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Phytochemicals from *Allium tuberosum* Rottler ex Spreng Show Potent Inhibitory Activity against B-Raf, EGFR, K-Ras, and PI3K of Non-Small Cell Lung Cancer Targets

Rajat Nath [1,†], Shreeta Singha [1,†], Deepa Nath [2], Gitishree Das [3], Jayanta Kumar Patra [3,*] and Anupam Das Talukdar [1,*]

[1] Department of Life Science and Bioinformatics, Assam University, Cachar, Silchar 788011, Assam, India
[2] Department of Botany, Guru Charan College, Cachar, Silchar 788004, Assam, India
[3] Research Institute Integrative Life Sciences, Dongguk University-Seoul, Goyangsi 10326, Republic of Korea
* Correspondence: jkpatra@dongguk.edu (J.K.P.); anupam@bioinfoaus.ac.in (A.D.T.); Tel.: +91-9401416452 (A.D.T.)
† These authors contributed equally to this work.

Abstract: The major cause of death around the world is cardiovascular disease, while cancer ranks second. Lung cancer stands out as a major cause of concern because it accounts for 12% of all cancer cases and is the leading cause of cancer-related death. Since prehistoric times, humans have relied on plants as a reliable resource for all three of these essentials: food, livestock, and healthcare. When it comes to treating human illness, plants have been relied on extensively. Researchers are becoming increasingly intrigued by the prospect of deciphering plant chemistry. The Alliaceae plant family has yielded many novel phytochemicals. To identify a potent phytocompound against lung cancer from the plant *Allium tuberosum* Rottler ex Spreng, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) were performed. Before that, total phenolic content (TPC), total flavonoid content (TFC), and DDPH free radicals scavenging activity were determined in order to select the best plant extract. Four targets for non-small cell lung cancer (NSCLC) were retrieved in mutated form by literature mining to carry out this work. EGFR and B-Raf were selected as cell proliferating proteins and K-Ras and PI3K were selected as antiapoptotic proteins. Molecular docking was performed against these targets with the 94 phytocompounds present in *Allium tuberosum*, which were identified by GC-MS and LC-MS. Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling was also conducted with the nine best-screened compounds. Americine, an alkaloid from this plant, showed inhibitory activity against all four selected targets and was bound more strongly than their respective positive controls in docking studies amongst all other phytocompounds. The ADMET study also confirmed the drug-like candidature of the compound. This study reveals the alternative therapeutic potential of americine against NSCLC by promoting apoptosis and inhibiting cell proliferation.

Keywords: lung cancer; *Allium tuberosum*; phytocompounds; americine; molecular docking; apoptosis; cell proliferation

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1. Introduction

Cancer is an uncontrollable division and growth of cells in the body that is believed to be the leading cause of mortality worldwide [1]. Lung cancer is one of the predominantly diagnosed cancer types common among patients. The alteration in genetic level due to recent changes in the environment and trends in lifestyle accounts for the increased number of cancer cases [2]. Lung cancer is further classified into Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) [3]. NSCLC is a heterogenous type of cancer, with 85 to 90% of the cases commonly associated with primary and secondary smokers; workers exposed to asbestos, nickel, chromium, arsenic, and beryllium; people with a

family history of lung cancer; people who live in a region with high air pollution. The class NSCLC has reduced sensitivity to radiation and chemotherapy [4]. The epithelial cells covering the central bronchi to alveoli at the terminal of the lung are the site of origin for the NSCLC [5]. NSCLC includes multiple types of cancer, viz., adenocarcinomas (LUADs), large cell cancers, and squamous cell cancers (LUSCs). LUSCs arise usually near the central bronchus and LUADs usually initiate from the periphery of lung tissue [6]. Lung carcinogenesis follows a complex multiple-step pathway to develop into a diseased condition. LUSCs and LUADs develop from a defined-premalignant lesion [7]. Gradually, the precursor lesions turn invasive and undergo morphological changes due to dysplasia, metaplasia, hyperplasia, and carcinoma [8]. Symptoms such as chest pain, weight loss, worsening cough, hoarseness, hemoptysis, and dyspnea are found to be associated with patients suffering from lung cancer [9].

Plants have been an unconditional support system for food, feed, and medicine since ancient civilizations. Plants have been profoundly used as a therapeutic source to heal a wide array of diseases in humans [10]. Following up on this knowledge, a high-fold interest is growing among researchers to unveil and study the chemical attributes of various compounds present in plants. Many novel phytochemicals having anticancer potentials have been identified from the various plant families. Alliaceae is one among them, having therapeutic potential against many human ailments. This family consists of approximately 700 members, distributed across Asia, Europe, North America, and Northern Africa [11]. The representatives vary in color, odor, taste, and phenotypic traits, while showing affinities in terms of phytochemical, nutraceutical, and biochemical value. *Allium tuberosum* Rottler ex Spreng is one of the significant therapeutic representatives of the family. Consumption and cultivation of *Allium tuberosum* are mainly reported from China (origin), Southern Asia, and in the Northeastern region of India [12–14]. This plant species is reported to have antidiabetic, hepatoprotective [15], cytotoxic, and antitumor activity [16]. Because the crude extract of this plant demonstrated antitumor and cytotoxic properties, we chose it for the identification of possible anti-NSCLC phytocompounds.

Taking a clue from the literature and the retrieval of data from KEGG pathways, four different targets for the progression of NSCLCs, viz., B-Raf, EGFR, K-Ras, and PI3K, were selected for in silico-based anticancer activity prediction [17]. The genome studies of most lung cancer patients reflect highly the presence of a mutation in the BRAF (a proto-oncogene that encodes B-Raf and belongs to the Raf kinase family) [18]. The BRAF mutation leads to the activation of the MAP kinase pathway and suppresses phosphorylation of the AKT-induced pathway inhibition, resulting in tumorigenesis [19]. EGFR (Epidermal Growth Factor Receptor) has become another significant target for the therapeutic process to check NSCLCs complications [20]. Somatic mutation of the EGFR gene was found commonly in populations of Japanese and U.S lung cancer patients [21]. Further, KRAS is a mutated oncogene common in NSCLC cases [22]. K-Ras plays a significant role in the signaling pathway that leads to cell division, growth, and differentiation. Therefore, it is an excellent target for NSCLC inhibition using effective plant-based drugs [23,24]. PI3K (phosphoinositide 3-kinases) is another potential target drawing the high attention of researchers working with the inhibition of lung tumorigenesis as it is a multifaceted player in the regulation of signaling, cell survival, proliferation, vesicle trafficking, and migration [25,26].

The in-practice therapeutics for cancer is very toxic for normal cells and the cost is out of reach for common peoples. An alternative strategy is highly in demand in medical science for the treatment of rapidly growing cancer cases. Among all the different types of cancer, lung cancer is the main matter of concern as it contributes to the highest number of deaths related to cancer. Thus, in our study, we focused on identifying the phytocompounds present in *Allium tuberosum* that have the capability to control the NSCLC. To kickstart the objective, we targeted mutated B-Rraf, EGFR, K-Ras, and PI3K with the phytochemicals identified from methanolic extract of *A. tuberosum* by aiding computational tools for in silico screening in an attempt to predict the anticancer activity (Figure 1) [27,28].

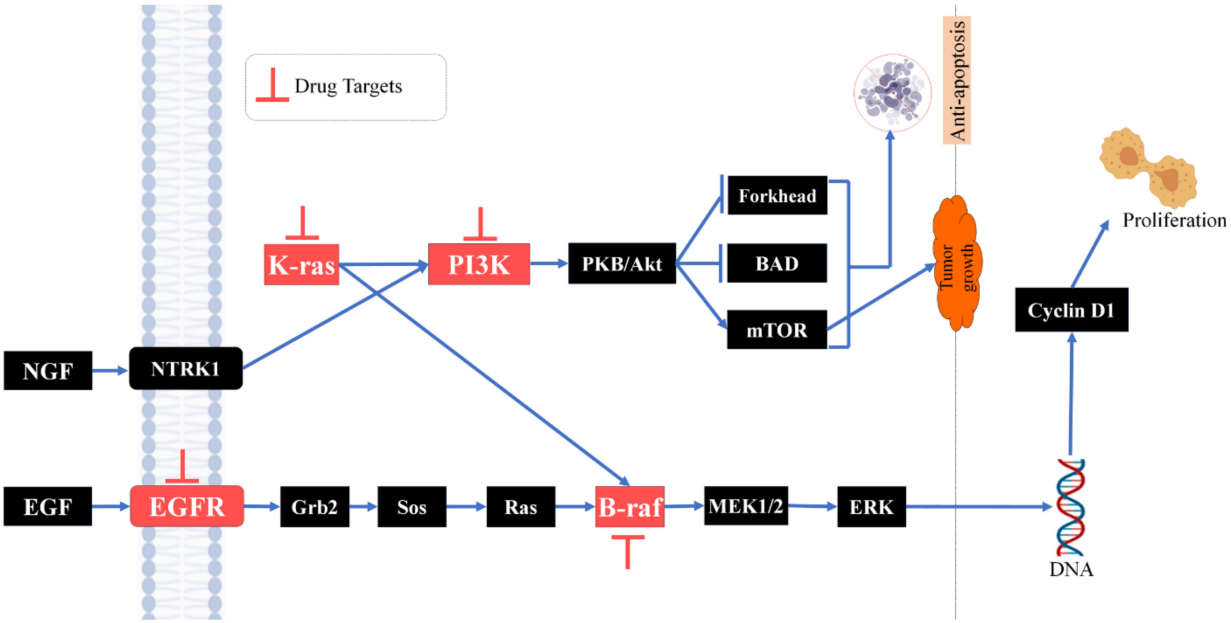


Figure 1. Figure showing the selected target molecules and their link with the progression of lung cancer. The targets in the red boxes are the targets selected by literature mining.

2. Methodology

2.1. Preparation of Plant Extracts

A mature fresh sample of *Allium tuberosum* plant leaves was collected from the local farmer. The samples were cut into small pieces, dried, and ground into fine powder. The maceration process [29] was employed to prepare the crude extract of the plant leaves following the low to high polar solvents, viz., petroleum ether, ethyl acetate, acetone, and methanol, keeping each solvent for 72 h. The extract was made completely solvent-free by using a rotary evaporator and lyophilizer.

2.2. Quantitative Phytochemical Screening

2.2.1. Determination of Total Phenolic Content (TPC)

A standard protocol for estimating total phenolic content [30] was used to determine the TPC of the plant extract. Briefly, 500 µL of extract dissolved in methanol (1 mg/mL) was taken in a test tube, and 100 µL of Folin–Ciocalteu solution (FC solution) and 2400 µL of distilled water were then added to it. It was kept for 3 min and then 2000 µL of 2% Na_2 CO_3 solution was added. The mixture was incubated for 1 h in the dark. At 750 nm, the absorbance was measured. The results were calculated in terms of gallic acid equivalents (GAE/mg) of plant extracts.

2.2.2. Determination of Total Flavonoid Content (TFC)

Quantification of total flavonoid content (TFC) was conducted by following the standard protocol [31]. Then, 1000 µL of plant extract was dissolved in methanol (1 mg/mL) and mixed with an equal volume of 2% $AlCl_3$ solution (dissolved in methanol). For 15 min in the dark, the mixture was incubated before its absorbance at 415 nm was measured. The final result was expressed as the amount of quercetin equivalent (QE/mg) in the plant extracts used.

2.3. Antioxidant Activity by DPPH

The antioxidant activity of the plant extracts was performed using the DPPH free radicals scavenging activity following the method described by Kumarasamy et al. [32]. First, by the serial dilution process, 5 different concentrations of the sample were prepared from 1 mg/mL of stock solution using methanol. The diluted solution and DPPH

(80 µg/mL in methanol) solution were mixed 2 mL each and kept in the dark for 30 min. Absorbance was measured at 517 nm. The control was DPPH mixed with methanol instead of extracts. Ascorbic acid was treated as standard. A concentration–response curve was used to determine the IC_{50} value for each extract. Percent invitations were determined using the formula:

DPPH free radical scavenging (%) = $\left\{\frac{(A_C - A_t)}{A_C} \times 100\right\}$, where A_C is the absorbance of the control and A_t is the absorbance of the test sample.

2.4. Metabolites Profiling

Metabolite profiling was conducted for the plant methanolic extract as it showed high TPC, TFC, and DPPH free radicals scavenging activity than the other extracts. For the identification of volatile compounds, GC-MS (Gas Chromatography-Mass Spectroscopy) and, for non-volatile compounds, LC-MS (Liquid Chromatography Mass Spectroscopy) were conducted.

GCMS was conducted at AIRF, Jawaharlal Nehru University, Delhi, with a GCMS-QP2010 Plus from Shimadzu, Kyoto, Japan. With an initial temperature of 60 °C, a 30 m long by 0.25 mm in diameter by 0.25 m thick RXi-5 Sil MS column was used. Helium was used as the carrier gas. Two libraries, NIST14.lib and WILEY8.lib, were used to find the compounds.

At IIT Bombay's SAIF, LCMS analysis was carried out using Varian Inc.'s 410 Prostar Binary LC with 500 MS IT PDA Detectors. The separation was carried out with an RRHT (rapid-resolution high-throughput) C18 column (2.1 mm 100 mm, 1.8 m column). For the mobile phase, two different solvent systems were used. Solvent A was water with 0.1% formic acid, and solvent B was acetonitrile with 10% water and 0.1% formic acid. The amount injected was maintained at 5 µL, the flow rate was maintained at 0.300 mL/min, and the temperature of the column was maintained at 40 °C. The described method was used to look at the standards and extracts. The gradient elution was optimized by performing the following (Table 1):

Table 1. Solvent gradient used for HR-LCMS analysis.

Time	Solvent A	Solvent B	Flow Rate	Pressure
1 Min	95%	5%	0.3 mL/min	1200 bar
20 Min	0%	100%	0.3 mL/min	1200 bar
25 Min	0%	100%	0.3 mL/min	1200 bar
26 Min	95%	5%	0.3 mL/min	1200 bar
30 Min	95%	5%	0.3 mL/min	1200 bar

2.5. Selection and Preparation of Receptor

Apoptosis plays an important role in combating cancer. It is the first and foremost strategy of cancer therapy to kill malignant cells. Cell proliferation is another important characteristic of malignant cells by which they increase their number rapidly. We selected 4 targets, 2 from each pathway, based on the literature mining for this study. These four targets were B-Raf, EGFR, K-Ras, and PI3K. As the mutated forms of these proteins are responsible for the development of malignancy, we retrieved the 3D structure of mutated forms of these proteins from the protein data bank (www.rcsb.org/pdb; Accessed on 30 September 2022). A total of 3 types of mutations were mainly found in EGFR protein: L858R, T790M, and C797S. Approximately 50% of lung cancer patients with acquired resistance have the T790M mutation, which is thought to increase the receptor's affinity for adenosine triphosphate compared to its affinity for tyrosine kinase inhibitors (TKIs) [33]. When EGFR-L858R was expressed in lung cancer cells, it led to enhanced invasiveness and malignant pleural effusion (MPE) formation, as well as upregulation of the CXCR4 chemokine receptor [34]. Patients with NSCLC, who are treated with the currently available EGFR TKIs, often have difficulties due to the presence of the C797S point mutation. Recent

clinical investigations show that almost 40% of patients who were treated with third-generation EGFR TKIs afterward had C797S mutations [35].

The common form of BRAF mutations that occurs in NSCLC patients is BRAF V600E [36]. The most prominent oncogene in NSCLC is KRAS, contributing to 23% of lung cancer cases. In total, 3 types of mutations are found in KRAS in patients with lung cancer, viz., G12C, G12V, and G12D; among them, 48% of G12C mutations are found in NSCLC [37]. For PI3K, no effective mutations have been reported to date [38]. The normal protein of PI3K has been targeted by researchers for its anticancer studies [39]. Details of all the target receptors are mentioned in Table 2.

Table 2. Details of the selected receptor proteins.

Sl No.	Receptor	PDB Id	Mutation	Resolution	R-Free	Experimental Methods
1	B-Raf	4R5Y	V600E	3.50 Å	0.306	XRD
2	EGFR	6LUB	L858R/T790M/C797S	2.31 Å	0.226	XRD
3	K-Ras	8DNI	G12C	1.50 Å	0.254	XRD
4	PI3K	3APC	-	2.54 Å	0.290	XRD

2.6. Preparation of Ligands

A total of 94 phytocompounds were identified from the plant *Allium tuberosum*. The smile format of all the compounds was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/; Accessed on 2 October 2022) and ACD/Labs chem sketch software (ACD/ChemSketch, version 2021.1.2, Advanced Chemistry Development, Inc. (ACD/Labs, Toronto, ON, Canada). For molecular docking, .mol format is required. Smile to .mol formation conversion of the ligand was conducted using open bubble software.

2.7. Molecular Docking

Molecular docking is the bioinformatics process by which we can predict the binding efficiency of the ligand with the active site of the target proteins. Along with the binding efficiency, binding conformation and orientation can also be predicted with this process. Molegro Virtual Docker (MVD) (v 6.0) was used to perform the molecular docking analysis. Receptor proteins were loaded into the MVD software by removing bound inhibitors/s, cofactor/s, and water molecules. The protonation state of the receptors' amino acids was corrected accordingly with the inbuilt protein preparation plugin available with the software. Active sites of the receptors were identified by MVD's detect cavities option and the docking sites of the receptors were defined. After post-processing steps such as minimizing energy and optimizing hydrogen bonds, the software determines the MolDock score, the hydrogen bond score, and the optical geometry of how the ligand binds to the active site(s) of the receptor(s) [40].

2.8. Prediction of ADME Profile and Drug-likeness

The swissADME server of the Swiss Institute of Bioinformatics was used for ADMET analysis. The smile format of the compounds was put in the server and the algorithm of swissADME generated all the physicochemical, lipophilicity, water solubility, pharmacokinetics, medicinal chemistry, and drug-likeness properties.

3. Result

3.1. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Our findings indicate that in the methanolic extract of the plant *Allium tubersosum*, TPC and TFC present highest compared to other extracts. TPC for the methanolic extract is 119 g GAE/mg extract, while TFC is 146 g quercetin/mg extract (Figure 2). To determine TPC, we used the following equation for the gallic acid standard curve: $y = 0.0045x + 0.4498$;

$R^2 = 0.9867$, and for quercetin, we used the following equation: $y = 0.0002x + 0.117$; $R^2 = 0.9999$.

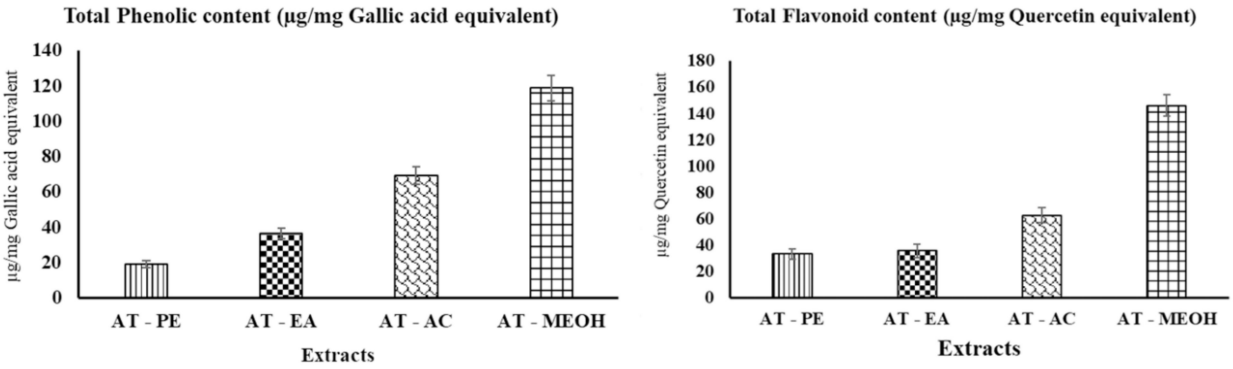


Figure 2. Comparative TPC and TFC in the different extracts of Allium tuberosum.

3.2. DPPH Free Radical Scavenging Assay

Allium tuberosum extracts and ascorbic acid, a reference antioxidant, were tested for their ability to quench DPPH free radicals, and the findings are displayed in Figure 3**.** Antioxidant activity is commonly measured by its ability to reduce free radicals by 50%. The DPPH radical was effectively scavenged by increasing quantities of both the plant extract and the standard in this study. The IC_{50} for DPPH radical scavenging activity is shown in Figure 3. Methanolic extract of *Allium tuberosum* has the lowest IC50 value (197 ± 15 µg/mL) compared to the other sample extracts.

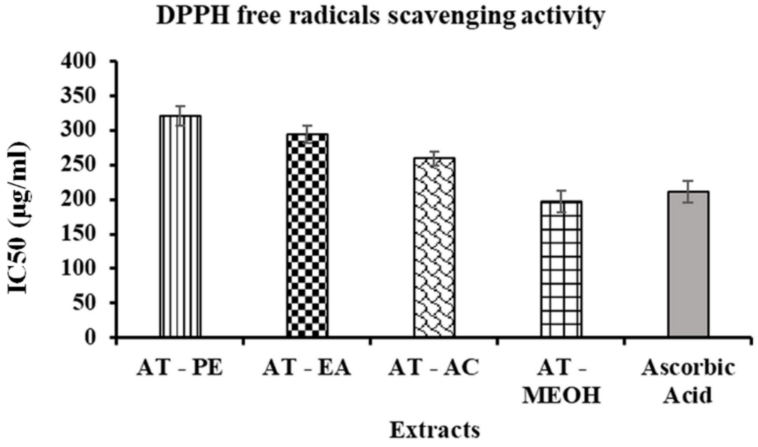


Figure 3. Comparative DPPH radical scavenging activity of extracts with standard ascorbic acid.

3.3. Metabolites Profiling

Taking the clue from the findings of TPC, TFC, and DPPH free radicals scavenging activity, methanolic extract of *Allium tuberosum* (AT—MeOH) was selected for the phytochemical profiling with GC-MS and LC-MS. The metabolite profiling provides a brief idea of phytochemicals present in the plant and based on which identification of phytochemicals can be undertaken. Compounds identified in the GC-MS and LC-MS chromatogram (Figure 4) of AT—MeOH are provided as Supplementary Files (Supplementary—I).

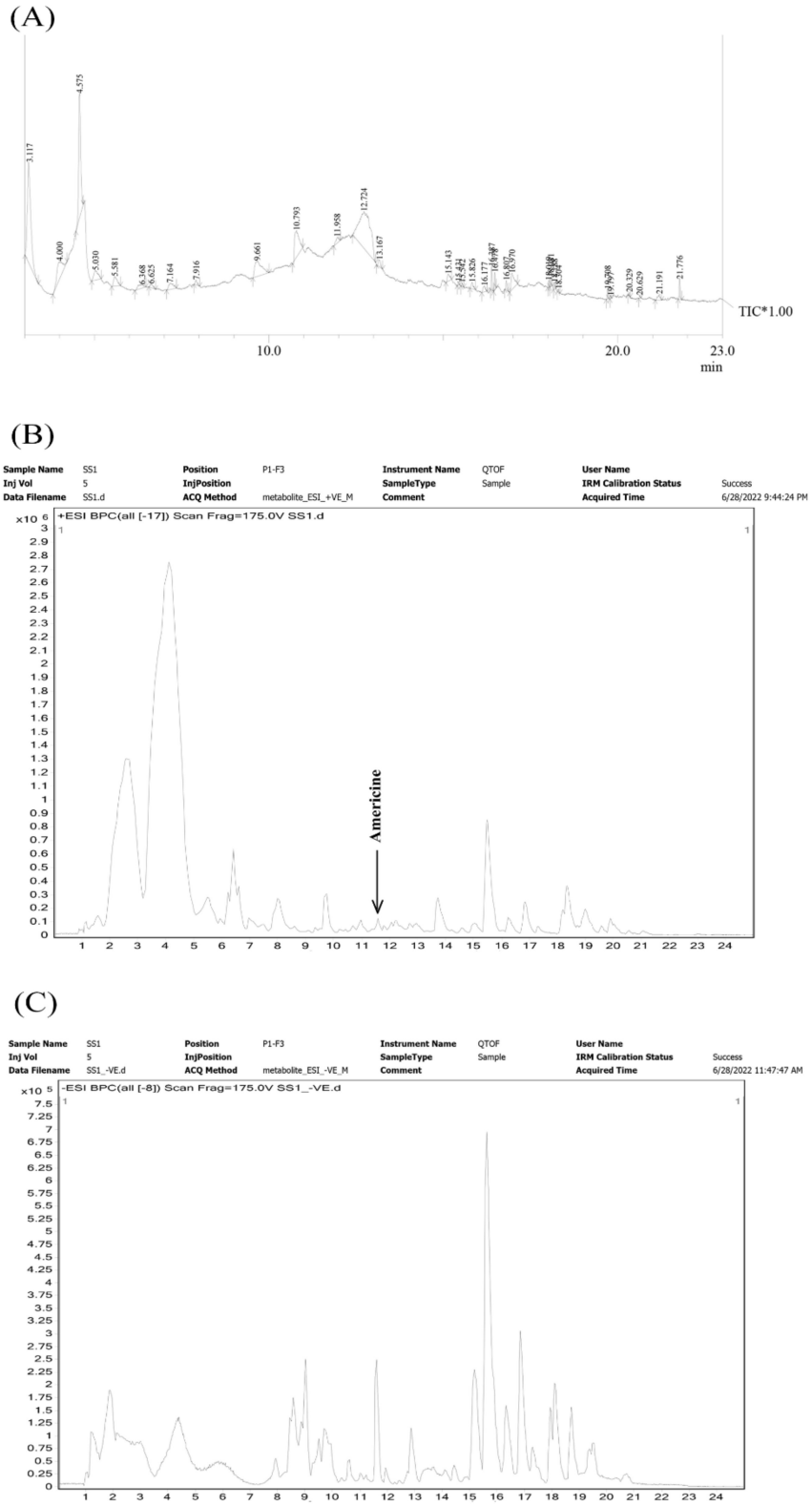


Figure 4. (**A**) Chromatogram of GC-MS of AT—MeOH; (**B**) chromatogram of HRLC-MS of AT—MeOH in +ve ESI; (**C**) chromatogram of HRLC-MS of AT—MeOH in −ve ESI.

3.4. Docking Scores and Inhibition of Receptors

In the four selected targets, some of the identified compounds show the best binding efficacy than the positive controls of these respective targets. In EGFR triple mutant protein, ethyl2E,4Z-hexadecadienoate shows the best binding efficacy with a MolDock

score of −124.85, whereas positive control gefitinib shows −103.14. Other than ethyl2E,4Z-hexadecadienoate, 19 more phytocompounds from this plant show better binding than its positive control (Table 3). The docking result with B-Raf V600E mutant protein reveals that the compound americine shows the best binding followed by 9-Hydroxy-9,11,15-octadecatrienoic acid compared to its positive control Dabrafenib (MolDock Score −138.89). Americine shows the binding score, i.e., MolDock score −144.45. These two proteins are involved in cancer cell proliferation. The binding minimizes the cancer cell proliferation and formation of the malignant tumor.

Table 3. Docking score ligands against the targets in comparison with the positive control, i.e., market-approved drugs against the target.

Compound Name	Docking Score (MolDock Score)			
	EGFR	**K-Ras**	**PI3K**	**B-Raf**
Gefitinib (Positive Control of EGFR)	−103.14	-	-	-
Adagrasib (Positive Control of K-Ras)	-	−136.77	-	-
Duvelisib (Positive Control of PI3K)	-	-	−113.52	-
Dabrafenib (Positive Control of B-Raf)	-	-	-	−138.89
Americine	−112.82	−139.02	−115.76	**−144.45 ***
9-Hydroxy-9,11,15-octadecatrienoic acid (9-HOTE)	−121.96	−144.61	−123.48	−140.27
Di-n-octyl phthalate	−110.60	−154.00	**−133.50 ***	
8-hydroxyoctadeca-9,12-dienoic acid (8S-HODE)	−111.91	**−140.70 ***	−127.40	
9-hydroperoxyoctadeca-10,12,15-trienoic acid [9(S)-HpOTrE]	−123.38	−142.13	−126.70	
α-Linolenic Acid	−118.29	−142.05	−122.03	
Fumaricaciddi(2-decyl)ester	−117.66	−167.84	−127.15	
3-ketostearic acid	−115.01	−143.54	−123.17	
1,2-Benzenedicarboxylicacidbutyloctylester	−103.81	Low Score	−114.19	
N,N′-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	−110.92	−150.51		
Ethyl2E,4Z-hexadecadienoate	**−124.85 ***	−144.21		
9,12-Octadecadien-1-Ol	−110.65	−142.31		
Eicosanoicacid, Methyl ester	−106.81	−141.91		
Petroselinicacid	−115.01	−138.96		
10,16-dihydroxy-palmitic acid	−120.06	−137.31		
Nonadecane,9-methyl	−114.58			
Methaphenilene	−109.04			
10,11-Epoxy-3,7,11-trimethyl-2E,6E-tridecadienoic acid	−105.83			
Glycerol1,2-diacetate	−105.46			
Leucyl-glutamate	−104.83			

* best binding shown among all compounds and positive control for this target.

Two more selected proteins involved in the antiapoptotic activity of carcinoma are also inhibited by the identified compounds of the selected plant. G12C mutant K-Ras protein is inhibited by 8-hydroxyoctadeca-9,12-dienoic acid (MolDock Score −140.70) along with more than 13 compounds, whereas the binding efficiency shown by its positive control Adagrasib is −136.77. Di-n-octyl phthalate (MolDock score −133.50) shows the best binding efficacy with the target protein PI3K than its positive control Duvelisib (MolDock score −113.52). In addition, eight more compounds show better binding efficiency than the positive control of PI3K.

After analyzing the data, it was found that the two compounds americine and 9-hydroxy-9,11,15-octadecatrienoic acid have the capability to inhibit all the four selected target proteins and may serve as an anti-NSCLC drug. If we put aside the target B-Raf, we found that nine compounds: 1,2-benzene dicarboxylic acid, butyl octyl ester; 3-ketostearic acid; (8S)-hydroxyoctadeca-9,12-dienoic acid; 9-hydroperoxy-10,12,15-octadecatrienoic acid; 9-hydroxy-9,11,15-octadecatrienoic acid; α-linolenic acid; americine; di-n-octyl phthalate; fumaric acid, di(2-decyl)ester (Figure 5), can inhibit the target protein EGFR, K-Ras, and PI3K. The docking pose and 2D interaction of the best compound americine and the positive controls are shown in Figures 6 and 7, respectively. The docking score of all 94 compounds is given in Supplementary File—II.

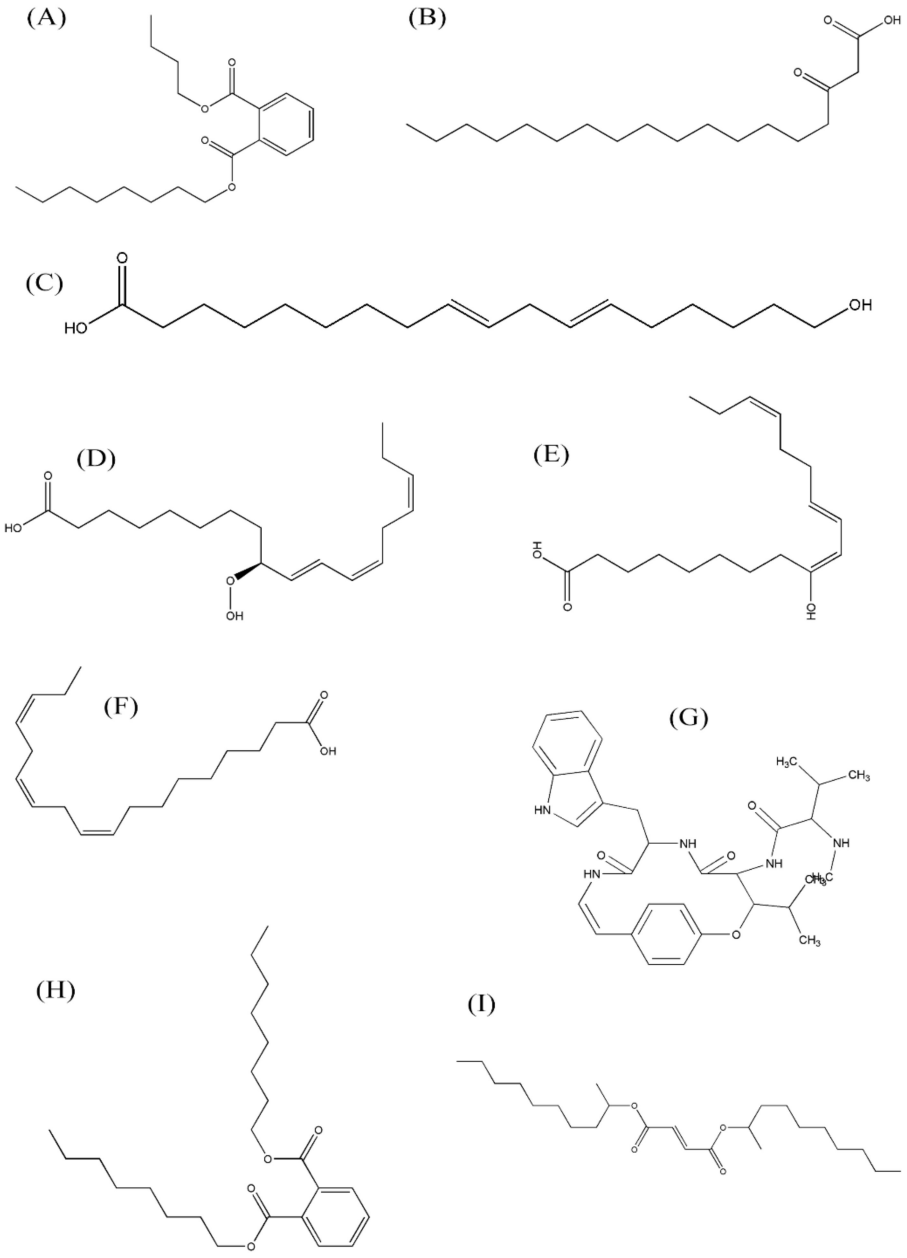


Figure 5. Structure of (**A**) 1,2-benzene dicarboxylic acid, butyl octyl ester; (**B**) 3-ketostearic acid; (**C**) (8S)-hydroxyoctadeca-9,12-dienoic acid; (**D**) 9-hydroperoxy-10,12,15-octadecatrienoic acid; (**E**) 9-hydroxy-9,11,15-octadecatrienoic acid; (**F**) a-linolenic acid; (**G**) americine; (**H**) di-n-octyl phthalate; (**I**) fumaric acid, di(2-decyl)ester.

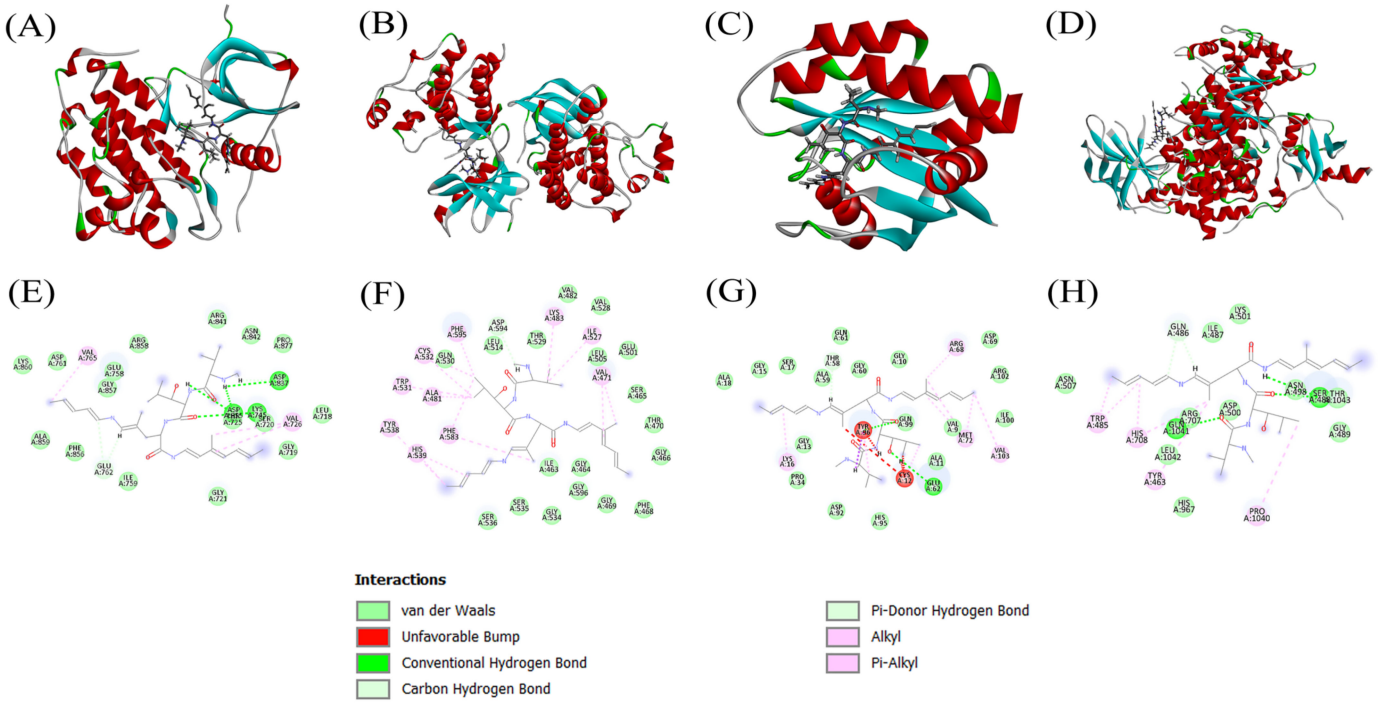


Figure 6. Docking pose of ligand americine with the target receptors: (**A**) EGFR, (**B**) B-Raf, (**C**) K-Ras, and (**D**) PI3K. 2D interaction of ligand americine with the target receptors: (**E**) EGFR, (**F**) B-Raf, (**G**) K-Ras, and (**H**) PI3K.

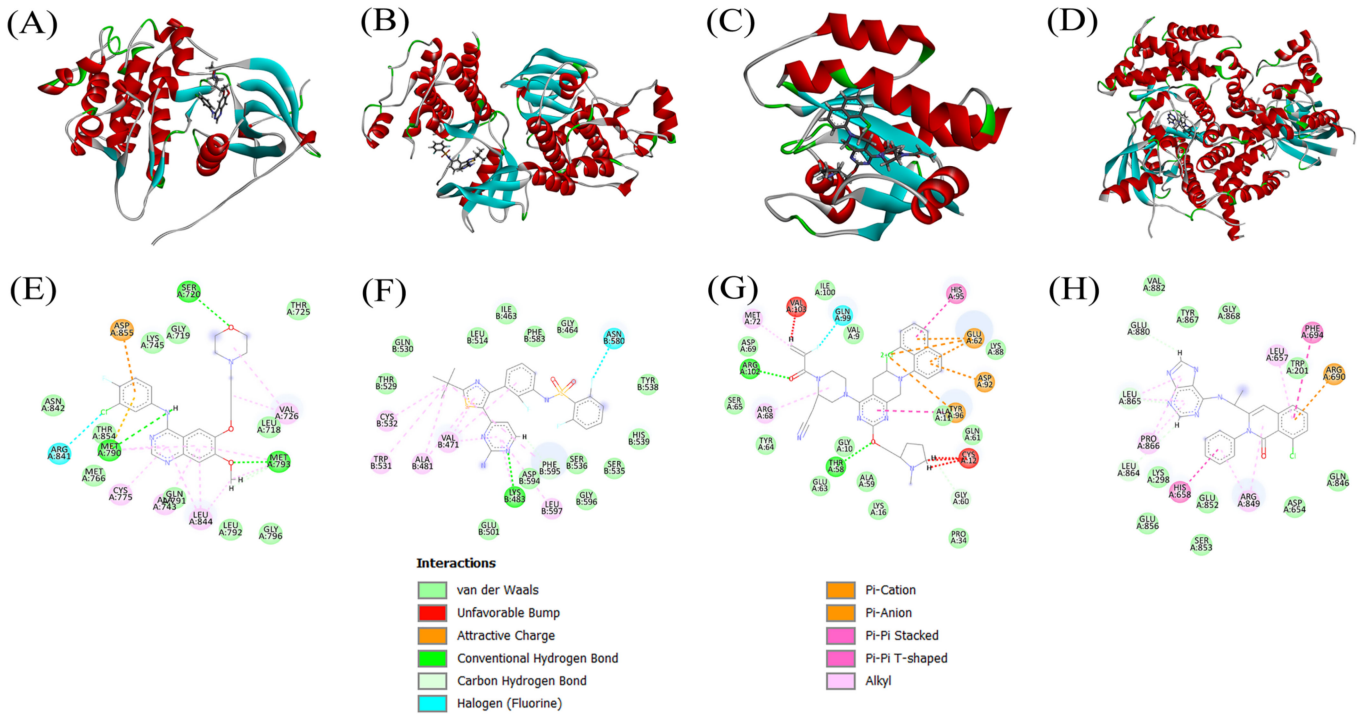


Figure 7. Docking pose of target proteins with their positive control: (**A**) EGFR with Gefitinib; (**B**) B-Raf with DaB-Rafenib; (**C**) K-Ras with Sotorasib; (**D**) PI3K with Duvelisib. 2D interaction of target proteins with their positive control: (**E**) EGFR with Gefitinib; (**F**) B-Raf with DaB-Rafenib; (**G**) K-Ras with Sotorasib; (**H**) PI3K with Duvelisib.

3.5. ADMET Profile Analysis

Nine compounds selected after molecular docking were considered for ADMET profiling by the SwissADME server. By the ADMET analysis, it was found that, except for americine and di-n-octyl phthalate, all the seven remaining compounds penetrate the blood–brain barrier, which is not good, as they may interact with the central nervous system. In addition, they are not effluated by the BBB's P-glycoprotein pumping, as predicted by the BOILED-Egg Model (Figure 8), implying that it may have passed through the BBB. However, all the compounds follow the drug-likeness profile except di-n-octyl phthalate and fumaric acid, di(2-decyl)ester. All eight compounds have high gastrointestinal absorption capacity except fumaric acid, di(2-decyl)ester. Considering all the parameters of ADMET, it was found that only one compound, i.e., americine, shows drug-like activity and may be suitable for further validations. The pharmacokinetics and drug-likeness score of all the compounds is recorded in Table 4.

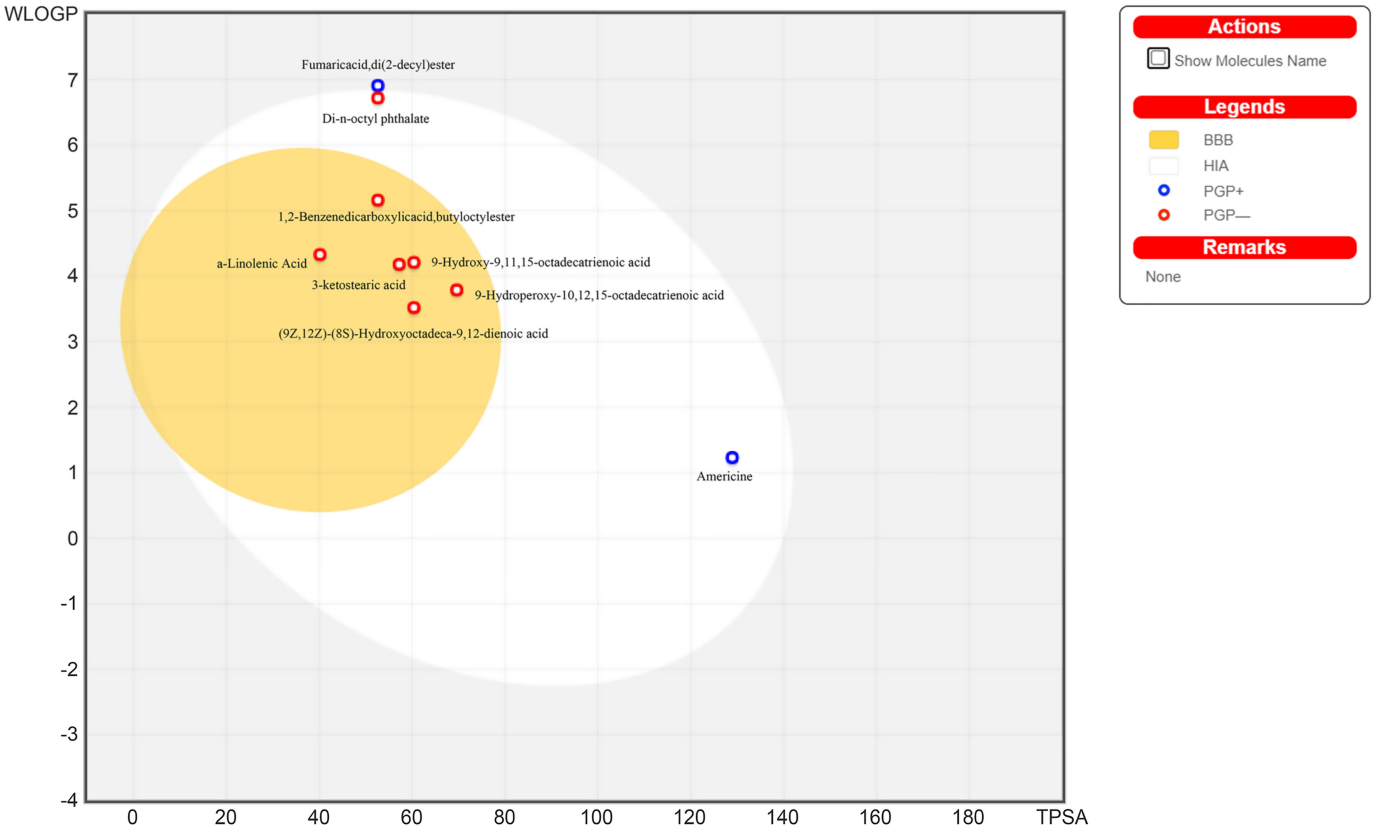


Figure 8. BOILED-EGG MODEL for Absorption in the Gastrointestinal tract and Penetration into the Brain: Molecules in the yolk of the BOILED-Egg are thought to passively pass through the blood–brain barrier (BBB). Molecules in the white of a Boiled Egg are thought to be absorbed passively by the digestive tract. The p-glycoproteins are thought to remove the blue-dotted molecules from the Central Nervous System (CNS).

Table 4. Calculated pharmacokinetics and drug-likeness parameters of the ligands.

	1,2-Benzenedicarboxylicacid, butyloctylester	**3-Ketostearic Acid**	**9-Hydroperoxy-10,12,15-octadecatrienoic Acid**	**9-Hydroxy-9,11,15-octadecatrienoic Acid**	**(9Z,12Z)-(8S)-Hydroxyoctadeca-9,12-dienoic Acid**	**α-Linolenic Acid**	**Americine**	**Di-n-octyl phthalate**	**Fumaricacid,di(2-decyl)ester**
Pharmacokinetics									
GI absorption	High	High	High	High	High	High	High	High	Low
BBB permeant	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
P-GP substrate	No	No	No	No	No	No	Yes	No	Yes
Drug-likeness									
Lipinski (Pfizer)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghose (Amgen)	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Veber (GSK)	No	No	No	No	No	No	Yes	No	No
Egan (Pharmacia)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Muegge (Bayer)	No	No	Yes	No	No	No	Yes	No	No
Bioavailability Score	0.55	0.85	0.85	0.85	0.85	0.85	0.55	0.55	0.55

4. Discussion

Secondary metabolites found in plants are promising research materials for drug discovery. A growing number of people are interested in phytochemical research as an alternative to the high cost and risk associated with conventional pharmaceuticals. Inhibition of non-small cell lung cancer with plant secondary metabolites has been widely reported [41–44]. The primary goal of this computational modeling study was to identify the key drug targets of non-small cell lung cancer and the phytochemicals from *Allium tuberosum* with the greatest potential to inhibit the disease.

The plant *Allium tuberosum* (Chinese Chives) is little known as compared to the other species present in the same genus, namely *Allium sativum* (Garlic) and *Allium cepa* (Onion). Many impactful works have already been performed in recent years [45]. The phytochemicals present in petroleum ether, ethyl acetate, acetone, and methanolic extracts were tested in a mouse model for bioactivity; those extracts were found to be anticancer agents, suppressing tumor development by triggering apoptosis [16]. The crude extract has been reported to be utilized to cure notable human disorders in the brain, eyes, lungs, liver, gastrointestinal tract, kidney, and other parts of the body [46]. However, for thorough lightening up of the functions, pathways, and the systematic mechanism for healing tumor growth, more in-depth accumulation of information, identification of phytoconstituents, and structure elucidation is required [47]. Identifying effective inhibitors and specific new phytochemicals that correctly regulate the malignant activity pathway would undoubtedly be a beneficial alternative to cancer treatment [48–50]. The plant extract of *Allium tuberosum* has been found to possess cytotoxic activity in many experiments with mice by various researchers [51–53]. Numerous compounds are believed to have potential bioactivity, identified from the plant parts that may be optimized to become a significant lead molecule against the target for NSCLC [54]. The GC-MS and LC-MS-based metabolite profiling of the methanolic extract of *A. tuberosum* leaves revealed the presence of 94 bioactive phytochemicals. Very little work has been reported with the compounds present in this plant. Two compounds, S-methyl 2-propene-1-thiosulfinate and S-methyl methanethiosulfonate, from these plants have been reported as anticancer potentials; they are capable of inducing apoptosis in breast cancer cell line MCF-7 [16]. These two compounds, also reported by Seo et al., show antibacterial activity [55]. Another study by Kim et al. reported the anti-prostate cancer activity of the compound thiosulfinate from the same plant [56]. Thiosulfinate also induces apoptosis in human colon cancer cell line HT-29, studied by Lee et al. [57]. Much work has yet to be carried out, and metabolic profiling of the plant extract may reveal the possession of many compounds showing effectiveness against cancer [58–60].

There are wide varieties of stimuli that could cause a normal cell to transform into a malignant one. When a cell's apoptosis process is blocked for some mechanism, the cell becomes immortal and may convert into a cancerous one. Apoptosis is an essential feature of normal cells by which aged and damaged cells proceed toward self-degradation [61]. Antiapoptotic activity in NSCLC is mediated by PI3K [62] and K-Ras [63]. In addition to its role in cell proliferation, K-Ras dysfunction is thought to contribute to the development of nearly 15% to 20% of cases of non-small cell lung cancer [64]. The growth factors B-Raf and EGFR are also to blame [64]. Blocking any of the aforementioned targets can result in the remission of NSCLC as some of the previous studies have validated [65–70].

Protein–ligand molecular docking is an efficient and essential tool for drug discovery, particularly for determining the drug-likeness and mechanism of action of novel compounds [71]. Studies of absorption, distribution, metabolism, and excretion (ADME) help us to predict the drug's efficacy [72].

The results of this computational analysis show that americine, an alkaloid, has the potential to interact with all four target proteins in the same way that their known inhibitors/drugs do. When the MolDock score is negative, it indicates that the ligand binds to its targets without being forced. A higher negative score implies stronger chemical bonding. Americine shows a docking score of −112.82 in EGFR, while positive control

gefitinib shows −103.14. Similarly, for K-Ras, americine shows −139.02 and positive control adagrasib shows −136.77. In the case of PI3K, docking scores of −115.76 and −113.52 are shown by americine and positive control duvelisib, respectively. Lastly, −144.45 was scored by americine against a score of −138.89 of positive control dabrafenib for the target B-raf. Mutations in K-Ras and PI3K make normal cells resistant to death via apoptosis [73,74], while mutations in EGFR and B-Raf cause unchecked cell proliferation [75,76]. Americine prevents cell growth and induces apoptosis to treat NSCLC as it inhibits all four of these targets. Similar studies by Trabalzini et al. [65] were based on molecular docking, with Oat Avenanthramides as EGFR inhibitors, which resulted in the inhibition of growth and migration in NSCLC cells. Another molecular docking analysis of B-Raf with linalool by Singh et al. [77] revealed the chemopreventive activity against lung cancer.

Through molecular docking, we know that the plant secondary metabolite americine has the potential to bind and inhibit the drug targets of NSCLC; however, this does not guarantee that it will be an effective drug. We have completed the ADMET for predicting the compound's activity to foretell its potential as a pharmaceutical candidate. These in silico toxicological analysis techniques are generally accepted by the pharmaceutical industry for use in decision-making throughout the medication development process for validation [72,78,79] and have been shown to drastically cut down on the number of chemicals tested on animals [80]. Adverse drug reactions, metabolic effects, and drug transport predicted that americine would be well absorbed by the human gastrointestinal tract [80]. BOILED-Egg analysis confirms the low risk of affecting regular CNS activity, demonstrating the compound's inactivity. Similar studies can be found in the work of Laskar et al. [72].

Some additional pharmacokinetic and drug-likeness profiling was performed with the help of the SwissADME server [72]. With only one infraction of the drug-likeness rule and a moderate Abbot bioavailability score, americine stands out as the most promising candidate for use as a structural template for the development of new drugs.

This research foretold that the anti-lung cancer benefits of the americine compounds would be exerted via a combination of mechanisms. With current therapies having strong side-effects and being too expensive to reach the hand of the common person, further research must be conducted with this potent phytocompound against such deadly diseases. Plant-based therapeutics offer a feasible replacement for conventional cancer treatments such as chemotherapy and radiation therapy, with the added benefits of reduced side-effects and lower treatment costs.

5. Conclusions

From these in silico experiments, it is concluded that americine, a phytocompound present in the methanolic extract of the plant *Allium tuberosum*, has the potential to control the NSCLC by inducing apoptosis and controlling cell proliferation. By molecular docking analysis, americine is found to be the best inhibitor for all the four selected target proteins, viz., mutated EGFR, B-Raf, K-Ras, and PI3K. In addition, ADME screening confirms the drug-like properties also exhibited in this compound. Thus, further studies may be continued that may lead this compound into a marketed drug for cancer therapeutics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122211749/s1, Supplementary—I: Compounds identified in the GC-MS and HRLC–MS chromatogram; Supplementary—II: Docking Results.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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