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SHORT COMMUNICATION / KISA BİLDİRİ

INTERACTION OF PTERIDOPHYTIC BIOACTIVE COMPOUNDS WITH FUNGAL DIHYDROFOLATE REDUCTASE ENZYME AS INHIBITOR

PTERİDOPİTİK BİYOAKTİF BİLEŞİKLERİN İNHİBİTÖR OLARAK MANTAR DİHİDROFOLAT REDÜKTAZ ENZİMİ İLE ETKİLEŞİMİ

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ABSTRACT

Objective: *Fungal infections which are relatively common mainly invades the body of an immunosuppressed patients and people undergoing therapy. These pathogens act through different pathways like the Dihydrofolate reductase (DHFR) has a role in the folate synthetic pathway which is responsible for DNA synthesis. Since the early ages herbal remedies were used and have been tested for treating these fungal infections. Previous studies have revealed the use of bioactive molecules of pteridophytes to demonstrate antifungal activity.*

Material and Method: *In the present study different pteridophytes were selected from available library which showed the presence of bioactive phytoconstituents. In-silico studies on DHFR target (PDB ID: 6DRS and PDB ID: 3QLW) was carried out using PyRx program (India) to determine the affinity of bioactive molecules against the fungal strain.*

Result and Discussion: *Molecular docking was performed with 11 bioactive molecules showing activity against the selected target proteins. So, we can conclude that the selected bioactive molecules are active against fungal strain and can be further investigated for both in-vivo and in-vitro studies.*

Keywords: *Dihydrofolate reductase (DHFR), fungal infection, molecular docking, pteridophytes*

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ÖZ

Amaç: *Nispeten yaygın olan mantar enfeksiyonları esas olarak immün sistemi baskılanmış hastaların ve tedavi gören kişilerin vücudunu istila eder. Bu patojenler, DNA sentezinden sorumlu olan folat sentezinde rol oynayan dihidrofolat redüktaz (DHFR) gibi farklı yolaklar üzerinden etki gösterir. İlk çağlardan beri bitkisel ilaçlara bu mantar enfeksiyonlarını tedavi etmek için kullanılmış ve test edilmiştir. Önceki çalışmalar, antifungal aktiviteyi göstermek için biyoaktif pteridofit moleküllerinin kullanıldığını ortaya koymaktadır.*

Gereç ve Yöntem: *Bu çalışmada, biyoaktif fito bileşenlerinin varlığını gösteren mevcut kütüphaneden farklı pteridofitler seçilmiştir. Biyoaktif moleküllerin mantar suşuna karşı afinitesini belirlemek için Pyrx (Hindistan) programı kullanılarak, DHFR hedefi (PDB ID 6DRS ve PDB ID 3QLW) üzerine in-siliko çalışmalar gerçekleştirilmiştir.*

Sonuç ve Tartışma: *Seçilen hedef proteinlere karşı aktivite gösteren 11 biyoaktif molekül ile molekler yerleştirme çalışması gerçekleştirilmiştir. Buna göre, seçilen biyoaktif moleküllerin mantar suşuna karşı aktif olduğu ve hem in-vivo hem de in vitro çalışmalar için daha fazla araştırılabileceği sonucuna varılmıştır.*

Anahtar Kelimeler: *Dihidrofolat redüktaz (DHFR), mantar enfeksiyonu, moleküler yerleştirme, pteridofitler*

INTRODUCTION

In humans, fungal infections vary from topical mild rashes and itching to systemic diseases such as fungal pneumonia, meningitis, and bloodstream infections which can be fatal in nature [1]. Dermatophytes such as *Tinea capitis* or *Tinea corporis* are mainly responsible for topical infections [2] whereas Candida species like *Candida albicans* or *Candida glabrata* and Aspergillus species, viz, *Aspergillus flavus* are major examples of causing invasive fungal infections [3]. Currently, there are three classes of antifungal drugs that are used, viz, azoles, polyenes, and allyl amines [4]. These drugs act on various targets such as P-450 demethylase, squalene epoxidase, ornithine decarboxylase, alpha and beta tubulin, Dihydrofolate reductase (DHFR), etc. [3]. DHFR is an important target in cancer, microbial infections, malaria, tuberculosis, fungal infections etc. [5]. DHFR is involved in the folate synthetic pathway responsible for DNA synthesis which is initiated by the cellular uptake of folic acid through a specialized mechanism followed by the reduction of dihydrofolate to tetrahydrofolate (THF) in the presence of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) [3]. THF is a major coenzyme that serves as a carrier of one carbon unit for different enzymes during their interconversion between several oxidative states which is required for the biosynthesis of purines, methionine, and other important metabolites [6]. Various studies have been carried out using *in-silico* methods for the inhibition of DHFR (an important enzyme involved in the folate pathway) [3].

Development of resistance and relapse of disease are the major drawback of antifungal drugs. Therefore, there is a requirement to discover new antifungal therapies derived from plant sources having lesser side effects than conventional synthetic medicines [7]. In the current study, many Himalayan pteridophytes have been reported to have various bioactive molecules with appreciable antifungal activity [8,9]. The purpose of this study is to evaluate the selected compounds for antifungal potential against DHFR of Aspergillus flavus and *Candida albicans* using CADD (Computer Aided Drug Design).

MATERIAL AND METHOD

Various Himalayan pteridophytes were identified through literature search from different databases such as PubChem, PubMed, Google Scholar, ScienceDirect, etc. Among these, 20 were selected whose chemical constituents were disclosed based on GCMS analysis [10-12]. These selected pteridophytes provided a total of 180 bioactive compounds which were further explored for their fungal activity prediction. Additionally, *in-silico* studies were performed on the identified phytoconstituents.

Protein and Ligand Preparation

The foremost step in molecular docking is protein preparation which was done using the BIOVIA Discovery studio visualizer [13]. In this study, the target protein, Dihydrofolate Reductase, i.e., DHFR (PDB ID- 6DRS and PBD ID- 3QLW) were selected from Prot ein Data Bank (https://www.rcsb.org/). Protein preparation involves removing water molecules, heteroatoms, and ligands from the active site and further it was saved in .pdb format. Furthermore, all the 180 phytocompounds were retrieved from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/). These ligands were converted into 3D structures using ChemDraw 16.0 [14] and their smiles were generated for docking studies.

Molecular Docking

Molecular docking is an effective tool for identifying the most appropriate binding site of the protein where the ligand fits energetically as well as geometrically [15]. It investigates important molecular events including ligand binding modes and intermolecular interactions of the protein-ligand complex [16]. This study uses PyRx software to conduct docking studies. The selected 19 bioactive compounds were docked with DHFR to generate docking scores to predict the binding energies of the protein-ligand complex. The scoring function gives score based on the best docked ligand complex which is represented as a negative value in kcal/mol. The compounds with promising binding affinity are chosen for further analysis and visualization.

Visualization of Protein-Ligand Complex

Protein-ligand interactions are visualized and analysed via BIOVIA Discovery studio visualizer. Visualization of the docked protein-ligand model provides 2D and 3D structures of the complex with interacting bonds, bond category, bonding distance, and so on [17]. 2D structures display the various interacting amino acid residues bonded by hydrogen and hydrophobic bonds between the ligand and target protein [18] whereas 3D structures help to understand the molecular arrangement and how protein and ligand are bonded to each other.

RESULT AND DISCUSSION

Previous research unfolded the significance of DHFR activity in DNA synthesis and inhibition of DHFR is a well-established mechanism of action. To discover new potent antifungal agents, 180 phytoconstituents were identified on which docking studies were conducted against the target DHFR proteins. Molecular docking provides the binding affinities of the selected phytoconstituents. The results of the docking studies have been mentioned in Table 1.

Docking studies revealed that only 11 compounds (structures depicted in Figure1A) were recognized with very good binding affinity towards DHFR (PDB ID – 6DRS) of *Aspergillus flavus.* All these compounds depicted binding affinities even higher than the reference compound, i.e., 3-{[(3R)-7,9-diamino-3-methyl-2,3-dihydrofuro[2,3-*f*]quinazolin-4-yl]oxy}benzonitrile (-6.3kcal/mol), represented in Figure 1B and 1C. Furthermore, docking studies of the same compounds on DHFR (PDB ID-3QLW) of *Candida albicans* revealed that these 11 phytoconstituents showed good binding affinities as depicted in and among them, 6 phytoconstituents (PC-1, PC-2, PC-6, PC-8, PC-9, PC-11) exhibited exceptionally good binding affinities which are even higher than the reference compound, 5-[3-(2,5-dimethoxyphenyl) prop-1-yn-1-yl]-6-ethylpyrimidine-2,4-diamine with binding affinity -6.9kcal/mol, represented in Figure 1D and 1E. The docking results are stated in Table 1. Visualization of the compounds was carried out using BIOVIA Discovery studio visualizer for which results of 3D interactions are shown in Figure 2 and molecular interactions with amino acid residues are mentioned in Table 1 for both target proteins. The findings of this study can be applied to future research on several pathways for both *in-vitro* and *in-vivo* analysis against the fungus.

Table 1. Molecular docking results and amino acid interactions of phytoconstituents

Phyto-Consituents	Source	PDB-6DRS		PDB-3QLW	
		Docking Score (kcal/mol)	Amino Acid Interactions	Docking Score (kcal/mol)	Amino Acid Interactions
PC-1	*Blechnum orientale*	-7.4	TRP A:34, ILE A:10, ALA A:12, TYR A:162, VAL A:11, LEU A:32, ILE A:26, THR A:66, PHE A:44, LEU A:77, VAL A:70, ILE A:156	-7.3	ILE A:62, ILE A:112, ARG A:72, LEU A:69, PHE A:66, LYS A:37, PRO A:63, ILE A:33, PHE A:36, MET A:25,
PC-2	*Blechnum orientale*	-7.8	ASP A:40, VAL A:11, ILE A:26, GLY A:27, LEU A:32, THR A:31, SER A:69, THR A:66, GLY A:157, GLY A:158, TYR A:162, ILE A:156, ALA A:12, ILE A:10, PHE A:44	-7.8	PRO A:63, SER A:61, LEU A:69, MET A:25, ILE A:62, THR A:58, PHE A:36, ILE A:112, ILE A:33
PC-3	*Blechnum orientale*	-7.5	GLY A:27, GLY A:158, GLY A:157, ILE A:26, ALA A:12, TYR A:162, VAL A:11, ILE A:10, PHE A:44, ILE A:156, LEU A:32, ASP A:40, THR A:66, TRP A:34	-5.8	ILE B:135, ILE B:33, ILE B:112, TYR B:118, PHE B:36, ILE B:9, VAL B:10, GLE B:32, ALA B:11, MET B:25, TRP B:27, LEU B:29
PC-4	*Blechnum orientale*	-6.4	LEU A:77, THE A:44, ASP A:40, ILE A:10, LEU A:32, GLY A:158, GLY A:157, ILE A:156, THR A:66, TYR A:162, ALA A:12, VAL A:11, VAL A:70	-6.2	ILE B:33, PHE B:36, MET B:25, ILE B:112, THR B:58, LEU B:69, PRO B:63, ILE B:62, PHE B:66, ARG B:72, PRO B:70, LYS B:37
PC-5	*Calaguala*	-6.6	GLY A:157, GLY A:158, TYR A:162, ILE A:10, VAL A:11, PHE A:44, ALE A:12, LEU A:32, VAL A:70, LEU A:78, THR A:31, THR A:197, LEU A:77, GLY A:27, GLY A:30, ASP A:196, ILE A:156, THR A:66, ILE A:26	-6.3	GLU A:32, VAL A:10, ILE A:112, PHE A: 36, ILE A:33, PHE A:66, LEU A:69, LYS A:37, ARG A:72, PRO A:70
PC-6	*Drynoria quercifolia*	-7.5	ASP A:40, ALA A:12, TRP A:34, ILE A:26, LEU A:32, SER A:69, THR A:31, GLY A:30, THR A:66, GLY A:158, GLY A:157, PHR A:197, GLY A:27, TYR A:162, ILE A:156, VAL A:11, ILE A:10, PHE A:44	-7.2	VAL B:10, ILE B:9, PHE B:36, ILE B:62, LEU B:69, ILE B:33, LEU B:29, MET B:25, GLU B:32, TRP B:27, ALA B:11, TYR B:118
PC-7	*Drynoria quercifolia*	-7.0	THR A:197, GLY A:157, GLY A:27, TYR A:162, ILE A:156, PHE A:44, ASP A:40, ILE A:10, VAL A:11, ALA A:12, ILE A:26, LEU A:32, THR A:66, THR A:31, GLY A:30, SER A:69, GLY A:158, ALA A:159	-6.6	PHE A:36, ILE A:112, ILE A:33, PHE A:66, ILE A:62, MET A:25, PRO A:63
PC-8	*Drynoria quercifolia*	-6.9	GLU A:160, GLY A:158, THR A:197, SER A:169, THR A:66, LEU A:32, GLY A:27, ILE A:26, ASP A:196, GLY A:30, LYS A:65, ALA A:159	-7.7	GLU A:32, TRP A:27, MET A:25, ILE A:33, ARG A:72, LEU A:69, LYS A:37, PHE A:66, ALA A:11, ILE A:112, PHE A:36, ILE A:9, TYR A:118, VAL A:10
PC-9	*Pteris vitata*	-6.9	VAL A:11, ASP A:40, GLY A:157, TYR A:162, SER A:69, THR A:66, ILE A:26, LEU A:32, ALA A:12, TRP A:34, ILE A:156, PHE A:44	-7.4	PRO A:63, MET A:25, ILE A:62, ILE A:9, TYR A:118, VAL A:10, PHE A:36, GLU A:32, ILE A:112, THR A:58, LEU A:69
PC-10	*Equisetum arvense*	-6.4	ILE A:26, ALA A:12, VAL A:11, TRP A:34, ILE A:10, TYR A:162, ASP A:40, ILE A:156, LEU A:32, PHE A:44, GLY A:157, GLY A:158, THR A:66	-6.3	LEU A:69, PHE A:36, ILE A:112, THR A:58, MET A:25, ILE A:62, VAL A:10, ILE A:9, ALA A:11, GLU A:32, ILE A:33
PC-11	*Equisetum arvense*	-6.8	MET A:41, PHE A:44, ASP A:40, ALA A:12, LEU A:32, VAL A:11, TRP A:34, TYR A:162, ILE A:26, GLY A:157, GLY A:158, THR A:66, ILE A:156	-7.2	LYS A:24, SER A:61, THR A:58, ILE A:112, PHE A:36, LEU A:69, ARG A:72, LYS A:37, PHE A:66, ILE A:33, ILE A:62, PRO A:63, MET A:25

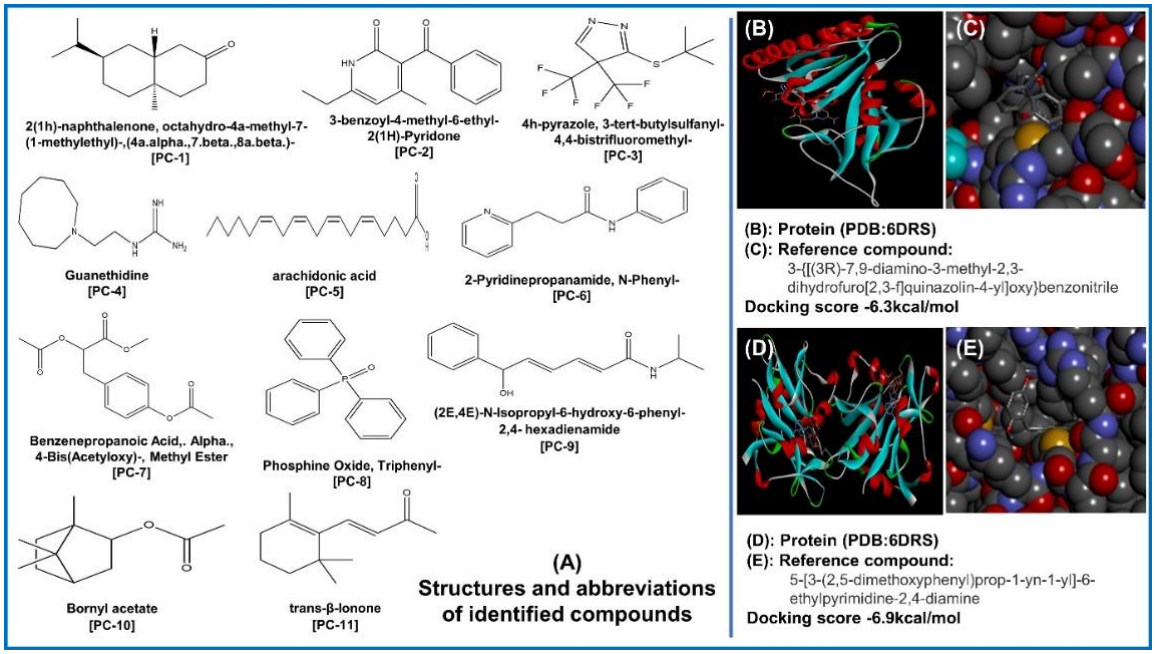

Figure 1. (A) Structure and abbreviations of identified pteridophytic molecules; (B) Protein structure of DHFR enzyme of *A. niger;*(C) Docking of reference molecule; (D) Protein structure of DHFR enzyme of *C. albicans*

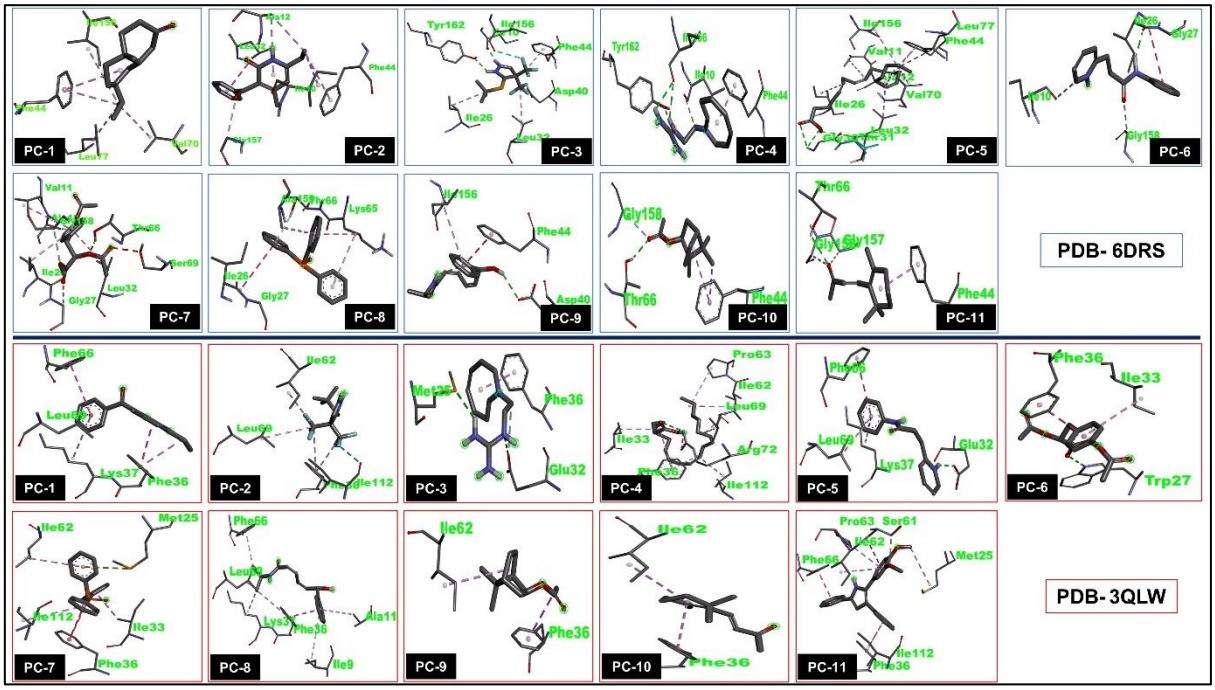

Figure 2. Pose view and binding scaffold of phytochemical compounds (PC-1 to PC-11) with DHFR enzyme (PDB: 6DRS and 3QLW)

DHFR inhibitors are a significant class of drugs, which is evident from their utilization as antibacterial, antimalarial, antifungal, and anticancer agents [19]. The DHFR protein plays a crucial role in the process of DNA synthesis during the development of bacterial and human cells [20]. To assess the potential antibacterial and anticancer properties, we performed docking studies of the selected ligands with the DHFR enzyme against *Aspergillus flavus* and *Candida albicans*. Surprisingly, we

observed docking profiles with significantly higher binding affinity compared to their respective standard molecules. The analysis of the docking study discovered that in the selected phytoconstituents, having large size and polar groups is essential for establishing favourable interactions with the proteins targeted for anti-fungal effects. The physical and chemical characteristics of these biologically active compounds, along with their intriguing binding interactions with specific proteins involved in treating fungal infections, can be utilized for combating fungal diseases and to progress the development of newer antifungal agents through *in-vitro* and *iv-vivo* studies targeting DHFR and other proteins.

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AUTHOR CONTRIBUTIONS

Concept: A.K., P.B.; Design: A.K., P.B.; Control: S.P., Sources: A.K., P.B.; Materials: M.S., M.R.; Data Collection and/or Processing: M.S., M.R.; Analysis and/or Interpretation: A.S., A.K.; Literature Review: M.S., M.R.; Manuscript Writing: M.S., M.R., A.S., A.K.; Critical Review: A.K., P.B., S.P.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Mujwar, S., Tripathi, A. (2022). Repurposing benzbromarone as antifolate to develop novel antifungal therapy for *Candida albicans*. Journal of Molecular Modelling, 28, 193. [CrossRef]
2. Hainer, B.L. (2003). Dermatophyte infections. American Family Physician, 67(1), 101-108.
3. Basak, T., Nath, V., Kumar, V., Goyal, A.K. (2021). *In silico* identification of antifungal compounds as mutant DHFRase inhibitors: structure-based approach, molecular dynamics simulation and structural integrity analysis. Journal of Computational Biophysics and Chemistry, 20(6), 589-602. [CrossRef]
4. Ghannoum, M.A., Rice, L.B. (1999). Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clinical Microbiology Reviews, 12(4), 501-517. [CrossRef]
5. Chawla, P., Teli, G., Gill, R.K., Narang, R.K. (2021). An insight into synthetic strategies and recent developments of dihydrofolate reductase inhibitors. Chemistry Europe, 6(43), 12101-12145. [CrossRef]
6. DeJarnette, C., Luna-Tapia, A., Estredge, L.R., Palmer, G.E. (2020). Dihydrofolate reductase is a valid target for antifungal development in the human pathogen *Candida albicans*. mSphere, 5(3), e00374-20. [CrossRef]
7. Kaushik, K., Agarwal, S. (2019). The role of herbal antifungal agents for the management of fungal diseases: A systematic review. Asian Journal of Pharmaceutical and Clinical research, 12(7), 34-40. [CrossRef]
8. Baskaran, X.R., Vigila, A.V.G., Zhang, S.Z., Feng, S.X., Liao, W.B. (2018). A review of the use of pteridophytes for treating human ailments. Journal of Zhejiang University-SCIENCE B, 19(2), 85-119.
9. Mimica-Dukic, N., Simin, N., Cvejic, J., Jovin, E., Orcic, D., Bozin, B. (2008). Phenolic compounds in field horsetail (*Equisetum arvense* L.) as natural antioxidants. Molecules, 13(7), 1455-1464.
10. Vastinge-Tuominen, M., Perera-Ivarsson, P., Shen, J., Bohlin, L., Rolfsen, W. (2004). The fern Polypodium decumanum, used in the treatment of psoriasis, and its fatty acid constituents as inhibitors of leukotriene b4 formation. Prostaglandins Leukotrienes and Essential Fatty Acids, 50(5), 279-284. [CrossRef]
11. Semwal, P., Painuli, S., Painuli, K.M., Antika, G., Tumer, T.B., Thapliyal, A., Setzer, W.N., Martorell, M., Alshehri, M.M., Taheri, Y., Daştan, S.D., Ayatollahi, S.A., Petkoska, A.T., Sharifi-Rad, J., Cho, W.C. (2021). *Diplazium esculentum* (Retz.) Sw.: Ethnomedicinal, phytochemical, and pharmacological overview of the himalayan ferns. Oxidative Medicine and Cellular Longevity, 2021, 1917890. [CrossRef]

12. Lin, L.J., Huang, X.B., Lv, Z.C. (2016). Isolation and identification of flavonoids components from pteris vittata l. SpringerPlus, 5(1), 1649. [CrossRef]
13. Chaturvedi, M., Kajal, N., Yadav, J.P. (2021). *In silico* approach for identification of natural compounds as potential COVID 19 main protease (Mpro) inhibitors. Virus Disease, 32, 325-329. [CrossRef]
14. Aliye, M., Dekebo, A., Tesso, H., Abdo, T., Eswaramoorthy, R., Melaku, Y. (2021). Molecular docking analysis and evaluation of the antibacterial and antioxidant activities of the constituents of *Ocimum cufodontii*. Scientific Reports, 11(1), 10101. [CrossRef]
15. Azam, S.S., Abbasi, S.W. (2013). Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. Theoretical Biology and Medical Modelling, 10, 63. [CrossRef]
16. Ferreira, L.G., Dos Santos, R.N., Oliva, G., Andicopulo, A.D. (2015). Molecular docking and structure-based drug design strategies. Molecules, 20, 13384-13421. [CrossRef]
17. Susmi, T.F., Khan, M.R., Rahman, A., Mahmud, S., Saleh, A., Reza, A., Rahi, S. (2022). *In vitro* antioxidant and cytotoxicity activities and *in silico* anticancer property of methanolic leaf extract of *Leucas indica*. Informatics in Medicine Unlocked, 31, 100963. [CrossRef]
18. Pathak, R.K., Gupta, A., Shukla, R., Baunthiyal, M. (2018). Identification of new drug-like compounds from millets as Xanthine oxidoreductase inhibitors for treatment of Hyperuricemia: A molecular docking and simulation study. Computational Biology and Chemistry, 76, 32-41. [CrossRef]
19. James, J.P., Ail, P.D., Crasta, L., Kamath, R.S., Shura, M.H., Sindhu, T.J. (2023). *In silico* ADMET and molecular interaction profiles of phytochemicals from medicinal plants in Dakshina Kannada. Journal of Health and Allied Sciences NU (accepted). [CrossRef]
20. Article, O., Markandeyan, D., Kannaiyan, S., Suresh, S., Nimmakayala, R., Ilamurugan, R., Santhalingam, K., Paul, B. (2016). Virtual screening of phytochemicals for methotrexate like dihydrofolate reductase and aminoimidazole-4-carboxamide ribonucleotide (aicar) transformylase inhibitory property using molegro virtual docker. International Journal of Pharmacy and Pharmaceutical Sciences, 8(5), 83-87.