Analyzing Efficacy of Allium sativum L. Phytoconstituents with Epidermal Growth Factor Receptor (PDB:1M17) Target Protein of Lung Adenocarcinoma: Molecular Docking Studies

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Abstract
Background: Lung cancer poses a significant challenge to modern society despite the availability of several treatment options, including surgery, chemotherapy, and immunotherapy. Factors such as late diagnosis, drug resistance, adverse effects, high cost of medication approval, and low drug efficacy further make the condition chaotic. Epidermal growth factor receptor (EGFR) (PDB ID: 1m17) is one of the target proteins of lung cancer and is preferred for determining the most effective and promising drugs from plant extracts. Allium sativum L., which has been used since ancient times in the treatment of various diseases, has been reported to have some sulfurous compounds having an anticancer effects. **Methods**: Herein, molecular docking was used to dock the phytochemicals of Allium sativum L. on EGFR (1m17) target proteins to determine know its exact efficacy. Auto Dock Vina or 4.2, BIOVIA Discovery Studio, and Pymol were used for docking and visualization of the docked complex to calculate the binding energy score and amino acid residues of the binding pocket. **Result:** Out of fifty-four compounds of garlic, only 21 compounds have drug-like properties used for further docking. Molecular docking revealed that out of a total of 21 docked compounds, only five phytoconstituents, allixin (−5.9), carvacrol (−5.8), alliin (−4.6), sally-cysteine (−4.2), and 2,6-Dimethylpyrazine (−4.2), have better efficacy for EGFR. **Conclusion:** Allixin and carvacrol have potential as anticancer drugs and can be used as therapeutic agents in in vitro and in vivo studies of drug design for lung cancer treatment. This research will serve as a strong foundation for future research and the development of therapeutic application techniques for medications made from various plant extracts.

Keywords: binding energy, garlic phytoconstituents, in-silico docking, lung cancer, nonsmall lung cancer, target protein

1. Introduction

Lung cancer is highly heterogeneous and is the second most common cause of death worldwide. Globacon 2020, estimated, total 19,292,789 new cancer cases in both sexes of all age in all over the world, from which lung cancer consist of 22,06,771 cases. The mortality rate of lung cancer is also high, accounting for 1,796,144 deaths from total deaths 9,958,133 from all types of cancer worldwide. Breast cancer surpasses lung cancer with an estimation of 2.3 million new cases (11.7%) of total death [1]. In Asia, both the incidence and death rates of non-small cell lung cancer have increased significantly over the last few decades, with approximately 80% of patients diagnosed with advanced stages. Adenocarcinoma is the most common type of lung cancer, accounting for approximately 40% of lung cancers present in the lining of the bronchi or outer region of the lung. Although it is associated with

tobacco use, it is also found in people who have never smoked [2]. Genetic mutations, smoking, ionizing radiation, and other factors cause abnormalities in some oncogenes such as Epidermal growth factor receptor (EGFR), KRAS, ALK, RET, MET, ROS, BRAF, HER2/ERBB2, and ROT [3]. Mutations in these genes cause abnormalities in cell signaling pathways. Furthermore, uncontrolled protein kinases of the signaling pathways, such as MAPK/ERK, JAK/STAT, and PI3K/AKT, are the leading causes of cancer; only genetic factors account for more than 8% of lung cancer [4,5]. However, most lung cancers are associated with EGFR and KRAS mutations. Cigarette smoking is the most significant risk factor for EGFR mutation [6]. Depending on the stage of the malignancy, the most commonly used lung cancer treatment modalities are surgery, radiation, chemotherapy, immunotherapy, and conventional drugs such as crizotinib, gefitinib, and erlotinib [7,8]. However, these treatments have limited efficacy against lung cancer and other limitations such as side effects, high costs, and poor five-year survival rates [9]. Therefore, there is a need for an alternative method, or a combined treatment method, targeted drugs, or natural products with chemotherapeutic drugs to overcome the problem of drug resistance [10]. Garlic consists of organosulfur compounds such as allixin, diallyl sulfide, diallyl disulfide, allicin, ajoene, and s-allyl cysteine, which have a wide range of health benefits, such as lowering of blood lipids, blood pressure, and microbial (viral, fungal, and bacterial) growth inhibitors and anticancer effects [11]. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfate or diallyl disulfide), and the most abundant sulfurous compound is alliin (S-allyl cysteine sulfoxide), which is present in fresh and dry garlic at 10 and 30 mg/g, respectively [12,13]. Evidence exists that Unani Hakims, Indian Vaidas, European and Mediterranean cultures, as well as indigenous cultures such as Rome, Egypt, China, India, Iran, Africa, and America, have used garlic in their traditional medical systems since ancient times [14]. Conventional drug discovery takes over 10–15 years for new drug synthesis and approval, which is very high, at approximately $880 million. Of all the drugs that reach the market, only a few are approved by the FDA. Moreover, more than half of the drugs failed in the last stage of the clinical trials. Furthermore, some new drugs are withdrawn from the market soon after their approval due to severe side effects and clinical risks that were not detected in Phase III trials. To avoid these problems related to conventional therapy or chemical drug development, researchers have focused on CAM therapy using herbal medicine with a higher success rate, negligible side effects, and low cost of synthesis [15]. Theoretically, when disease targets have been identified, candidate pharmaceuticals can be prepared by choosing from a list of already approved medications or by designing the candidate drug at the molecular level using a computer-aided design programme. While identifying disease and medication targets is vital, it is also essential for drug development to understand how the targets interact [16].

Various studies have found that Allium sativum L. has anticancer activity against lung cancer, but the mechanism of action or efficacy of its phytoconstituents is not clear until now. In view of that, to explore the potential of garlic phytoconstituents as anticancer agents, we focused on one of the target proteins, EGFR (PDB:1M17), which is responsible for most adenocarcinoma types in lung cancer [17]. The aim of this study was to analyze the efficacy of garlic phytoconstituents on specific receptors, such as EGFR (PDB:1M17). Molecular docking studies were used to assess the binding affinity of each of the phytoconstituents of garlic with drug-like properties against EGFR, which further helped to explore the mechanism of anticancer activity. Understanding their binding site and target–ligand interactions shortens the time duration and animal models of drug finding experiments [18]. Using computational methods, we identified just five ligands with the ability to fit geometrically and energetically into the binding pocket of the target protein and have regulatory roles in lung cancer. These ligands can be exploited in the future for the development of novel lung cancer drugs.

2. Materials and methods

The hardware and software used in this work for molecular docking study includes: laptop (11th Gen Intel(R) Core (TM) i3-1115G4, 3.00 GHz processor, 8.00 GB RAM, Windows 11 operating system.) Our methodology consisted of a few sets of parts, such as 1) Ligand selection and preparation, 2) Screening of Promising compound, 3) Target selection, and 4) Docking, and their detailed descriptions are provided below:

2.1 Ligand selection and preparation

Phytoconstituents of Allium sativum L. showing medicinal properties were retrieved from Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT), a curated database, as well as from several published studies on phytochemical ids and canonical smiles. The initial data set included 51 phytoconstituents with medicinal value and was used in screening to identify the most promising compounds [19]. The 2D SDF structures were retrieved from the PubChem database [20], and imported into the workspace for ligand preparation to remove the heteroatoms. Open Babel, a chemical toolbox, was then used to convert the SDF file of each molecule into PDB format [21], which was further read by Swiss ADME.

2.2 Screening of promising compound

We filtered the retrieved ligands to remove compounds that produced false positive results using Pan Assay Interference Compounds (PAINS) Remover [22], and the resulting compounds were further refined by Swiss

ADME based on their ADMET properties [23] DruLiTo is an open-source virtual screening tool downloaded and installed from the website [24] used to evaluate drug-likeness properties and bioactivity of all phytocompounds based on Lipinski's rule of five. Table 1 shows the results of ligand screening, which included only 21 phytochemicals. These were then used to dock on the active binding sites of the target protein.

2.3 Target selection

For the selection of target proteins of lung cancer, primarily of the adenocarcinoma type, Google Scholar, Medline, PubMed, and literature were searched. In this study, EGFR was selected for docking from numerous target proteins, such as EGFR, BRAF, and KRAS, which play the greatest role in lung cancer causing and docked with garlic phytoconstituents. Their X-ray crystallographic and NMR solved 3D structures were downloaded from the RCSB Protein Data Bank (PDB ID: 1M17) with a good resolution of 2.60 Å as shown in figure 1. The downloaded PDB structure of the EGFR tyrosine kinase domain was present in complex with the 4-anilinoquinazoline inhibitor erlotinib [25]. Proteins were prepared for docking by removing all heteroatoms and water molecules.

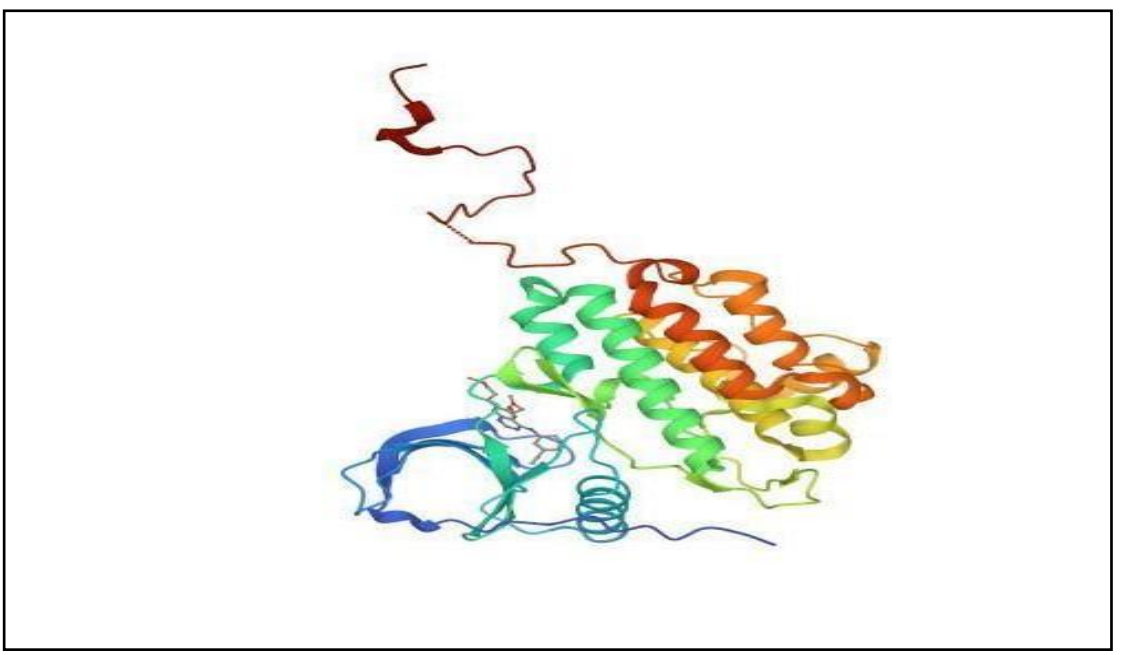

Fig. 1: The 3D pdb structure of EGFR (1M17) target protein of lung cancer (https://www.rcsb.org/).

The Swiss PDB viewer 4.1, downloaded and installed from the website [26], was used for energy minimization of proteins by removing cocrystallized ligands and water molecules to reduce bad van der Waals forces. This energy-minimized peptide was further used to predict the active site and xyz coordinates of binding sites involved in receptor–ligand interactions.

2.4 Molecular docking

The target protein consists of various binding sites on which ligands can bind. Active sites and xyz coordinates of the active site in the form of configure files were predicted using the BIOVIA Discovery Studio. The top-ranked 10 active sites were generated, but site 1 was used to calculate the grid size dimension and XYZ coordinates, which were further used in docking. The binding mode, affinity, and selectivity of the phytoconstituents to the target protein were determined using Auto Dock Vina. Auto Dock Vina is a source tool used that is more accurate and easier to use for molecular docking and novel drug designing. Herein, 21 ligands were docked on the predicted xyz coordinates (x-24.951366, y-1.083000, and z-54.454000) of the target protein. A grid map was formed around the active site by using 60 × 60 × 60 points using an auto grid program, and the grid was placed in the center of the active site with xyz coordinates (x-24.951366, y-1.083000, and z-54.454000). Ligands were clustered on the receptor's active site in different orientations, and docking results were retrieved in the form of binding energy ranked according to the auto-dock scoring function. Pymol was further used for visualization of ligand–receptor complex and first orientation was selected for retrieval of amino acid residues of binding site. Amino acid residues, type of interactions, and distance between them were determined for each ligand using the first ligand–receptor complex in BIOVIA Discovery Studio. By comparing the binding affinities of docked molecules, the best conformation pose with the highest scoring value was selected for future studies.

3. Results and Discussion

3.1 Molecular docking

The growing interest in anticancer drug development from natural products indicates the potential role of garlic as an anticancer drug in lung cancer treatment. EGFR was selected as the target protein in this study for docking to search for promising phytoconstituents of garlic with anticancer properties. Fifty-one phytoconstituents of Allium sativum L. were retrieved from IMPPAT, and some others, such as S-allyl-l-cysteine sulfoxide (alliin), γ-Glutamyl-L-cysteine peptides, S-allyl-l-cysteine, and z-ajoene, were taken from the literature and have medicinal value in lung cancer treatment. To find a new compound to reduce cancer progression, EGFR receptor protein (PDB: 1m17) was docked with only 21 phytoconstituents with good ADME properties, as listed in Table 1. The binding site and xyz coordinates of the active site (x-24.951366, y-1.083000, and z-54.454000) of the target protein were predicted and docked with each of the 21 promising phytoconstituents of garlic. The molecular docking method was used to determine the binding affinity of all 21 ligands. The results were examined based on binding energy and hydrogen bonds or other bonds and amino acid residues of the binding site of the target protein with each ligand. As displayed in table 1. The binding affinity of each target–receptor complex is expressed in terms of (kJ/mol), and this shows that complexes with a higher negative score were thought to be more relevant for future research.

Table 1: Binding energy of garlic phytoconstituents docked on lung cancer target protein EGFR (1m17)

S. no.	Phytochemical id	Phytochemical name	Binding energy (kJ/mol)
1.	CID:65036	Allicin	−3.8
2.	CID:86374	Allixin	−5.9
3.	CID:16590	Diallyl disulfide	−3.5
4.	CID:7938	2,6-dimethylpyrazine	−4.2
5.	CID:5386591	E-ajoene	−3.8
6.	CID:10451	1,3-dithiane	−2.8
7.	CID:10364	Carvacrol	−5.8
8.	CID:8103	1-hexanol	−3.4
9.	CID:12514	2,5-dimehylthiophene	−3.8
10.	CID:31252	2,5-dimethylpyrazine	−4.1
11.	CID:5352855	(E)-1-Propenyl 2-propenyl disulfide	−3.6
12.	CID:536652	Disulfide, methyl 1-propenyl	−2.8
13.	CID:19310	Dimethyl trisulfide	−2.2
14.	CID:16315	Diallyl trisulfide	−3.6
15.	CID:11617	Diallyl sulfide	−3.1
16.	CID:16591	Allyl propyl disulfide	−3.2
17.	CID:61926	Allyl methyl trisulfide	−3.0
18.	CID:150636	3-Vinyl-4H-1,2-dithiin	−4.0
19.	CID:87310	Alliin	−4.6
20.	CID:9881148	Z-ajoene	−4.1

21.	CID:9793905	S-allyl cysteine	−4.2

From 21 compounds, we selected the five best compounds with more negative binding affinity, allixin (−5.9), carvacrol (−5.8), alliin (−4.6), s-allyl cysteine (−4.2), and 2, 6-Dimethylpyrazine (−4.2), which showed the highest ranks as compared to other compounds. These five docked ligands were used for further visualization and investigation of the binding sites and amino acid residues, interactions, and the distance between them. The types of interactions, bond lengths, and amino acid residues of the selected five ligands are shown in Table 2.

Table 2: Bonding pattern of allixin, carvacrol, alliin, s-allyl cysteine and 2,6-dimethylpyrazine with EGFR 1m17 target protein

S.no.	Phytochemicals	Types of interaction	Amino acid Residue of binding site Of EGFR (1M17)	Ligand atom	Distance (Å)
1.	Allixin	Conventional H-bond	Met769: HN Thr766: HG1	UNL1:C UNL1: O	1.89 2.40
		Carbon-H Bond	Met769: O	UNL1:C	3.60
		Alkyl	Lys721 Lys721 Leu764 Val702 Ala719	UNL1: O UNL1 UNL1:C UNL1:C UNL1	3.88 5.47 4.56 3.93 4.79
		Pi-alkyl	Ala719 Val702	UNL1 UNL1	4.00 5.19
		Pi-sigma	Leu820: CD1	UNL1	3.48
2.	Carvacrol	Alkyl	Met742 Lys721 Leu764 Val702 Leu820	UNL1:C UNL1 UNL1 UNL1 UNL1	4.88 4.22 3.84 3.99 5.37
		Pi-alkyl	Lys721 Val702 Ala719	UNL1 UNL1 UNL1	4.14 5.10 4.86
		Pi-sulfur	Met742: SD	UNL1	5.92
3.	Alliin	Conventional H-bond	Asp831: HN Thr766: HG1 Thr766: OG1	UNK1:O UNK1:O UNK1:H	2.40 2.72 2.58
		Alkyl	Met769 Leu820	UNK1:C UNK1:C	5.26 4.23
		Unfavorable acceptor-acceptor	Glu738: OE2	UNK1:O	2.82
4.	S-ally cysteine	Conventional H-bond	Asp831: NH	UNK1:O	2.25
		Alkyl	Ala719 Ala719 Val702 Leu820	UNK1:C UNK1:C UNK1 UNK1:C	4.05 4.25 4.87 4.63

5.	2,6-Dimethylpyrazine	Carbon-H- Bond	Glu738: OE2	UNL1:C	3.71
		Alkyl	Leu764	UNL1:C	4.15
			Met742	UNL1:C	4.87
			Leu820	UNL1:C	5.05
			Ala719	UNL1:C	3.77
			Lys721	UNL1:C	5.00

3.2 Allixin-EGFR complex

Molecular docking of Allixin on EGFR had the greatest binding affinity (i.e., −5.9 kj/mol) among the 21 screened compounds, which had shown drug-like properties. The binding interaction of allixin with wild-type EGFR (1m17), a human lung cancer protein, is shown in Figure 2. The amino acid residues involved in the highest binding affinities and drug-like qualities in the binding site of the target proteins were Lys721, Leu764, Leu820, Val702, Ala719, Met769, and Thr766. The Met769 and Thr766 amino acid residues form conventional hydrogen bonds with the oxygen of Allixin at a distance of 1.89 and 2.40 Å. Similarly, the Oxygen of Met769 was observed to form a carbon–hydrogen bond at distance of 3.60 Å increasing the oral bioavailability according to Lipinski's rule of five. Lys721, Leu764, Val702, and Ala719 had alkyl interactions at distances of 3.88, 4.56, 3.93, and 4.79 Å, respectively, establishing stability of ligand–receptor complex. This provided strong evidence about ligand (Allixin) blocking the mutation site such as leucine, methionine, and Threonine of EGFR protein involved in cell proliferation and cell signaling pathway activation [27]. Ala719 and Val702 form pi–alkyl interaction with bond length of 4.00 and 5.19 Å and leu820 exhibited a pi–sigma bond with a length 3.48 Å. The result of the complex stability can be linked to the extra pi–sigma interactions associated with (leu820) and pi–alkyl interactions (Ala719 and Val702).

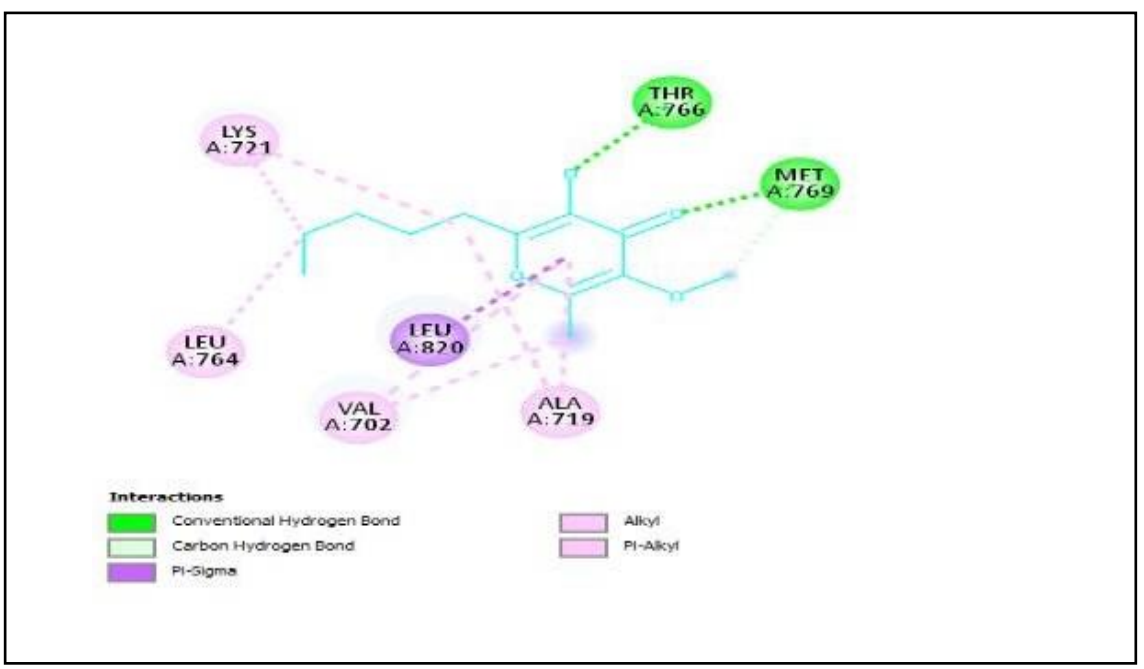

Fig. 2: The 2D structure of docked complex of Allixin with EGFR (1m17) target protein

3.3 Carvacrol-EGFR complex

Carvacrol docked on the EGFR binding site had the second highest position when compared on the basis of binding affinity (−5.8 kj/mol), as shown in Table 3. The 2D structure of the carvacrol–EGFR complex is shown in figure 3, showing alkyl interactions with amino acid residues such as Lys721, Ala719, Leu764, Val702, and Leu820, which play a role in carvacrol binding at the binding pocket of EGFR. Unlike Allixin, carvacrol does not have any hydrogen bonds but establishes only electrostatic and alkyl interactions with residues Lys721, Leu764, Val702, and Leu820 with bond lengths of 4.22, 3.84, 3.99, and 5.37 Å, respectively, which helped to increase binding affinity and stability of the complex. Some of them also had shown pi–alkyl and pi–sulfur interactions. Met742 establishes pi–sulfur interactions with bond length 5.92 Å and Ala719, lys721, and val702 formed pi–alkyl interaction with distance 4.14 and 5.10 Å. complex stability can be linked to other interactions such as pi–cation (Lys833), pi–sulfur (Met742), and pi–alkyl interactions with amino acids Ala719, lys721, and val702 [28].

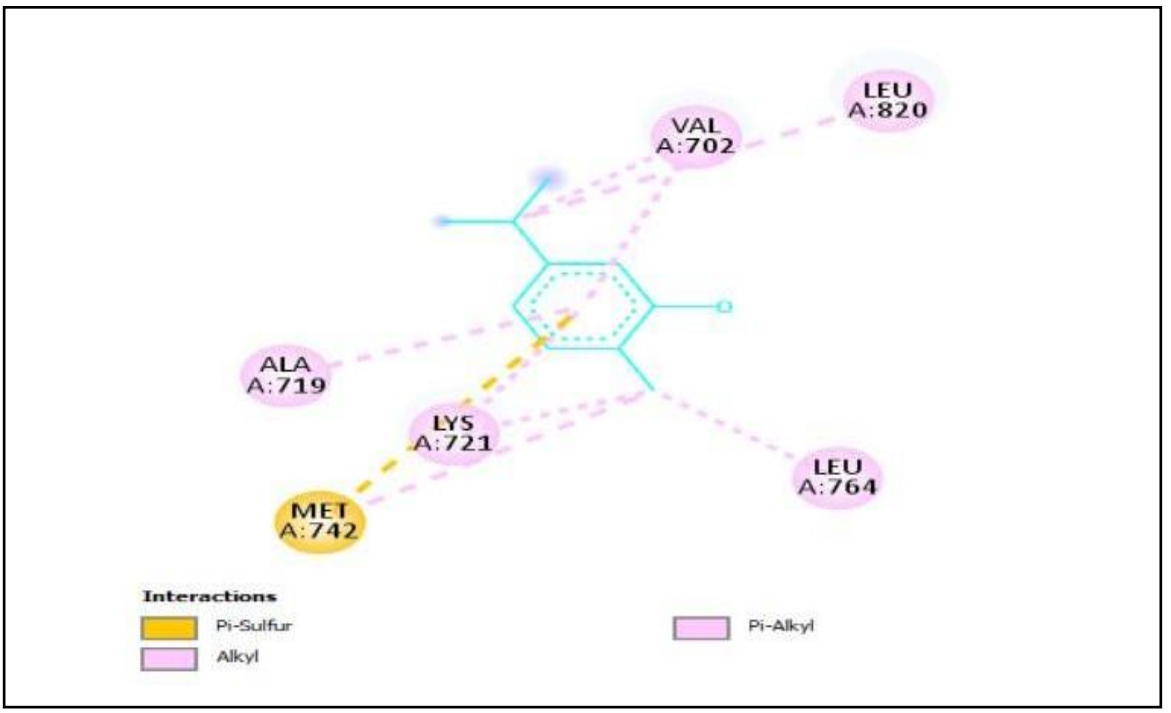

Fig. 3: Two-dimensional structure showing interactions between carvacrol–EGFR complexes

3.4 Alliin

As shown in Table 2, the binding affinity of alliin for the receptor active site is −4.6 kj/mol and figure 4 illustrates the two-dimensional structure of the alliin–EGFR receptor complex, which shows all of the interactions between the ligand and amino acid residues. Alliin formed two conventional hydrogen bonds with amino acid like Thr766 with a distance of 2.72 and 2.58 Å and one with Asp831 at a bond length of 2.40 Å. Met769 and leu820 have alkyl interactions with alliin at distance of 5.26 and 4.23 Å providing binding strength, affinity, and increased ligand efficiency. Figures 5 and 6 depict the binding patterns of 2,6-Dimethylpyrazine and sally-cysteine as two additional ligands with more negative binding affinities.

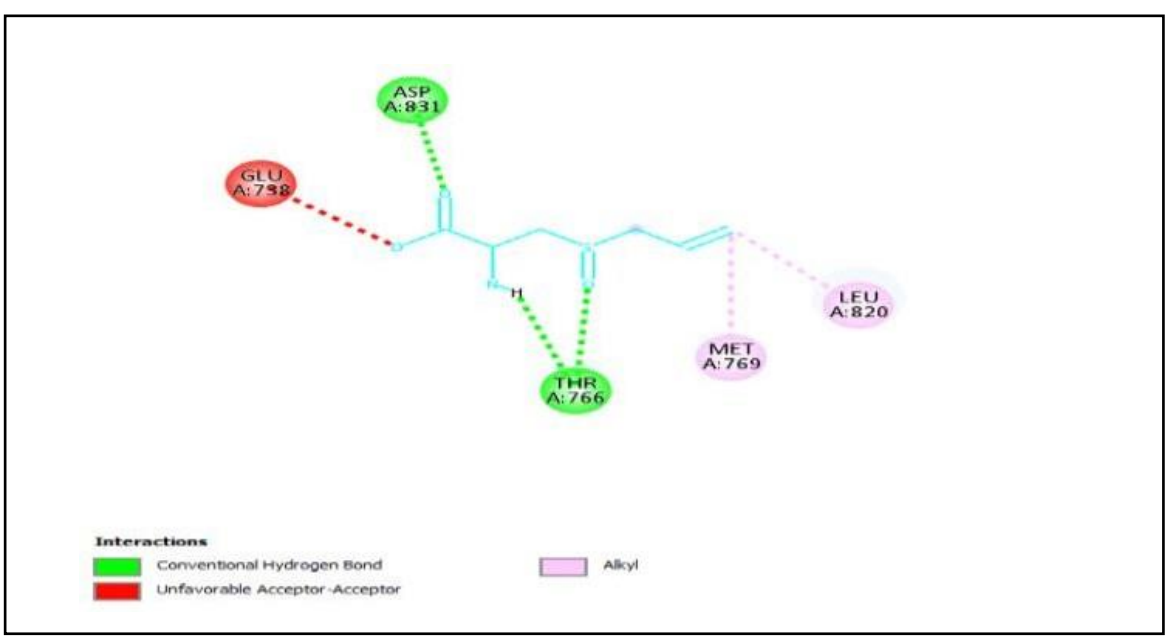

Fig. 4: Interactions between amino acid residues of EGFR (1m17) and alliin

3.5 2,6-Dimethylpyrazine

When 2,6-Dimethylpyrazine docked on EGFR had a negative binding affinity i.e., −4.2 kj/mol. It establishes a single carbon-hydrogen bond with glutamine738 at a length of 3.71 Å. In addition, there were five alkyl bonds with Ala719, leu820, met742, and leu764 with

distances of 3.77, 5.05, 4.87, and 4.15 Å. One pi–alkyl interaction was also present between lys721 and 2,6-Dimethylpyrazine, with a bond length of 5.00 Å.

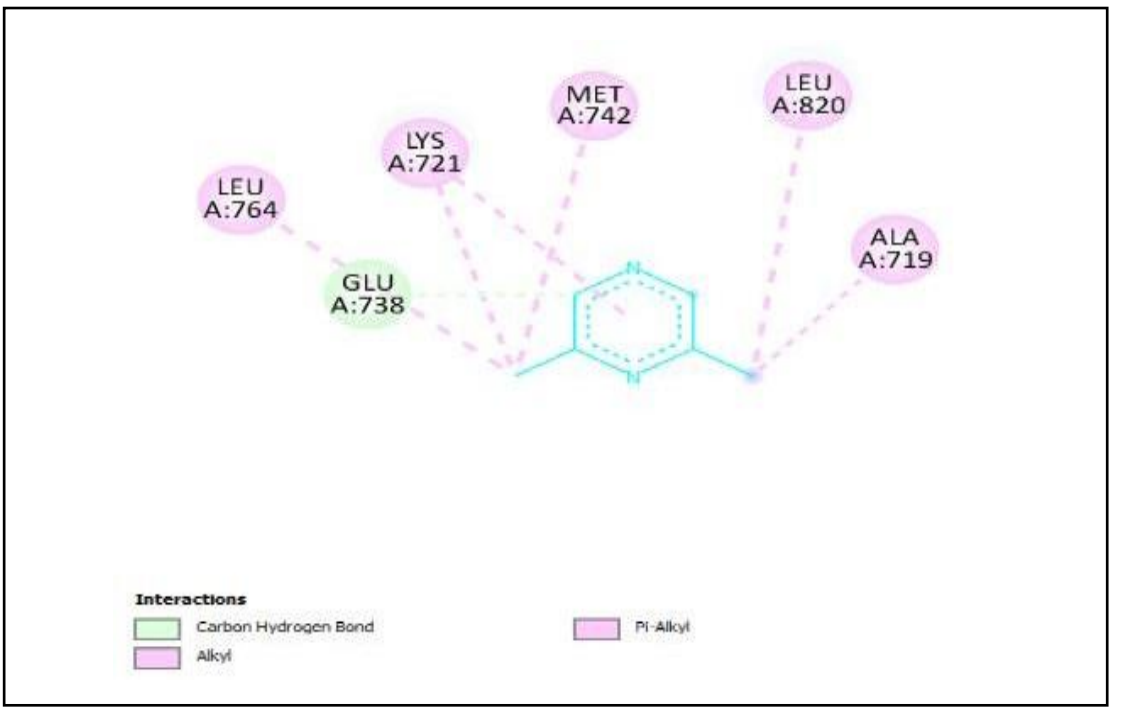

Fig. 5: Docked complex of 2,6-Dimethylpyrazine on EGFR receptor showing hydrogen and alkyl bond interactions

3.6 S-allyl cysteine

S-allyl cysteine docked on the binding cavity of the EGFR target protein with a binding energy of (−4.2kj/mol) and has one hydrogen bond with Asp831 at a distance of 2.25 Å. Three alkyl bonds were observed to be present between s-allyl cysteine and leu820, Ala719, and val702, with distances of 4.90, 4.25, 4.87 Å, respectively, as shown in figure 5.

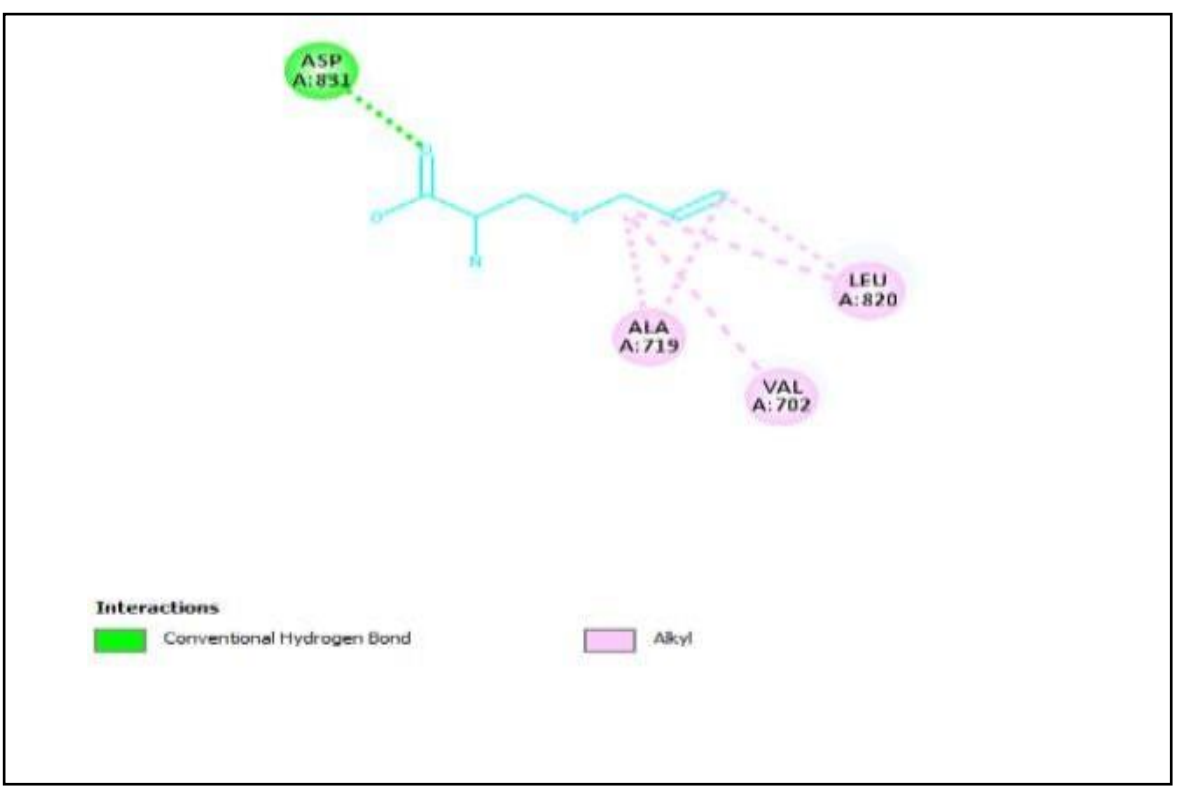

Fig. 6: Docked complex of S-allyl cysteine interacting with EGFR (1m17) amino acid residues.

These interactions increase the binding affinity in favor of drug likeness. The highest docking score and lowest binding affinity of the docked complex revealed a greater possibility of inhibiting the target proteins at different levels of lung cancer progression. These molecular docking studies indicate that these five phytoconstituents of Allium sativum L., having minimum binding energy and great efficacy against lung cancer protein, wild 1m17 (EGFR), can be used as drugs or have therapeutic value. A molecular docking approach was used in this study to identify promising compounds from natural products, such as Allium sativum, against lung cancer target proteins. Similar studies have been conducted by other researchers to calculate the binding affinity and amino acid residues of the binding pocket because all inhibitors work by binding to active sites. Arthur and Uzairu (2019) examined the role of PI3K in cancer treatment using a similar docking study. In this study, a dataset of 119 NCI anticancer analogs was used to study the role of interaction and binding affinity, and their inhibitory mechanism on PI3K target proteins through molecular docking. The results of this study revealed the role of hydrogen bonds in the enhancement of binding affinity and van der Waals forces in the stabilization of the ligand–target complex [29]. Durga and Julius (2020) reported that the thymoquinone phytoconstituent of Nigella sativum and an analog of poloxime have therapeutic value against lung cancer target proteins, such as GTPase KRAS, Sir2 protein, ALK5, and Beta-catenin. This docking study revealed that a high negative binding score was more likely to inhibit the target protein [30]. In 2021, Fadaka used docking approaches to develop therapeutic molecules from natural products against SARS-CoV-2 M^{pro}, an important target in covid 19 treatment. This study identified some flavonoids such as Quercetin-3-O-Neohesperidoside, Myricetin 3-Rutinoside, Quercetin 3-Rhamnosid, Rutin, and Myricitrin based on their binding energy and amino acid residues [31]. In all of these studies, computational techniques were used to reduce the experimental cost and use of animal models in conventional drug discovery. After analyzing the docking results of the present study, we believe that these five garlic compounds can accommodate the binding site of the target. This study provides evidence that these phytoconstituents can be employed as therapeutic agents against lung cancer disorders because they can regulate the cell signaling pathway of cell proliferation and target binding sites to inhibit protein–protein dimerization of tyrosine kinase. These selected compounds must have diverse functions that can be further investigated via in vitro and in vivo tests. This will serve as a crucial platform for the development of new medications and will help formulate a logical strategy for their use as therapeutic agents.

4. Conclusion

Our in silico study identified only five compounds, including allixin, carvacrol, alliin, 2,6-dimethylpyrazine, and s-allyl cysteine, based on their interactions and binding affinities with the target protein EGFR (1M17). Allixin and carvacrol have good binding affinities, that is (−5.9 kj/mol) and (−5.8 kj/mol), and alliin formed two conventional H-bond with (−4.6 kj/mol) binding affinity. Conventional hydrogen and alkyl interactions with the binding activity of the target protein give strength and stabilityto all inhibitors as they bind to active sites. The resultsfrom our study, such as comprehensive structural insight, binding modes, and fundamental consequences on binding free energies investigated through computational approaches, will be extremely helpful for the rational structure-based design of innovative and powerful inhibitors in the future.

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References

1. Cancer today.iarc. 2020. International agency for research on cancer, WHO; GLOBOCAN Lung Fact Sheet 2020. Available from: https://gco.iarc.fr/.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49. doi: 10.3322/caac.21660.
3. Bethune G, Bethune D, Ridgway N, Xu Z. Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. J Thorac Dis 2010;2:48–51.
4. Mustafa M, Azizi A, IIIzam E, Nazirah A, Sharifa S, Abbas S. Lung cancer: risk factors, management, and prognosis. IOSR J Den Medical Sciences 2016;15:94–101.
5. Tseng CH, Chiang CJ, Tseng JS, Yang TY, Hsu KH, Chen KC, et al. EGFR mutation, smoking, and gender in advanced lung adenocarcinoma. Oncotarget 2017;8:98384–93. doi: 10.18632/oncotarget.21842.
6. Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res 2015;4:36–54. doi: 10.3978/j.issn.2218-6751.2014.05.01.
7. Zhu C, Zhuang W, Chen L, Yang W, Ou WB. Frontiers of ctDNA, targeted therapies, and

immunotherapy in non-small-cell lung cancer. Transl Lung Cancer Res 2020;9:111–38. doi: 10.21037/tlcr.2020.01.09.
8. Kumarakulasinghe NB, van Zanwijk N, Soo RA. Molecular targeted therapy in the treatment of advanced stage non-small cell lung cancer (NSCLC). Respirology2015;20:370–78.doi: 10.1111/resp.12490.
9. Nakonieczna S, Grabarska A, Kukula-Koch W. The potential anticancer activity of phytoconstituents against gastric cancer: a review on in vitro, in vivo, andclinical studies. Int J Mol Sci 2020;21:8307. doi: 10.3390/ijms21218307.
10. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. Avicenna J Phytomed 2014;4:1–14.
11. Lawson LD. Garlic: a review of its medicinal effects and indicated active compounds. In: Lawson LD, Bauer R, eds. Phytomedicines of Europe: Chemistry and Biological Activity. Washington, D. C.: American Chemical Society; 1998. p. 177–209.
12. Satti NM, A-Elbasit, Ishraga Eltayeb Mohamed, Ahmed FA, Eltahir SE. Comparative analysis on the levels of some bioactive constituents of Asian and African garlic types. Bioscience Biotechnology Research Communications 2018;11:556–62. doi: 10.21786/bbrc/11.4/4.
13. Myneni AA, Chang SC, Niu R, Liu L, Swanson MK, Li J, et al. Raw garlic consumption and lung cancer in a Chinese population. Cancer Epidemiol Biomarkers Prev 2016;25:624–33. doi: 10.1158/1055-9965.EPI-15-0760.
14. Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK, et al. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evidence Based Complement Alternat Med 2013;627375.
15. Berger SI, Iyengar R. Network analyses in systems pharmacology. Bioinformatics 2009;25:2466–72. doi: 10.1093/bioinformatics/btp465.
16. Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol 2008;21:16–22. doi: 10.1038/modpathol.3801018.
17. Khalilullah H. Identification of anti-cancer agents targeting aldo-keto reductase (AKR) 1C3 protein by pharmacophore modeling, virtual screening and molecular docking. ChemistrySelect 2021;6:12820–33. doi: 10.1002/slct.202103151.
18. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand B. Aparna SR, Mangalapandi P, et al. IMPPAT: a curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. Sci Rep 2018;8:4329. doi: 10.1038/s41598-018-22631-z.
19. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: an open chemical toolbox. J Cheminform 2011;3:1-4. doi: 10.1186/1758-2946-3-33.
20. PubChem. National Center for Biotechnology Information. U.S. National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/.Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem 2010;53:2719–40. doi: 10.1021/jm901137j.
21. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717. doi: 10.1038/srep42717.
22. Stamos J, Sliwkowski M X, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. J Biol Chem 2002;277:46265–72. doi: 10.1074/jbc.M207135200.
23. Drulito. Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research. Available from: https://niper.gov.in/pi_dev_tools/DruLiToWeb/DruLiTo_index.html.
24. Protein Data Bank. Available from: https://www.rcsb.org/.
25. Guex N, Peitsch MC. (1997) SWISS-MODEL and the Swiss-PdbViewer. Available from: https://spdbv.unil.ch/disclaim.html.
26. Zhao Z, Xie L, Bourne PE. Structural insights into characterizing binding sites in epidermal growth factor receptor kinase mutants. J Chem Inf Model 2019;59:453–62. doi: 10.1021/acs.jcim.8b00458.
27. Patil R, Das S, Stanley A, Yadav L, Sudhakar A, Varma AK. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. PLOS ONE 2010;5:e12029. doi: 10.1371/journal.pone.0012029.
28. Arthur DE, Uzairu A. Molecular docking studies on the interaction of NCI anticancer analogues with human Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit. J King Saud Univ Sci 2019;31:1151–66. doi: 10.1016/j.jksus.2019.01.011.
29. Durga B, Julius A. In-silico docking studies of thymoquinone as potential anti-cancer drug target on Lung Cancer Cells. Eur J Mol Clin Med 2020;07:1706–16.
30. Fadaka AO, Sibuyi NRS, Martin DR, Klein A, Madiehe A, Meyer M. Development of effective therapeutic molecule from natural sources against coronavirus protease. Int J Mol Sci 2021;22:9431. doi: 10.3390/ijms22179431.