-4.01
5	Genestein	-2.79
6	Quercitin	-1.07
7	Punicic acid	3.28
8	Linoleic acid	7.18
9	Catechin	16.97
10	Cyanidin-3-diglucoside	28.31

ADME PREDICTION

Table 5-ADME prediction of selected phytochemicals.

S. no	Phytochemicals	BBB Permeability (logBB)	CNS permeability (logPS)	Total clearance (log(ml/min/kg))	Human intestinal absorption (%)	Skin permeability (logKp)	Bioavailability score	Synthetic Accessibility score	LD50 (mg/kg)
1	Catechin	-1.054	-3.298	0.183	68.829	-2.735	0.55	3.50	1000
2	Ellagic acid	-1.272	-3.533	0.537	86.684	-2.735	0.55	3.17	2991
3	Punicic acid	-0.115	-1.547	1.979	92.836	-2.722	0.55	3.02	3200
4	Kaempferol	-0.939	-2.228	0.477	74.29	-2.735	0.55	3.14	3919
5	Luteolin	-0.907	-2.251	0.495	81.13	-2.735	0.55	3.02	3919
6	Quercitin	-1.098	-3.065	0.407	77.207	-2.735	0.55	3.23	159
7	Cyanidin-3-diglucoside	-1.374	-4.213	0.548	29.927	-2.735	0.17	5.30	1190
8	Gallic acid	-1.102	-3.74	0.518	43.374	-2.735	0.56	1.22	2000
9	Linoleic acid	-0.142	-1.6	1.936	92.329	-2.723	0.55	2.87	1190
10	Genestein	-0.71	-2.048	0.151	93.387	-2.735	0.55	2.87	2500

The main location of absorption in humans is the intestinal tract. Table 5 shows the percentage of phytochemicals absorbed in the gut. A chemical is deemed poorly absorbed if its absorbance is less than thirty percent. Skin permeability has a significant role in the effectiveness of many consumer goods and is relevant to the advancement of transdermal medication delivery. The skin permeability constant, logKp (cm/h), is used to forecast the likelihood that a given substance would be skin permeable. If a compound has a logKp >

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-2.5, it is regarded as having a relatively low skin permeability. In order to lessen toxicities and side effects or increase the effectiveness of medications whose potencies are already compromised, it is crucial to take into account a drug's capacity to enter the brain. The logarithmic ratio of brain to plasma drug concentrations, or logBB, is used to quantify blood-brain permeability in vivo in animal models. Molecules with a logBB < −1 are poorly distributed to the brain, but those with a logBB > 0.3 are thought to penetrate the blood-brain barrier with ease. A significant barrier to medication delivery to the central nervous system (CNS) is the blood-brain barrier (BBB), which makes it difficult to treat many CNS illnesses, including brain cancer, effectively. In the realm of compounds, those with a logPS > -2 are deemed capable of penetrating the Central Nervous System (CNS), whilst those with a logPS < -3 are deemed incapable of doing so. Drug clearance is primarily achieved by a combination of hepatic clearance (liver metabolism and biliary clearance) and renal clearance (kidney excretion). The proportionality constant CLtot is used to evaluate drug clearance. For a given drug, the estimated total clearance log (CLtot) is expressed in log(ml/min/kg).The LD50 values for toxic dosages are commonly expressed as mg/kg body weight. The dose at which 50% of test subjects pass away after being exposed to a substance is known as the median lethal dose, or LD50. The globally harmonized system of categorization and labeling of substances (GHS) is used to define toxicity classes. The units of LD50 are [mg/kg]. For a compound, when LD50 is less than 5 or in the range of 5 to 50, it will be fatal if swallowed and when LD50 is in the range of 50 to 300, it is toxic if swallowed and when LD50 is in the range of 300 to 2000,it will be harmful if swallowed and when LD50 is in the range of 2000 to 5000,it may be harmful if swallowed and when LD50 is greater than 5000,it is non toxic if swallowed. Synthetic Accessibility score achieves values from 1 (easy to synthesize) to 10 (hard to synthesize). A bioavailability score greater than 0.55 is assigned to any compound complying with Lipinski's rules and is considered ideal as it indicates the compound's optimal absorption.

DISCUSSION

In this study, we evaluated the antifungal potential of kaempferol and ellagic acid against Aspergillus fumigatus with a focus on their effects on tryptophan synthetase. Our results demonstrate that both compounds exhibit significant antifungal activity, with kaempferol showing binding energy -5.61 and ellagic acid displaying binding energy of -5.16. These findings are consistent with previous research on the antifungal properties of these compounds and provide new insights into their mechanisms of action. The practical implications of our study suggest that kaempferol and ellagic acid could be promising candidates for developing novel antifungal therapies. However, our research also has limitations, such as our study is specific to *Aspergillus fumigatus* . Future studies should address these limitations and explore other species also.In conclusion, our research contributes to the growing body of evidence supporting the use of plant-derived compounds as antifungal agents and paves the way for further exploration of their therapeutic potential.

CONCLUSION AND FUTURE PROSPECTS

Targeting tryptophan synthetase in Aspergillus fumigatus offers several promising future prospects. Developing novel antifungal agents that inhibit this enzyme could lead to new treatments effective against A. fumigatus and potentially other fungi. Such inhibitors might help overcome drug resistance issues associated with existing treatments. Additionally, combining tryptophan synthetase inhibitors with other antifungals could enhance overall efficacy and reduce resistance development. Investigating the enzyme’s role in fungal virulence could also provide valuable insights into controlling fungal growth and pathogenesis. Future research might focus on validating tryptophan synthetase as a therapeutic target through structural and functional studies, exploring alternative approaches like allosteric modulation, and discovering biomarkers for diagnostics and treatment monitoring. These advancements could significantly impact the management of fungal infections.

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REFERENCES

[1]. Vahedi-Shahandashti, R., & Lass-Flörl, C. (2020). Novel antifungal agents and their activity against Aspergillus species. *Journal of Fungi*, *6*(4), 213.

[2]. Latgé, J. P. (1999). Aspergillus fumigatus and aspergillosis. *Clinical microbiology reviews*, *12*(2), 310-350.

[3]. Malekzadeh, S., Sardari, S., Azerang, P., Khorasanizadeh, D., Amiri, S. A., Azizi, M., ... & Khalaj, V. (2017). Identification and evaluation of novel drug targets against the human fungal pathogen Aspergillus fumigatus with elaboration on the possible role of RNA-binding protein. *Iranian biomedical journal*, *21*(2), 84.

[4]. Srivastava, S., Maurya, N. S., Kushwah, S., & Mani, A. (2021). Current Promising Therapeutic Targets for Aspergillosis Treatment. *Journal of Pure and Applied Microbiology*..

[5]. de Andrade Monteiro, C., & dos Santos, J. R. A. (2019). Phytochemicals and their antifungal potential against pathogenic yeasts. *Phytochemicals in human health*, 1-31.

[6]. Lopez, M. J., & Mohiuddin, S. S. (2024). Biochemistry, essential amino acids. In *StatPearls [Internet]*. StatPearls Publishing

[7]. Kuplińska, A., & Rząd, K. (2021). Molecular targets for antifungals in amino acid and protein biosynthetic pathways. *Amino Acids*, *53*(7), 961-991.

[8]. Aviram, M., & Dornfeld, L. (2001). Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis*, *158*(1), 195-198.

[9]. Hartman, R. E., Shah, A., Fagan, A. M., Schwetye, K. E., Parsadanian, M., Schulman, R. N., ... & Holtzman, D. M. (2006). Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiology of disease*, *24*(3), 506-515.

[10]. Althunibat, O. Y., Al-Mustafa, A. H., Tarawneh, K., Khleifat, K. M., Ridzwan, B. H., & Qaralleh, H. N. (2010). Protective role of Punica granatum L. peel extract against oxidative damage in experimental diabetic rats. *Process Biochemistry*, *45*(4), 581-585.

[11]. Seeram, N. P., Henning, S. M., Zhang, Y., Suchard, M., Li, Z., & Heber, D. (2006). Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. *The Journal of nutrition*, *136*(10), 2481-2485.

[12]. Prashanth, D., Asha, M. K., & Amit, A. (2001). Antibacterial activity of Punica granatum. Fitoterapia, 72(2), 171-173.

[13]. Toi, M., Bando, H., Ramachandran, C., Melnick, S. J., Imai, A., Fife, R. S., ... & Lansky, E. P. (2003). Preliminary studies on the anti-angiogenic potential of pomegranate fractions in vitro and in vivo. Angiogenesis, 6, 121-128.

[14]. Lansky, E. P., & Newman, R. A. (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. Journal of Ethnopharmacology, 109(2), 177-206. https://doi.org/10.1016/j.jep.2006.09.006

[15]. Jurenka, J. S. (2008). Therapeutic applications of pomegranate (Punica granatum L.): A review. Alternative Medicine Review, 13(2), 128-144. https://www.altmedrev.com/archive/publications/13/2/128.pdf

[16]. Huang, T. H., et al. (2005). Pomegranate flower improves cardiac lipid metabolism in a diabetic rat model: Role of lowering circulating lipids. British Journal of Pharmacology, 145(6), 767-774. https://doi.org/10.1038/sj.bjp.0706249

[17]. Afaq, F., et al. (2005). Pomegranate fruit extract modulates UVB-mediated phosphorylation of mitogen-activated protein kinases and activation of nuclear factor kappa B in normal human epidermal keratinocytes. Photochemistry and Photobiology, 81(1), 38-45. https://doi.org/10.1562/2004-08-24-RA-296

[18]. Türk, G., Sönmez, M., Aydin, M., Yüce, A., Gür, S., Yüksel, M., Aksu, E. H., & Aksoy, H. (2008). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clinical nutrition (Edinburgh, Scotland), 27(2), 289–296. https://doi.org/10.1016/j.clnu.2007.12.006

[19]. Martí-Renom, M. A., Stuart, A. C., Fiser, A., Sánchez, R., Melo, F., & Šali, A. (2000). Comparative protein structure modeling of genes and genomes. Annual review of biophysics and biomolecular structure, 29(1), 291-325.

[20]. Kopp, J., & Schwede, T. (2004). The SWISS-MODEL Repository of annotated three-dimensional protein structure homology models. Nucleic acids research, 32(suppl_1), D230-D234.

[21]. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of cheminformatics, 3, 1-14.

[22]. Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced drug delivery reviews*, *23*(1-3), 3-25.

[23]. Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. Methods in molecular biology (Clifton, N.J.), 443, 365–382. https://doi.org/10.1007/978-1-59745-177-2_19

[24]. Shen, M. Y., & Sali, A. (2006). Statistical potential for assessment and prediction of protein structures. *Protein science*, *15*(11), 2507-2524.