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Research Article

MOLECULAR DOCKING OF PHYTOCHEMICALS AS INHIBITORS OF EGFR IN NON-SMALL CELL LUNG CANCER

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ABSTRACT

Objective: The epidermal growth factor has a predominant role in the pathogenesis of non-small cell lung cancer. The current is focused on addressing the phytocompounds for Ashwagandha as a potential therapeutic target for non-small cell lung cancer.

Methods: ADME analysis and PyRx were used to screen the phytochemicals selected from IMPPAT (Indian Medicinal Plants, Phytochemistry, And Therapeutics). The EGFR protein was retrieved from PDB (5ZWJ). The ligands with poor binding affinity were removed, and the remaining ligands were advanced to docking with AutoDock Tools.

Results: The assessment of the ligands with that of proteins portrayed that the ligands Withaferin A, Withanolide Q, Withanolide R, and Casuarinin have a better binding affinity with the protein.

Conclusion: Withaferin is a useful phytochemical found in ashwagandha and has a specific inhibitory effect on EGFR; therefore, it can be utilized as a therapeutic target for non-small cell lung cancer.

Keywords: EGFR, Phytocompounds, Non-small cell lung cancer, Medicinal plants, Docking, Cancer.

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INTRODUCTION

Even though lung cancer has been eclipsed by breast cancer as the most often occurring cancer worldwide, it remains a primary cause of cancer death. In the year 2020, a projected 2,206,771 new lung cancer cases will be diagnosed worldwide, with 1,796,144 deaths attributable to lung cancer [1]. Small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two most common kinds of lung cancer (NSCLC). Approximately 85% of all lung cancer cases are caused by the latter [2]. The creation of small-molecule inhibitors of target proteins involved in apoptosis, proliferation, and angiogenesis has led to the development of small-molecule inhibitors of target proteins involved in apoptosis, proliferation, and angiogenesis. The EGFR superfamily, which includes the four different receptors EGFR/erbB-1, HER2/erbB-2, HER3/erbB-3, and HER4/erbB-4, was discovered as a possible therapeutic target in solid tumors early in on. These receptors homo and heterodimerize after ligand binding, and their tyrosine kinase domain is activated, triggering a cascade of events implicated in cancer initiation and progression through impacts on cell cycle progression, apoptosis, angiogenesis, and metastasis. Small molecule inhibitors have been discovered to sensitize tumor cells to apoptosis when these kinases are inhibited [3-7].

Natural phytochemicals can serve as a small-molecule inhibitor, which could be beneficial to cancer patients both financially and psychologically. Plants contain not just the required nutrients for life, but also bioactive phytochemicals that aid in disease prevention and health promotion [8]. Plants create phytochemicals, also known as secondary metabolites, which are non-nutritive chemical molecules produced by a variety of chemical routes. A vast variety of phytochemicals has been shown in recent studies to be advantageous to the function of human cells [8]. Several of these phytochemicals are physiologically active substances found in nature with high anticancer potential. Natural phytochemicals can serve as a small-molecule inhibitor, which could be beneficial to cancer patients both financially and psychologically.

METHODS

Plants and their phytochemicals

Ocimum tenuiflorum

In India, *Ocimum tenuiflorum* is known as Holy Basil or Tulsi. It is a traditional Southeast Asian medicine that is commonly utilized in India. It has anti-inflammatory, analgesic, antipyretic, antidiabetic, hepatoprotective, hypolipidemic, antistress, and immunomodulatory properties, according to scientific studies [9]. Some of its phytochemicals, such as eugenol, rosmarinic acid, apigenin, myretenal, luteolin,–sitosterol, and carnosic acid, have been shown in preclinical studies to prevent chemical-induced skin, liver, oral, and lung cancers and to mediate these effects by increasing antioxidant activity, altering gene expressions, inducing apoptosis, and inhibiting angiogenesis and metastasis [9].

Withania somnifera

In India, *W. somnifera*, often known as Ashwagandha, is a *Solanaceae* shrub. Some proteins produced from *W. somnifera* have been revealed to have anticancer action in recent scientific research [10]. Withaferin A (WA) is a cell-permeable steroidal lactone that was first isolated from Withaferina somnifera as a potential anti-lung cancer drug [11].

Terminalia arjuna

The Terminalia arjuna tree belongs to the Terminalia genus. In English, it is known as arjuna or Arjun tree, and it may be found all over the Indian Subcontinent. Casuarinin, a hydrolyzable tannin derived from the bark of Terminalia arjuna and inhibits human non-

small cell lung cancer A549 cells by preventing cell cycle progression in the G0/G1 phase and triggering apoptosis, according to scientific investigations [12].

Ramachandran plot

The Ramachandran plot is a method for determining the secondary structure of proteins. The Zlab tool is used to check whether protein residues are legitimate or not, and it generates a plot called a Ramachandran plot. The alpha-helix and beta sheets make up the secondary structure of a protein; its orientation is computed using the torsion angle and indicates whether it is in the favored, allowed, or prohibited zone.

ADME analysis

Potential phytochemicals found in *O. tenuiflorum, W. somnifera,* and *Terminalia arjuna* were analyzed using SwissADME (http://www.swissadme.ch/index.php). Compounds that followed the "Lipinski Rule of Five" (showed no infractions) were screened.

Screening of ligands

PyRx-Virtual Screening Tool was used to testing possible phytochemicals. UFF (Universal Force Field) was used as the force field for Energy Minimization, with conjugate gradient as the optimization procedure. The ligands with the highest binding affinity were docked even more.

Molecular docking

The docking studies were carried out using AutoDock Tools (version 1.5.6) and the Lamarckian genetic algorithm. The following are the parameters that were taken into account: The population size is 150, the rate of gene mutation is 0.02, the rate of crossover is 0.8, the GA Crossover mode, and the maximum number of generations is 27000. PyMOL (version 2.4.2) was used to show the docking findings, and the files were in.pdbqt format.

RESULTS AND DISCUSSION

Docking results

A green dense zone or green cross in the Ramachandran plot reflects highly favored observations, while preferred observations are depicted in brown triangles. The red circles show questionable observations.

In the supplied Ramachandran plot (Fig. 1), it was discovered that the allowed zone has a large number of alpha-helices in the third quadrant. The same is true for the beta-strand; they cover the first quadrant and may be observed in the permissible region because they are both represented by a green cross. We can say that the EGFR protein is of acceptable quality and that it can be docked with phytochemicals because our protein residues fall within the authorized range.

Because our protein residues are within the allowed range, we can declare that the EGFR protein (Fig. 2) is of acceptable quality and that it can be docked with phytochemicals. When Eugenol, Plumbagin, and Luteolin were screened using PyRx were screened using PyRx (Table 1), it was discovered that they have a low binding affinity, with −5.8, −6.2, and −8.1, respectively. The strength of a ligand's binding to a protein is calculated using binding affinity. As a result, these phytochemicals were removed, leaving just Apigenin, Withaferin A, Withanolide Q, Withanolide R, and Casuarinin for docking.

Binding energies ranged from −2.44 to −9.90 when ligands were docked with proteins.

In general, high-affinity ligand binding is characterized by a higher intermolecular force between the ligand and its receptor, whereas low-affinity ligand binding is characterized by a lower intermolecular force. Withaferin A and Casuarinin had the lowest and highest binding energies, respectively (Table 2) [13]. There were four hydrogen bonds and one salt bridge found in withaferin (Fig. 3). Withaferin A may be a possible small molecule inhibitor with a crucial role in non-small cell lung cancer due to its low binding energy, hydrogen bonds, amino acids implicated in interaction, and RMSD value.

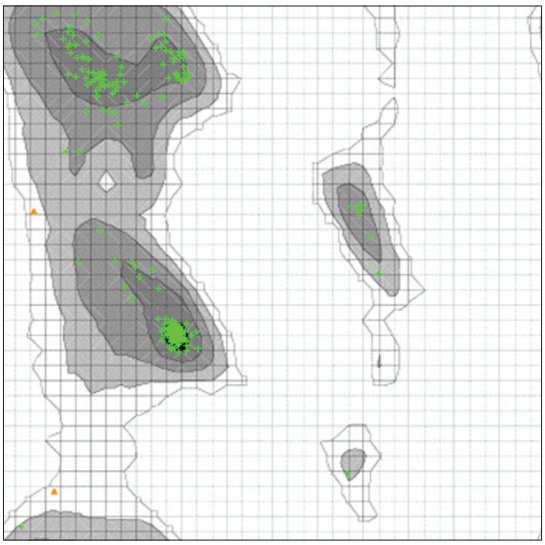

Fig. 1: Ramachandran Plot visualized in Zlab

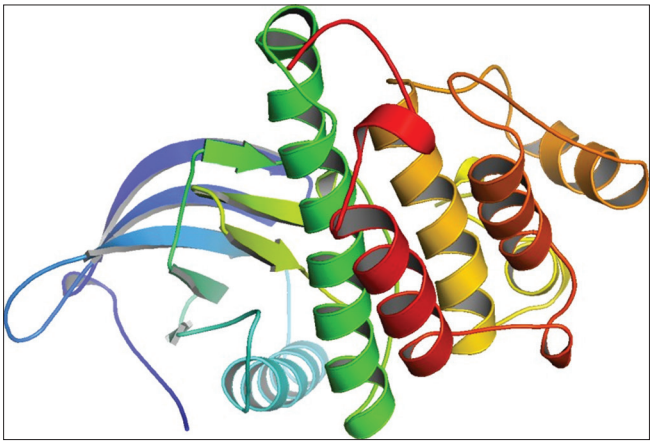

Fig. 2: Purified EGFR protein

Table 1: Docking of selected ligands with EGFR protein

S. No.	Plant Name	Phytochemicals	Binding affinity
1	*Ocimum tenuiflorum* (holy basil or tulsi)	1a. eugenol 1b. apigenin	−5.8–8.0
2	*Withania somnifera (Ashwagandha)*	2a.Withaferin A 2b. Withanolide Q 2c. Withanolide R	−8.4 −8.8 −9.2
3	*Plumbago zeylanica (doctorbush* or *wild leadwort)*	3a. Plumbagin	−6.2
4	*Terminalia arjuna (arjuna or arjun tree)*	4a. Casuarinin 4b. Luteolin	−9.4–8.1

(Red color score indicates the ligands that are selected for docking)

Hydrogen bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
1	855A	ASP	2.69	3.45	138.85	Yes	Yes	1542 [O3]	32 [O2]
2	855A	ASP	3.36	3.83	110.68	Yes	No	1536 [Nam]	33 [O3]
3	856A	PHE	2.78	3.41	121.30	Yes	No	1545 [Nam]	33 [O3]
4	856A	PHE	2.17	2.80	120.85	No	No	33 [O3]	1548 [O2]

Table 2: Amino acid interactions for the selected ligands

S. No.	Compound	RMSD	Binding energy (Kcal/Mol)	Inhibition constant (Ki)	No. of H bonds	Interacting amino acids
1	Casuarinin	18.71	−2.44	16.40 mM	7	Ile918(A), Ala920(A), Lys879(A), Val876(A), Arg803(A), Arg841(A)
2	Apigenin	23.28	−7.30	4.49 uM	6	Glu762(A), Asp855(A), Thr854(A), Leu788(A), Met790(A)
3	Withaferin A	19.18	9.90	55.61 nM	4	Asp855(A), Phe856(A)
4	Withanolide Q	35.58	−8.43	659.94 nM	1	Glu762(A)
5	Withanolide R	20.76	−9.04	234.79 nM	1	Lys (745)

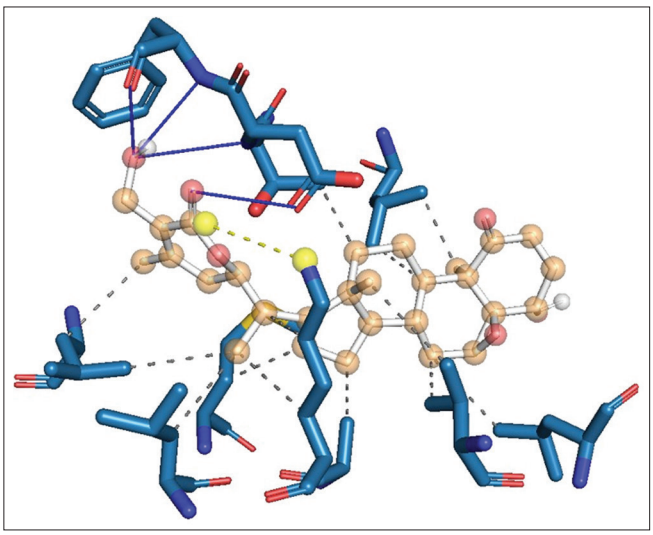

Fig. 3: Docked EGFR with Withaferin A (ball and stick) showing 4 Hydrogen bonds (blue), salt bridge (yellow), and hydrophobic interactions (grey-dotted)

CONCLUSION

Ashwagandha is used to cure a variety of ailments in Indian traditional Ayurvedic medicine, including tumors, inflammations, conjunctivitis, and tuberculosis. We have established that Withaferin is a useful phytochemical. A compound found in ashwagandha has a specific inhibitory effect on EGFR, a therapeutic target for non-small cell lung cancer. It has the potential to aid in the treatment of lung cancer. Ashwagandha has been found in preclinical trials to be useful in slowing the growth of lung cancer cells. People are now accepting ayurvedic medicines and attempting to live a healthy lifestyle. Cancer patients can benefit from naturally occurring phytochemicals if they are eaten properly.

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AUTHORS CONTRIBUTION

All the authors have contributed equally to the paper

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Nil.

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