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IN-SILICO AND *IN-VITRO* ANALYSIS OF POTENTIAL PLANT EXTRACTS AGAINST ASPERGILLOSIS

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Keywords:

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ABSTRACT: *Cutaneous aspergillosis* is a rare locally invasive disease in immunocompromised patients caused primarily by *Aspergillus species* which induce primary infections through injured skin, burns, or locally infect via fungal contaminated adhesive dressings or intravenous catheter insertion, resulting in lesions to erythematous papules. About 80% of aspergillosis is caused by the ubiquitous and fastest-growing airborne fungi *Aspergillus fumigatus* leads to mortality in immunocompromised patients. Thioredoxin systems are one of the two main antioxidant systems in maintaining their cellular redox homeostasis essential for cell viability and occurring in all life forms. Thioredoxin reductase is one of the components of Thioredoxin system responsible for the normal growth of *Aspergillus fumigatus*. The present study deals with the *in-silico* analysis of binding efficiencies between phytochemicals from different medicinal plants with the target protein thioredoxin reductase of *Aspergillus fumigatus* using bioinformatics tools. The phytochemicals and target protein structure were retrieved from PubChem and PDB (ID:6BPY) databases, respectively. Qikprop module was used for ADME profiling of phytochemicals and the selected phytochemicals were subsequently docked with protein using Schrodinger software. Docking studies revealed that 3'Acetyl lycopsamine from *Heliotropium indicum* scored the significant glide score of -6.64 Kcal/mol. Based on the docking studies, antimicrobial screening was carried out for different extracts of plants with phytochemicals that scored best with the target protein. Among the extracts, Ethyl acetate extract of *Heliotropium indicum* showed better antifungal activity against *Aspergillus fumigatus* suggesting that *Heliotropium indicum* can be used as a potential drug molecule against Aspergillosis.

INTRODUCTION: *Cutaneous aspergillosis* is a rare locally invasive disease commonly caused by *Aspergillus fumigatus* and *Aspergillus flavus* that can occur commonly in immunocompromised patients [1, 2].

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The infection originates as primary cutaneous *via* injured skin, burns or as a local infection generally associated with adhesive dressings or intravenous catheter insertion contaminated with fungus by forming single or multiple lesions, which in extreme conditions leads to the appearance of erythematous papules followed by becoming pustular, necrotic central ulceration having elevated border with black eschar [3, 4, 5].

However, predominantly these infections appear secondary to systemic or disseminated Aspergillosis of lungs [6].

The airborne fungi *A. fumigatus* are the major causative agent for about 80% of invasive aspergillosis and mortality in humans, especially in patients with compromised immune system. They are ubiquitous in nature, leads to dark eschars on reddish plaques or at the site of injuries [7, 8]. They are responsible for the fungal keratitis that are capable of inducing blindness [9, 10, 11] and are considered as one among the most common airborne pathogens as they develop stress-tolerant airborne spores as a part of their lifecycle [12, 13, 14]. Hydrophobic cell wall can withstand a wide range of pH, temperature and are thereby efficiently dispersed in the air. These fastest-growing fungal species promote the colonization of multiple niches with small hydrophobic spores facilitating penetration [15, 16].

Generally, Fungi exhibit various mechanisms to withstand antifungal resistance by the host immune system. The sulfur-based cellular system is important as they possess unique redox properties [17, 9, 18]. Thioredoxin systems are one of the two main antioxidant systems having Trx, Trx reductase (TrxR), and NADPH as components in maintaining cellular redox homeostasis, which is essential for the viability of cells and occurs in all forms of life [19, 20, 21]. The low molecular weight form of these dimeric flavoenzymes exhibits specificity for Trx substrate in fungi and catalyze the electron transfer from NADPH to dithiol, which is a redox-active of Trx and is also responsible for the antioxidant defense and synthesizes DNA [22, 23, 24].

Although various drug combinations and antifungal agents have been used against these infections, most lead to multidrug resistance and other side effects on prolonged or uncontrolled usage, suggesting the need for novel drugs [25, 26]. Since ancient times, medicinal plants have been extensively used for a wide spectrum of diseases and infections due to their rich phytochemicals and are considered pharmaceutical lead [27]. Ethnopharmacological studies have shown that a plant's phytochemicals can exhibit activities synergistically or by a single compound [28, 29]. Plants like *Heliotropium indicum* were used in folklore medicine with its leaf paste against various ailments, and they possess excellent wound healing activity [30, 31].

Some plants like *Grona triflora* have anticonvulsant, antinociceptive, antibacterial, anti-inflammatory, and antioxidant activities [32, 33]. Although plant-based medicines are effective against diseases, they may sometimes lead to adverse health effects. These side effects are associated with poor drug target interactions or increased compound toxicity [28]. *In-silico* studies help to overcome these by predicting the protein target interactions, and prior optimization of the drug can be carried out at an early stage of development from the most suitable drug compound [34]. The present study focuses on the structure-based identification of plants whose phytocompounds can actively target the protein thioredoxin reductase of *Aspergillus fumigatus* followed by their *in-vitro* study.

MATERIALS AND METHODS:

Databases: Protein Data Bank (www.rcsb.org) database was utilized to retrieve the 3D structure of the target protein Thioredoxin reductase, and their active site residues were predicted *via* Ligsite online tool (http://projects.biotec.tu-dresden.de/pocket/) [35, 36]. Structure of phytocompounds from plants like *Heliotropium indicum, Grona triflora, Evolvulus alsinoides, Ziziphus mauritiana, Commelina benghalensis, Aristolochia bracteolata, Pyrus communis* and *Coccinea grandis* were retrieved from PubChem database (http://www.ncbi.nlm.nih.gov/pccompound) and scrutinized for docking studies. Their pharmacological profile was verified with PASS online tool [37].

Prediction of Drug Likeliness: Initially, LigPrep module (Schrodinger, LLC, NY, USA, 2009) of Maestro 9.0.211 version of the Schrodinger suite was employed to optimize the ligands *via* converting the two-dimensional structure of the ligands into the three-dimensional structure by the addition of hydrogen atoms. The geometry of the ligands can be optimized with processes like correction, energy minimization, and ionization [38, 39, 40]. After preparing the ligands, the pharmacological properties of phytocompounds can be validated by ADME profiling with various parameters of QikProp module of Schrodinger [41]. Parameters like donor hydrogen bond, acceptor hydrogen bond, molecular weight, skin

permeability, blood brain barrier coefficient and Lipinski's rule of three and five were analyzed. Lipinski's rule of five helps discerning drug-like compounds from non-drug-like compounds [42]. Phytocompounds exhibiting sensible results were considered for docking studies with target protein [43].

Protein Preparation and Receptor Grid Generation: Protein Preparation Wizard of Schrodinger software performed the protein preparation that adds hydrogen atoms to rectify missing side chains and residues and stabilize the charges [44].

The X-Ray crystallographic structure of proteins may be bound to water molecules that affect the docking process thus the water molecules were removed to increase the entropy of target molecules followed by optimization and minimization [38].

The protein's active site was identified by generating a grid box where water molecules and hetero atoms are removed *via* the receptor grid generation module of Maestro. The area of interaction between the ligands and protein was defined with the help of the grid generated [45, 46].

Molecular Docking: Based on the ADME profile, the compounds that passed the drug-like parameters were docked against the target protein via GLIDE module of Schrodinger suite. GLIDE scores provided will represent the binding free energy of the interactions [47].

Plant Collection and Extract Preparation: Plants like *Heliotropium indicum* and *Grona triflora* were collected from Kanyakumari district and their identity was confirmed by The Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2022/Tech/517and BSI/SRC/5/23/2022/Tech/516). Leaves of the collected plants were shade dried and powdered separately. About 25 g of each powdered sample was placed in the thimble, and sequential Soxhlet extraction was carried out with 250ml of solvents liken-Hexane, Ethylacetate and Methanol. The solvents were then evaporated and the concentrated extracts were taken for further study [48].

Antifungal Activity: The fungicidal activity of the extracts was screened against *Aspergillus fumigatus* clinical isolates procured from Bioline Laboratory, Coimbatore. Agar well diffusion method was performed using Sabouraud dextrose agar medium where overnight cultures were prepared by inoculating the fungal strains in the SDA broth and incubated at 28°C.

The cultures were then swabbed on to the agar medium and 6mm wells were punched using sterile cork and borer. Each well was loaded with 50µl (2mg/ml) of each extract and incubated at 28°C. The diameter of zone of inhibition was measured in mm with standard Ketoconazole [49, 50].

RESULTS AND DISCUSSION:

Protein Structure Retrieval: The 3D structure of protein Thioredoxin reductase was retrieved from PDB with PDB ID: 6BPY as shown in **Fig. 1.**

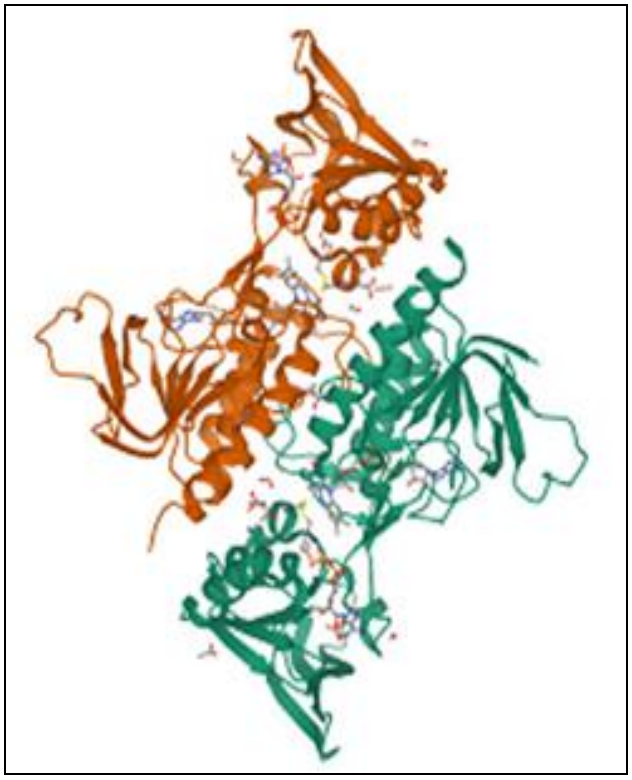

FIG. 1: 3D STRUCTURE OF THIOREDOXIN REDUCTASE FROM PDB

ADME Analysis: Phytocompounds structures were retrieved and analyzed for their ADME properties. The parameters of drug ability like rotatable bonds, acceptor and donor hydrogen atoms, molecular weight and Lipinski's rule of five limits were fulfilled by these 18 compounds as shown in **Table 1.**

TABLE 1: ADME PROFILE OF PHYTOCOMPOUNDS USING QIKPROP MODULE

Molecule Name	No. of rotatable bonds	Molecular weight	Dipole moment	SASA	Donor Hydrogen bonds	Acceptor Hydrogen bonds	QPlogP for Octanol/gas
Normal Range	**0.0-15.0**	**130.0-725.0**	**1.0-12.5**	**300.0-1000.0**	**0.0-6.0**	**2.0-20.0**	**8.0-35.0**
Indicine-N-oxide	8	315.366	8.382	553.78	2	8.15	17.388
3'-Acetyl lycopsamine	8	341.403	4.667	622.994	1	7.45	16.35
p-Coumaric acid	4	164.16	6.69	381.465	2	2.75	10.645
Ferulic acid	5	194.187	6.295	420.153	2	3.5	11.367
Caffeic acid	5	180.16	7.175	392.531	3	3.5	12.706
Syringic acid	4	198.175	3.44	400.033	2	4.25	10.721
Vanillic acid	3	168.149	4.027	360.214	2	3.5	9.897
Melilotic acid	4	166.176	7.038	381.362	2	2.75	10.725
Phloretic acid	4	166.176	5.872	385.788	2	2.75	10.427
Kaempferol	4	286.24	5.622	501.402	3	4.5	16.695
Quercetin	5	302.24	3.533	512.235	4	5.25	18.32
Cytidine	5	243.219	3.514	437.255	5	10.8	20.154
Luteolin	4	286.24	4.716	503.697	3	4.5	16.593
Clindamycin	10	424.982	4.393	664.829	4	11.8	24.779
2,3 Dimethoxycinnamic acid	5	208.213	4.739	414.151	1	3.5	9.744
21 Diazoprogesterone	3	340.464	7.087	595.173	0	7	16.395
Ethisterone	2	312.451	5.802	554.641	1.5	2.75	15.057
Arbutin	8	272.254	4.239	478.271	5	10	20.369

Molecule Name	QPlogP Water / Gas	QPlogP Octanol /Water	QPlog BB for Brain / Blood	QPlogKp for skin permeability	No. of Metabolic reactions	Human Oral absorption	Rule of Five	Rule of Three
Normal Range	**4.0-45.0**	**-2.0-6.5**	**-3.0-1.2**	**-8.0 to -1.0**	**1.0-8.0**	**1,2 (or)3 L, M, H**	**Max 4**	**Max 3**
Indicine-N-oxide	11.471	1.258	-1.149	-3.214	6	3	0	0
3'-Acetyl lycopsamine	9.182	1.917	-0.66	-4.995	5	3	0	0
p-Coumaric acid	7.787	1.443	-1.096	-3.621	1	3	0	0
Ferulic acid	8.031	1.378	-1.189	-3.697	2	3	0	0
Caffeic acid	9.871	0.558	-1.569	-4.524	2	2	0	1
Syringic acid	8.374	0.971	10.721	-3.848	3	3	0	0
Vanillic acid	8.12	1.046	9.897	-3.776	2	2	0	0
Melilotic acid	7.56	1.416	-0.891	-3.27	3	2	0	0
Phloretic acid	7.564	1.329	-1.021	-3.576	3	2	0	0
Kaempferol	12.28	1.06	-1.803	-4.533	4	3	0	0
Quercetin	14.36	0.387	-2.309	-5.422	5	2	0	1
Cytidine	19.957	-1.965	-1.886	-5.489	4	2	0	0
Luteolin	12.30	0.96	-1.947	-4.851	4	3	0	0
Clindamycin	19.536	2.121	-0.671	-5.019	6	2	0	0
2,3-Dimethoxycinnamic acid	6.237	2.135	-0.625	-2.601	2	3	0	0
21-Diazoprogesterone	8.821	1.94	-1.14	-4.263	4	3	0	0
Ethisterone	6.598	3.117	-0.312	-2.724	3	3	0	0
Arbutin	18.704	-0.995	87.374	-4.251	5	2	0	0

Molecular Docking: Docking studies revealed that from the 18 compounds taken only 17 compounds successfully docked as shown in **Table 2** with the target in which 3'Acetyllycopsamine showed the best G score of -6.64 Kcal/mol with the residues ALA124, ASP294, GLN45 and THR49 having

bond lengths 1.9, 2.1, 2.5 and 2.0 Å respectively was visualized using PYMOL software as given in **Fig. 2**. This was followed by Indicine-N-Oxide and Quercetin with significant G score of -6.54 and -6.36 Kcal/mol. Compounds like 3'Acetyl-lycopsamine, Indicine-N-Oxide, Caffeic acid, Syringic acid and Vanillic acidfrom *Heliotropium indicum* exhibited good GLIDE scoresof -6.64, -6.54, -5.30, -4.64 and -4.30Kcal/mol respectively. Followed by this, Compounds like Quercetin and Melilotic acid from *Grona triflora* showed promising interactions with G score -6.36 and -5.28 Kcal/mol. In addition to that, Arbutin from *Pyrus communis*also exhibited a good interaction of -6.02 Kcal/mol. Clindamycin from the plant *Ziziphus mauritiana* exhibits a significant interaction of -5.33 Kcal/mol. From the plant *Evolvulus alsinoides* the compound Cytidine bounds to the target with a score of -5.20 Kcal/mol whereas Luteolin from the same plant exhibited good interactions by forming seven bonds with the target protein together with a G score of -3.47 Kcal/mol. Compounds like 3'Acetyllycopsamine and Arbutin effectively bound to the active site pockets GLN45, SER11 and ASN 125 amino acid residues of target protein. Similarly, Indicine-N-Oxide and Caffeic acid bound to the active sites SER 11 where Indicine-N-Oxide also bound to the active site ASN 125 and Caffeic acid towards ASP 294 active sites. Apart from these compounds, Melilotic acid and syringic acid also effectively bound to the active site pockets of the target protein GLN 45, ASN 125, ASP 294 and SER 11. However, Ethisterone from *Coccinea grandis* alone showed no particular interactions with the target protein.

TABLE 2: INTERACTION OF PLANT COMPOUNDS WITH 6BPY

S. no.	Name of The Ligand (Pubchem ID)	Residues Interaction	Bond Length (Å)	No. of Hydrogen Bonds	G-Score (Kcal/mol)
1	3'Acetyllycopsamine (586647)	ALA124(O-H)	1.9		
		ASP294(H-O)	2.1	4	-6.64
		GLN45(O-H)	2.5		
		THR49(O-H)	2.0		
2	Indicine-N-Oxide (280564)	ASN125(O-H)	2.1		
		THR122(H-O)	2.4		
		THR40(H-O)	2.3	6	-6.54
		ALA41(H-O)	2.8		
		SER11(O-H)	2.4		
		ALA121(H-O)	2.1		
3	Quercetin (5280343)	ASP149(H-O)	1.8		
		ASP149(H-O)	2.6	3	-6.36
		SER306(H-O)	1.8		
4	Arbutin (440936)	SER11(O-H)	2.5		
		THR40(H-O)	2.1		
		ALA37(H-O)	2.2		
		ASN125(O-H)	2.4	7	-6.02
		ASN125(H-O)	1.9		
		GLN45(O-H)	2.7		
		SER306(H-O)	2.5		
5	Clindamycin (446598)	ASP149(H-O)	2.3		
		THR49(H-O)	2.2	3	-5.33
		ARG301(H-O)	2.6		
6	Caffeic acid (689043)	ASP294(H-O)	2.4		
		ASP294(O-H)	2.0		
		SER11(O-H)	1.9	5	-5.30
		SER306(H-O)	2.4		
		SER306(H-O)	1.7		
7	Melilotic acid (873)	ASN125(O-H)	1.9		
		ASP294(H-O)	2.3	3	-5.28
		GLN45(O-H)	2.4		
8	Cytidine (6175)	ASP294(H-O)	1.7		
		ALA144(O-H)	2.6		
		THR48(O-H)	2.0		
		SER143(O-H)	2.5	8	-5.20

		ASP149(H-O)	1.8		
		TRP139(O-H)	2.7		
		GLN139(O-H)	2.9		
		ALA144(O-H)	2.5		
9	Syringic acid (10742)	SER11(O-H)	2.0		
		GLY12(O-H)	2.6	4	-4.64
		GLN45(O-H)	2.1		
		ASP294(H-O)	2.4		
10	Vanillic acid (8468)	GLN45(O-H)	2.0		
		ASP294(H-O)	2.5	4	-4.30
		GLY12(O-H)	2.6		
		SER11(O-H)	2.0		
11	Luteolin (5280445)	GLN45(O-H)	2.2		
		ASP294(O-H)	2.5		
		SER11(O-H)	2.2		
		GLU33(H-O)	2.5	7	-3.47
		ALA37(H-O)	2.2		
		THR40(H-O)	2.1		
		ALA121(O-O)	2.6		
12	p-Coumaric acid (637542)	SER11(O-H)	2.8		
		SER11(O-H)	1.7	4	-3.18
		ASP294(H-O)	2.4		
		GLY12(O-H)	2.7		
13	Kaempferol (5280863)	ALA303(O-H)	2.7		
		ASP149(H-O)	1.9	4	-2.93
		ASP149(H-O)	2.5		
		SER302(H-O)	1.9		
14	21-Diazoprogesterone (104633)	ASN130(O-H)	2.1	1	-2.33
15	2,3 Dimethoxycinnamic acid (735842)	GLN45(O-H)	2.1	3	-2.02
		SER11(O-H)	1.9		
		SER11(O-H)	2.3		
16	Ferulic acid (445858)	GLN45(O-H)	1.8	2	-0.59
		ARG301(H-O)	1.8		
17	Phloretic acid (10394)	ALA14(O-H)	2.2	2	2.33
		ASP294(O-H)	1.8		

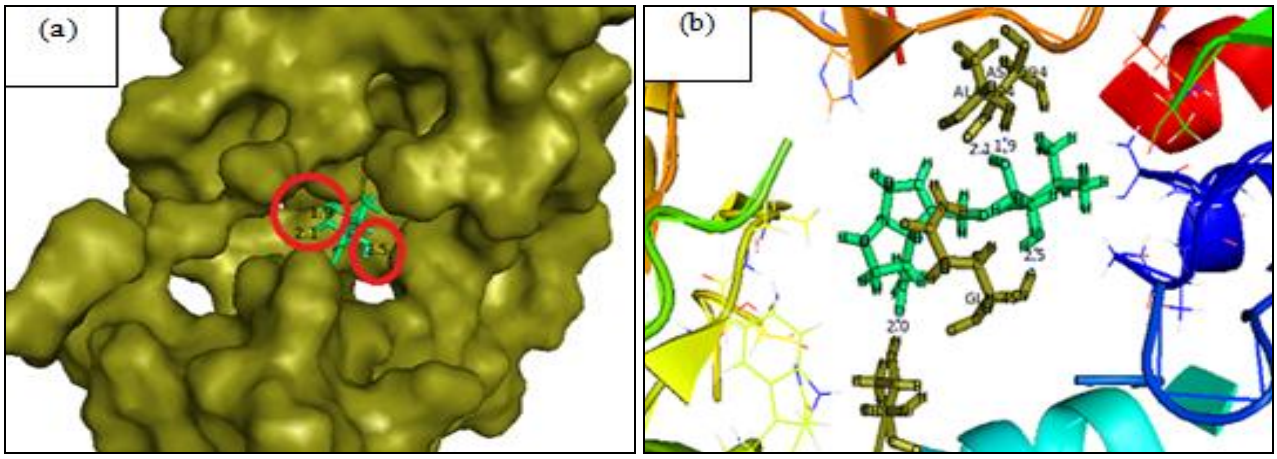

FIG 2: DIAGRAMMATIC REPRESENTATION OF INTERACTION BETWEEN 3'ACETYLLYCOPSAMINE WITH THIOREDOXIN REDUCTASE (6BPY). (a) represents the surface image of protein in which the pale green colour compound bounds to the active pocket of the target protein in deep olive colour. (b) represents the cartoon image of the interaction with amino acid residues with which the compound binds with bond lengths.

***In-vitro* Antifungal Studies:** From the docking results, it was validated that the greatest number of phytocompounds from *Heliotropium indicum* and *Grona triflora* showed better ADME and docking results. Thus, the results of antifungal study conducted for different extracts of these plants are shown in **Table 3.**

TABLE 3: ANTIFUNGAL ACTIVITY OF EXTRACTS AGAINST *ASPERGILLUS FUMIGATUS*

S. no.	Sample		Zone of Inhibition (mm)
1	*Heliotropium indicum*	Hexane	-
		Ethyl acetate	34
		Methanol	14
2	*Grona triflora*	Hexane	-
		Ethyl acetate	32
		Methanol	16
3	Ketoconazole		20

Results revealed that Ethyl acetate leaf extract of *Heliotropium indicum* effectively inhibits the growth of *Aspergillus fumigatus* with a maximum of 34mm zone of inhibition when compared with *Grona triflora* extracts and standard drug as given in **Fig. 3.**

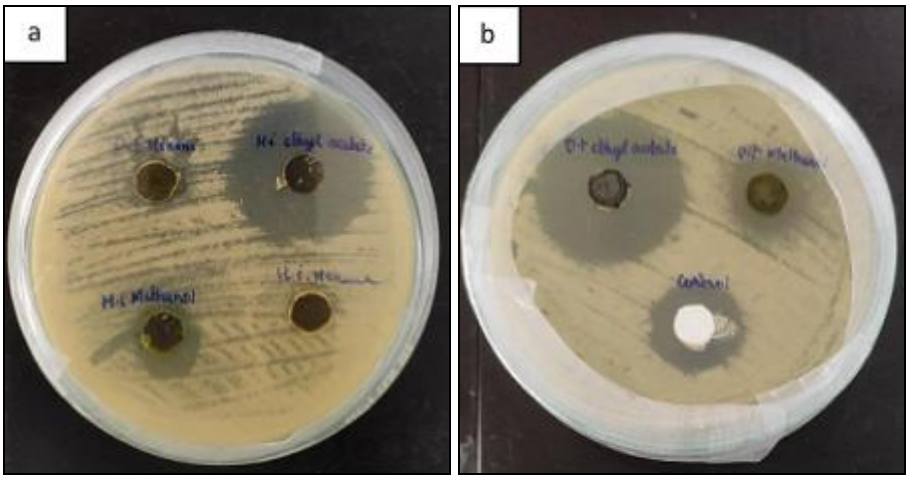

FIG 3: ANTIFUNGAL ACTIVITY OF PLANT EXTRACTS AGAINST *ASPERGILLUS FUMIGATUS.* (a) represents the antifungal activity of different extracts of *Heliotropium indicum* and hexane extract of *grona triflora* (d.t). b) represents the antifungal activity of ethyl acetate and methanol extracts of *Grona triflora* (d.t) and standard ketoconazole.

DISCUSSION: Aspergillus species are ubiquitous in nature that are capable to cause infections in human body like skin, lungs and even the central nervous system [51]. Azole-based drugs are extensively used against these infections as they inhibit the ergosterol synthesis pathway, thereby affecting the fungal cell membrane. However, due to prolonged exposure to these drugs during treatment results in drug resistance that are responsible for the increase in mortality rate [52, 53].

Thus, the current study is targeted against *Aspergillus fumigatus* to identify efficient drug molecules using several bioinformatic tools. Molecular docking is one of the most widely used strategies in the structure-based drug designing (SBDD) technique, which exploits the structural properties of targets for drug development and extensively studies the ligand-target interaction with their binding energies [54, 55]. Since, studies have revealed that targeting Thioredoxin reductase fungal proteins can reduce the growth of fungi, they were chosen as a target to evaluate their interactions with potential therapeutic candidates. They also showed limited similarities with human proteins and are shown to be substantially expressed during aspergillosis [56, 57]. They significantly impact thioredoxin's redox reaction, which is important for various physiological processes and pathological conditions in various organisms, leading to conditions like apoptosis and even autoimmune diseases [58]. As a potential methodology that may be used in the early phases of drug development, *in-silico* approach was used in this research as a preliminary step to determine the potent anti-fungicidal plants from the selected medicinal plants followed by *in-vitro* analysis [59]. Plants primarily produce bioactive components or secondary metabolites that possess various pharmacological properties [60].

The presence of these metabolites provides medicinal properties like antimicrobial properties to plants where these compounds might have disrupted the cell wall or inhibited biofilm formation [61]. The structures of phytocompounds

collected from PubChem databases were subjected to ADME analyses 62 to balance the important features of a drug-like molecule. It was evident from the ADME profiling data that the majority of compounds from plants that passed the test were in the order of *Heliotropium indicum, Grona triflora, Evolvulus alsinoides, Ziziphus mauritiana, Commelina benghalensis, Aristolochia bracteolata, Coccinea grandis* and *Pyrus communis.* Due to the widespread belief that natural remedies are safe, the use of herbal medicines is rising dramatically nowadays. However, natural products can occasionally cause adverse effects by modifying conventional drugs' performance [28].

Therefore, the compounds' biological activities have been understood and validated using the PASS online programme, which confirmed that all the compounds that passed the ADME exhibit drug-like properties. Molecular docking experiments have been performed to anticipate potential drug molecule binding orientations with the target protein [42, 63]. According to docking experiment results from **Table 3**, more phytocompounds from Heliotropium indicum and Grona triflora plants have substantial G scores for binding to the target protein's active residues. Studies also revealed that *Heliotropium indicum* and *Grona triflora* extracts have been extensively used against skin infections for years [63, 64].

The compound 3'Acetyllycopsamine from *Heliotropium indicum* is the most effective compound when compared to other compounds suggesting that it could be an excellent lead against the organism *Aspergillus fumigatus*. From **Table 2** it is evident that apart from 3'acetyllycopsamine, the compound Indicine-N-Oxide from *Heliotropium indicum* also possesses significant interactions towards the target whose anticancer activity was already reported [65]. Quercetin also exhibited a G score of -6.36 Kcal/mol with three bonds whose antifungal activity has already been reported [66]. However, *in-silico* interaction with Thioredoxin reductase of *Aspergillus fumigatus* was not reported early. **Table 2** indicates that Arbutin from *Pyrus communis*, in addition to the aforementioned compounds, also exhibited high binding activity towards the target; nevertheless, plants with more ADME passed docked compounds were taken into consideration for the following investigations. In order to validate the results of docking, *in-vitro* antifungal screening was conducted with different extracts of plants that ranked first and second, with the greatest number of compounds having significant docking scores. Different parts of the plants were considered for the *in-vitro* screening as the phytochemical profile of a plant varies with different parts of the plant [67].

Ethyl acetate leaf extracts of *Heliotropium indicum* and *Grona triflora* inhibited the fungal growth effectively when compared with other solvent extracts indicating the effect of sequential solvent extraction [48]. Hence, the results suggest that the leaf extracts of *Heliotropium indicum* can be an excellent source of antifungal agent as they possess several active phytocompounds that was observed to be a potential drug compound.

CONCLUSION: Primary cutaneous aspergillosis caused *via* skin injury is often connected with cutaneous invasive aspergillosis, which sometimes leads to mortality. Development of multidrug resistance and prolonged usage of drugs result in side effects disseminating the need for novel medicines. The present study suggests that *Heliotropium indicum* and *Grona triflora* possess activity against the growth of tested fungi. Further studies will be carried out to understand the antifungal profile of extracts from these plants, thereby analyzing their stability.

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REFERENCE:

1. Zhang R, Zhang Y, Xu W, Han X and Zhao J: Primary *Cutaneous aspergillosis* Due to *Aspergillus fumigatus* in an Immunocompetent Patient with Diabetes Mellitus after Tattooing: A Case Report and Review of Literature. Infection and Drug Resistance 2023; 791-7.
2. Zhang QQ, Li LI, Zhu M, Zhang CY and Wang JJ: Primary *Cutaneous aspergillosis* due to *Aspergillus flavus*: a case report. Chinese Medical J 2005; 118(03): 255-7.
3. Que AT, Nguyen NM, Do NA, Nguyen NL, Tran ND and Le TA: Infection of burn wound by *Aspergillus fumigatus* with gross appearance of fungal colonies. Medical Mycology Case Reports 2019; 24: 30-2.
4. Walsh TJ, Anaissie EJ, Denning DW, Herbrecht R, Kontoyiannis DP, Marr KA, Morrison VA, Segal BH, Steinbach WJ, Stevens DA and van Burik JA: Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clinical Infectious Diseases 2008; 46(3): 327-60.
5. Stevens DA, Kan VL, Judson MA, Morrison VA, Dummer S, Denning DW, Bennett JE, Walsh TJ, Patterson TF and Pankey GA: Practice guidelines for diseases caused by Aspergillus. Clinical Infectious Diseases 2000; 696-709.
6. Tatara AM, Mikos AG and Kontoyiannis DP: Factors affecting patient outcome in primary *Cutaneous aspergillosis*. Medicine 2016; 95(26).
7. Mada PK, Koppel DA, Al Shaarani M and Chandranesan AS: Primary Cutaneous *Aspergillus fumigatus* infection in immunocompetent host. BMJC R CP 2020; 13(2): 233020.
8. Arastehfar A, Carvalho A, Houbraken J, Lombardi L, Garcia-Rubio R, Jenks JD, Rivero-Menendez O, Aljohani R, Jacobsen ID, Berman J and Osherov N: *Aspergillus fumigatus* and aspergillosis: From basics to clinics. Studies in Mycology 2021; 100(1): 100115.
9. Leal SM, Vareechon C, Cowden S, Cobb BA, Latgé JP, Momany M and Pearlman E: Fungal antioxidant pathways promote survival against neutrophils during infection. The Journal of clinical investigation 2012; 122(7): 2482-98.
10. Cheng M, Lin J, Li C, Zhao W, Yang H, Lv L and Che C: Wedelolactone suppresses IL-1β maturation and neutrophil infiltration in *Aspergillus fumigatus* keratitis. International Immunopharmacology 2019; 73: 17-22.
11. Ratitong B and Pearlman E: Pathogenic Aspergillus and Fusarium as important causes of blinding corneal infections the role of neutrophils in fungal killing, tissue damage and cytokine production. Current Opinion in Microbiology 2021; 63: 195-203.
12. Ibrahim-Granet O, Dubourdeau M, Latgé JP, Ave P, Huerre M, Brakhage AA and Brock M: Methylcitrate synthase from *Aspergillus fumigatus* is essential for manifestation of invasive aspergillosis. Cellular microbiology 2008; 10(1): 134-48.
13. Dijksterhuis J: Fungal spores: Highly variable and stress-resistant vehicles for distribution and spoilage. Food Microbiology 2019; 81: 2-11.
14. Paulussen C, Hallsworth JE, Álvarez-Pérez S, Nierman WC, Hamill PG, Blain D, Rediers H and Lievens B: Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other Aspergillus species. Micro Biotech 2017; 10(2): 296-322.
15. Kwon-Chung KJ and Sugui JA: *Aspergillus fumigatus* what makes the species a ubiquitous human fungal pathogen?. PLoS Pathogens 2013; 9(12): 1003743.
16. Van De Veerdonk FL, Gresnigt MS, Romani L, Netea MG and Latge JP: *Aspergillus fumigatus* morphology and dynamic host interactions. Nature Reviews Microbiology 2017; 15(11): 661-74.
17. Brown SM, Campbell LT and Lodge JK: Cryptococcus neoformans, a fungus under stress. Current Opinion in Microbiology 2007; 10(4): 320-5.
18. Elias SJ. Arnér ESJ and Holmgren A: Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267(20): 6102-9.
19. Toledano MB, Delaunay-Moisan A, Outten CE and Igbaria A: Functions and cellular compartmentation of the thioredoxin and glutathione pathways in yeast. Antioxidants & Redox Signaling 2013; 18(13): 1699-711.
20. Deng Z: Anti-microbial and Anti-cancer activity of Traditional Chinese Medicine Extracts 2020.
21. Lu J and Holmgren A: The thioredoxin antioxidant system. Free Radical Biology and Medicine 2014; 66: 75-87.
22. Holmgren A and Bjornstedt M: [21] Thioredoxin and thioredoxin reductase. Meth in Enzym 1995; 252: 199-08.
23. Bjørklund G, Zou L, Wang J, Chasapis CT and Peana M: Thioredoxin reductase as a pharmacological target. Pharmacological Research 2021; 174: 105854.
24. Oliveira MA, Discola KF, Alves SV, Medrano FJ, Guimaraes BG and Netto LE: Insights into the specificity of thioredoxin reductase− thioredoxin interactions. A structural and functional investigation of the yeast thioredoxin system. Biochemistry 2010; 49(15): 3317-26.
25. Warren LR, Bhattacharjya S, Abrol N, Russell C and Antony-Olakkengil S: Treatment of invasive aspergillus-associated wound infection incorporating topical amphotericin B. Wound Practice & Research: J of the Australian Wound Manag Assoc 2017; 25(2): 98-100.
26. Sanglard D and White TC: Molecular principles of antifungal drug resistance. Molecular Principles of Fungal Pathogenesis 2006; 17: 197-212.
27. McChesney JD, Venkataraman SK and Henri JT: Plant natural products: back to the future or into extinction?. Phytochemistry 2007; 68(14): 2015-22.
28. Süntar I: Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry Reviews 2020; 19(5): 1199-209.
29. Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G, Karakasiliotis I, Kampa M and Castanas E: From traditional ethnopharmacology to modern natural drug discovery: A methodology discussion and specific examples. Molecules 2022; 27(13): 4060.
30. Sarkar C, Mondal M, Khanom B, Hossain MM, Hossain MS, Sureda A, Islam MT, Martorell M, Kumar M, Sharifi-Rad J and Al-Harrasi A: *Heliotropium indicum* L.: from farm to a source of bioactive compounds with therapeutic activity. Evidence-Based Complementary and Alternative Medicine 2021; 2021: 1-21.
31. Dash GK and Murthy PN: Studies on wound healing activity of *Heliotropium indicum* Linn. leaves on rats. International Scholarly Research Notices 2011; 2011.
32. Thankachan AK, Chandran M and Krishnakumar K: Pharmacological activity of Desmodium triflorum-A review. Asian Journal of Phytomedicine & Clinical Research 2017; 5(1): 33-41.
33. Daya RW, Chandra JA and Dayendra RC: Antinociceptive activity of cold water extract of *Desmodium triflorum* in rats. Int Res J Pharm 2011; 2(7): 120-3.
34. Xia X: Bioinformatics and drug discovery. Current Topics in Medicinal Chemistry 2017; 17(15): 1709-26.
35. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chen L, Crichlow GV, Christie CH, Dalenberg K, Di Costanzo L,

Duarte JM and Dutta S: RCSB Protein Data Bank: powerful new tools for exploring 3D structures of biological macromolecules for basic and applied research and education in fundamental biology, biomedicine, biotechnology, bioengineering and energy sciences. Nucleic Acids Research 2021;49(D1): 437-51.

36. Zhao J, Cao Y and Zhang L: Exploring the computational methods for protein-ligand binding site prediction. Computational and Structural Biotechnology Journal 2020; 18: 417-26.
37. Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, Pogodin PV and Poroikov VV: Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds 2014; 50: 444-57.
38. Vijayakumar S, Manogar P, Prabhu S and Singh RA: Novel ligand-based docking; molecular dynamic simulations; and absorption, distribution, metabolism, and excretion approach to analyzing potential acetylcholinesterase inhibitors for Alzheimer's disease. Journal of Pharmaceutical Analysis 2018; 8(6): 413-20.
39. Shah B, Modi P and Sagar SR: *In-silico* studies on therapeutic agents for COVID-19: Drug repurposing approach. Life Sciences 2020; 252: 117652.
40. Hashem H: *In-silico* approach of some selected honey constituents as SARS-CoV-2 main protease (COVID-19) inhibitors 2020.
41. Qikprop, module 4.4 Schrodinger suite, New York 2012.
42. Chaudhary KK and Mishra N: A review on molecular docking: novel tool for drug discovery. Databases 2016; 3(4): 1029.
43. Yusof I and Segall MD: Considering the impact drug-like properties have on the chance of success. Drug Discovery Today 2013; 18(13-14): 659-66.
44. Rolta R, Salaria D, Kumar V, Patel CN, Sourirajan A, Baumler DJ and Dev K: Molecular docking studies of phytocompounds of Rheum emodi Wall with proteins responsible for antibiotic resistance in bacterial and fungal pathogens: *in-silico* approach to enhance the bio-availability of antibiotics. Journal of Biomolecular Structure and Dynamics 2022; 40(8): 3789-803.
45. Sahayarayan JJ, Rajan KS, Vidhyavathi R, Nachiappan M, Prabhu D, Alfarraj S, Arokiyaraj S and Daniel AN: *In-silico* protein-ligand docking studies against the estrogen protein of breast cancer using pharmacophore based virtual screening approaches. Saudi JBS 2021; 28(1): 400-7.
46. David TI, Adelakun NS, Omotuyi OI, Metibemu DS, Ekun OE, Inyang OK, Adewumi B, Enejoh OA, Owolabi RT and Oribamise EI: Molecular docking analysis of phyto-constituents from Cannabis sativa with pfDHFR. Bioinformation 2018; 14(9): 574.
47. Ahmed S and Shohael AM: *In-silico* studies of four anthraquinones of *Senna alata* L. as potential antifungal compounds. Pharmacology 2019; 2: 259-68.
48. Alara OR, Abdurahman NH, Ukaegbu CI and Kabbashi NA: Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. J of Taibah Univ for Sci 2019; 13(1): 414-22.
49. Gizaw A, Marami LM, Teshome I, Sarba EJ, Admasu P, Babele DA, Dilba GM, Bune WM, Bayu MD, Tadesse M and Abdisa K: Phytochemical screening and in vitro antifungal activity of selected medicinal plants against *Candida albicans* and *Aspergillus niger* in west shewa zone, Ethiopia. Advances in Pharmacological and Pharmaceutical Sciences 2022; 2022.
50. Baskaran C, Velu S and Kumaran K: The efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by well diffusion method. Asian Pacific Journal of Tropical Disease 2012; 2: 658-62.
51. Mohapatra S, Xess I, Swetha JV, Tanveer N, Asati D, Ramam M and Singh MK: Primary cutaneous aspergillosis due to *Aspergillus niger* in an immunocompetent patient 2009.
52. Verweij PE, Chowdhary A, Melchers WJ and Meis JF: Azole resistance in *Aspergillus fumigatus*: can we retain the clinical use of mold-active antifungal azoles?. Clinical Infectious Diseases 2016; 62(3): 362-8.
53. Duong TM, Le TV, Tran KL, Nguyen PT, Nguyen BP, Nguyen TA, Nguyen HL, Nguyen BN, Fisher MC, Rhodes J and Marks G: Azole-resistant *Aspergillus fumigatus* is highly prevalent in the environment of Vietnam, with marked variability by land use type. Environmental Microbiology 2021; 23(12): 7632-42.
54. Salum LB, Polikarpov I and Andricopulo AD: Structure-based approach for the study of estrogen receptor binding affinity and subtype selectivity. Journal of Chemical Information and Modeling 2008; 48(11): 2243-53.
55. Kalyaanamoorthy S and Chen YP: Structure-based drug design to augment hit discovery. Drug discovery today 2011; 16(17-18): 831-9.
56. Marshall AC, Kidd SE, Lamont-Friedrich SJ, Arentz G, Hoffmann P, Coad BR and Bruning JB: Structure, mechanism, and inhibition of *Aspergillus fumigatus* thioredoxin reductase. Antimicrobial Agents and Chemotherapy 2019; 63(3): 02281-18.
57. Binder J, Shadkchan Y, Osherov N and Krappmann S: The essential thioredoxin reductase of the human pathogenic mold *Aspergillus fumigatus* is a promising antifungal target. Frontiers in Microbiology 2020; 11: 1383.
58. Saccoccia F, Angelucci F, Boumis G, Carotti D, Desiato G, E Miele A and Bellelli A: Thioredoxin reductase and its inhibitors. Current Protein and Peptide Science 2014; 15(6): 621-46.
59. Wu F, Zhou Y, Li L, Shen X, Chen G, Wang X, Liang X, Tan M and Huang Z: Computational approaches in preclinical studies on drug discovery and development. Frontiers in Chemistry 2020; 8: 726.
60. González Mera IF, González Falconí DE and MoreraCórdova V: Secondary metabolites in plants: Main classes, phytochemical analysis and pharmacological activities. Bionatura 2019; 4(4): 1000-9.
61. Mickymaray S: Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens. Antibioti 2019; 8(4): 257.
62. Bencheikh B, Cheriet M, Djemil R and Khatmi D: *In-silico* study of selected natural products as Sars-Cov-2 Mpro binder: molecular docking and molecular dynamics simulation. Polycyclic Aromatic Compounds 2022; 1-3.
63. Muthu C, Ayyanar M, Raja N and Ignacimuthu S: Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine 2006; 2(1): 1-0.
64. Singh S, Parmar N and Patel B: A review on Shalparni (*Desmodium gangeticum* DC.) and Desmodium species (*Desmodium triflorum* DC. & *Desmodium laxiflorum* DC.)–Ethnomedicinal perspectives. J Medicinal Plants 2015; 3(4): 38-43.
65. Appadurai P and Rathinasamy K: Indicine N-oxide binds to tubulin at a distinct site and inhibits the assembly of microtubules: a mechanism for its cytotoxic activity. Toxicology Letters 2014; 225(1): 66-77.

66. Yin J, Peng X, Lin J, Zhang Y, Zhang J, Gao H, Tian X, Zhang R and Zhao G: Quercetin ameliorates *Aspergillus fumigatus* keratitis by inhibiting fungal growth, toll-like receptors and inflammatory cytokines. International Immunopharmacology 2021; 93: 107435.

67. Bhimaneni S, Sharma R, Dey P and Kumar A: Pharmacovigilance of herbal medicines: An overview. Evidence Based Validation of Traditional Medicines: A comprehensive Approach 2021; 513-35.

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