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In Silico Analysis of Phytoconstituents from Ethanolic Extract of Plumeria obtusa as Potential EGFR-Targeting Compounds in Lung Cancer

Mishika Ahuja[1], Mohammad Salman Akhtar[2], Mudra Sikenis[3,] Raed A. Alharbi[4], Abdulmajeed A A Sindi[5], Mohammed A. Shanawaz[6], Nargis Begum Javed[7], Tintu Thomas Uthup[8], Aisha Farhana[9], Meshal Naif Nasser Alshammari[10], Subhabrata Kar[3*], Mohammad Zeeshan Najm[1*],

[1,*] School of Bioscience, Apeejay Stya University, Gurugram,
[2]Department of Basic Medical Sciences, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia,
[3,*]Department of Life Science, Sharda University, Noida,
[4]Department of Laboratory Medicine, Faculty of Applied Medical sciences, Al-Baha University, Al-Baha Saudi Arabia,
[5]Department of Basic Medical Sciences, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia,
[6]Department of Public Health, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia,
[7]Department of Public Health, College of Health Science, Saudi Electronic University, Dammam,
[8]Department of Public Health, College of Health Science, Saudi Electronic University, Riyadh,
[9]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka 72388, Aljouf, Saudi Arabia,
[10]Department of Radiology, University Medical Clinics, University of Hail, Hail, Saudi Arabia.
Corresponding Author: Mohammad Zeeshan Najm1*
Email: biotechzeeshan@gmail.com
Co-Corresponding Author: Subhabrata Kar3*
Email: kar.subha@gmail.com

ABSTRACT: Non-small cell lung cancer (NSCLC) remains one of the leading causes of cancer-related deaths worldwide, where aberrant epidermal growth factor receptor (EGFR) signaling and activating mutations play a central role in tumor progression. Targeted EGFR inhibition has emerged as an effective therapeutic strategy for the management of EGFR-driven NSCLC. Natural products provide a diverse source of chemical scaffolds that may act as potential anticancer agents. Plumeria obtusa is a medicinal plant reported to contain diverse secondary metabolites with potential pharmacological activities. The present study was designed as a preliminary exploratory investigation of phytoconstituents from the ethanolic extract of P. obtusa and their potential interaction with EGFR. GC–MS profiling tentatively identified 42 chromatographic peaks corresponding to multiple phytochemical constituents. The

identified compounds were subsequently subjected to drug-likeness and ADME screening using SwissADME, and selected compounds were evaluated by molecular docking against five experimentally determined EGFR kinase-domain structures (PDB IDs: 1XKK, 2ITX, 2GS6, 3VJO, and 4I23). Among the evaluated compounds, 2-Methoxy-5-phenylbenzoic acid (PubChem ID 17834343) exhibited the most favorable docking scores across the five EGFR structures (−6.2 to −7.1 kcal/mol), whereas 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- (PubChem ID 5363777) showed scores ranging from −5.5 to −6.7 kcal/mol. The results indicate that 2-Methoxy-5-phenylbenzoic acid has a comparatively favorable predicted interaction profile with the EGFR kinase domain and may warrant further investigation.

INTRODUCTION

Lung cancer continues to be the leading cause of cancer-related death worldwide. The World Health Organisation (WHO) predicts that increased tobacco use globally, especially in Asia, will significantly increase lung cancer mortality rates in the future, increasing this public health burden.

The primary risk factor for this illness is still tobacco use, but air pollution and radon exposure also play a part. Due to insufficient screening programs and delayed development of clinical symptoms, the majority of patients are diagnosed with advanced-stage disease; as a result, their prognosis is quite bad. NSCLC can be diagnosed using a variety of methods, such as X-ray, CT, and PET imaging, as well as histological analysis of tumour specimens (1). In 2020, over 20% of the world's population smoked, with a prevalence of 15.4% among younger people (15–24 years old). Despite the declines in smoking prevalence over the previous 20 years, the incidence of lung cancer in non-smokers is increasing globally, highlighting the need for more research in this area (2).

The characteristics of malignant neoplasms include invasion of their surroundings, metastasis to other sites, persistent angiogenesis, and immune system evasion. Uncontrolled cell proliferation, which is imbalanced by the ability to cause cell loss (cell death by apoptosis and necrosis) and independent of normal tissue homeostasis, is inherent in these traits (2,3). Modification of the normal genome and its expression, which results in altered cellular morphology and abnormal cell behaviour through changed protein expression, is a basic feature of malignancy (4). Numerous oncogenic changes that impact TSGs and oncogenes can be found in NSCLC. The majority of therapeutically relevant oncogenic drivers are kinases that are activated by mutation, such as receptor tyrosine kinases (RTKs) like EGFR, ERBB2 (also known as HER2), and MET; or by forming fusion proteins with constitutively activated kinase domains, such as fusions involving ALK, RET, ROS1, NRG1, NTRK1, NTRK2, or NTRK3. (5,6). The majority of these driver mutations and fusions are not linked to tobacco carcinogens; for instance, air pollution may be linked to the proliferation of lung epithelial cells carrying EGFR mutations.

There has been a major push toward the development of targeted medicines since these NSCLC tumours became extremely dependent on these particular altered pathways for survival—a concept known as "oncogene addiction." Tyrosine kinase inhibitors (TKIs) are the main therapy in this field. These are usually small-molecule medications designed to attach directly to the active kinase domain's ATP-binding pocket, hence blocking the growth signal of the malignancy (2). Non-small cell lung cancer (NSCLC) is largely caused by important oncogenic drivers, including mutations in KRAS and BRAF, in addition to alterations in receptor tyrosine kinase (RTK). Rather than altering the surface receptors themselves, these modifications hyperactivate intracellular signalling cascades downstream of the cell membrane (7,8). Key characteristics of these downstream mutations include the activation of RAS–RAF–MEK and PI3K–AKT–mTOR signalling pathways, as well as mutations in KRAS and BRAF.

Tyrosine kinase inhibitors (TKIs) are unquestionably effective in treating oncogene-addicted non-small cell lung cancer (NSCLC), the development of acquired drug resistance often compromises their long-term effectiveness. For instance, first- and second-generation TKIs may become ineffective due to subsequent mutations in the EGFR kinase domain, such as the T790M or C797S mutations, which can result in a resurgence of the disease (9). Furthermore, the therapeutic efficacy of synthetic targeted medicines is severely limited by their systemic toxicity, unfavourable side effects, and high cost. This

emphasises how urgently new, highly selective treatment agents with improved safety profiles and the capacity to circumvent existing resistance mechanisms must be found and developed.

Natural products have historically been a rich source for the development of anti-cancer medications. Because of their enormous structural diversity and multitargeted modes of action, phytoconstituents—bioactive chemicals originating from plants—are appealing prospects for the creation of new kinase inhibitors with potentially lower toxicity (10).

Plumeria obtusa (family *Apocynaceae*) is one of the many therapeutic plants that has attracted a lot of ethnopharmacological attention. Extracts from the Plumeria genus are known to have a broad range of biological actions, including antibacterial, anti-inflammatory, antioxidant, and possibly anti-neoplastic qualities. They have long been used in numerous indigenous medicine systems (11). Alkaloids, flavonoids, terpenoids, and glycosides are among the several secondary metabolites found in *P. obtusa's* ethanolic extract that may be useful lead molecules against molecular targets unique to cancer.

However, it takes a lot of time and resources to evaluate a wide range of phytochemicals using conventional in vitro and in vivo techniques. Computational or *in silico* methods are now essential for speeding up drug discovery. Before laboratory testing, researchers can quickly screen compounds, predict their binding affinities to target proteins like EGFR, and assess their drug-likeness and safety profiles using methods like molecular docking, molecular dynamics (MD) simulations, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling (12).

Thus, using an *in silico* method, the current work seeks to close the gap between targeted cancer therapy and conventional medicine. In particular, this work examines the phytoconstituents found in *Plumeria obtusa's* ethanolic extract in order to assess their molecular interactions and binding effectiveness as possible EGFR inhibitors in non-small cell lung cancer. This study aims to discover prospective lead compounds that require additional in vitro and in vivo validation for lung cancer treatment by analysing these interactions.

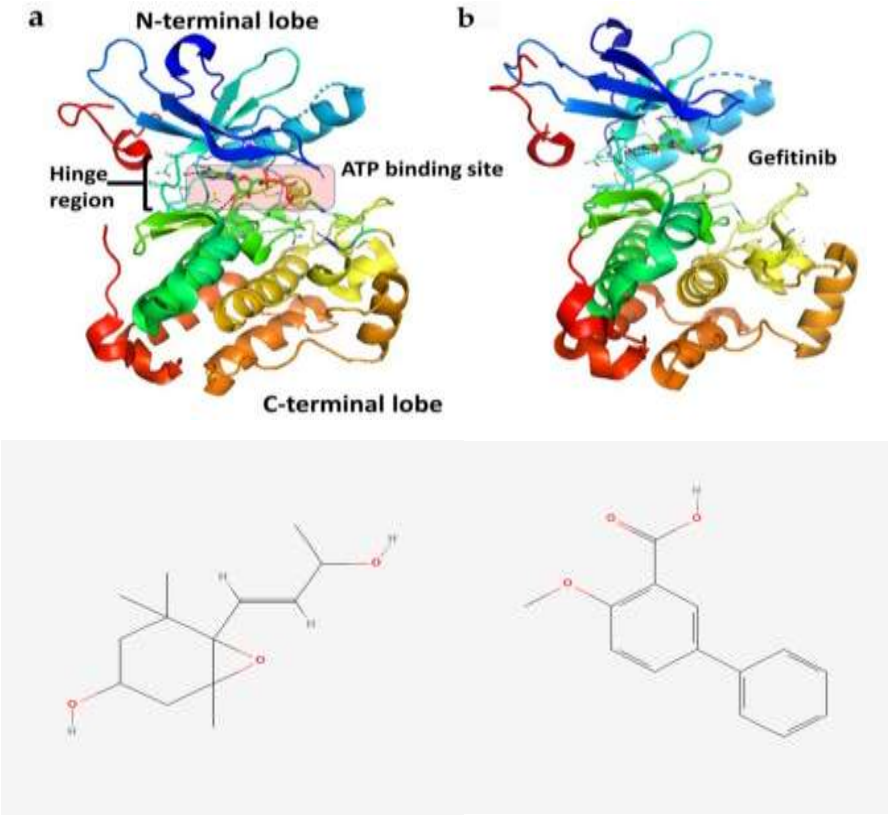


Figure 1 A. EGFR kinase domain crystal structure with the ATP binding site shown (PDB ID: 2GS6). In the ATP-binding pocket, TKIs of EGFR attach to EGFR and create one to three hydrogen bonds with the hinge area. two dimensional structures of compounds **B.** 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- and 2-Methoxy-5-phenylbenzoic acid.

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METHODOLOGY

Gas chromatography –mass spectrometry of crude Ethanoic extract of *Plumeria obtusa* L.

The phytoconstituents present in the ethanolic extract of *Plumeria obtuse* were analyzed using a Shimadzu GCMS-QP-2010 Plus equipped with an Rtx®-5MS low-bleed capillary column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness). The mass scan's range was adjusted to 50–600 m/z. To identify and analyse the identified substances, their spectrum patterns were compared to those in the NIST and Wiley databases (13,14). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 0.5 µL sample was injected in splitless mode, with a purge flow of 3 mL/min. The injector and detector temperatures were maintained at 250°C and 280°C, respectively. The oven temperature was initially maintained at 60°C for 2 min and subsequently increased at a rate of 5°C/min to a final temperature of 280°C. Compounds were ionised by electron impact (EI) at 70 eV, and mass spectra were acquired over an m/z range of 50–600. The detected constituents were tentatively identified by comparing their mass spectral patterns with those available in the NIST and Wiley spectral libraries.

ADMET analysis

The identified phytoconstituents obtained from the GC–MS analysis were subjected to drug-likeness and predicted ADME screening using the SwissADME web-based platform. The corresponding compound structures and PubChem identifiers were obtained from the PubChem database, and the canonical SMILES of the 39 unique tentatively identified phytoconstituents were retrieved and submitted to SwissADME. The compounds were evaluated for their physicochemical properties, Lipinski's rule of five, predicted gastrointestinal (GI) absorption, bioavailability score, and PAINS alerts. Based on the overall drug-likeness and predicted ADME profiles, compounds meeting the study's selection criteria were prioritised for subsequent molecular docking analysis against the EGFR kinase domain (15).

Molecular Docking

The binding affinity and interaction of ADMET-screened plant-derived compounds with the target EGFR protein were analysed using AutoDock Vina v1.2.7. To account for structural variability of the receptor, five experimentally determined crystal structures of the EGFR kinase domain (PDB IDs: 1XKK, 2ITX, 2GS6,3VJO, and 4I23) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/). Protein structures were prepared by removing co-crystallized ligands and water molecules, followed by the addition of polar hydrogen atoms and Kollman charges using AutoDockTools v1.5.7. Ligand structures were obtained from the PubChem database were converted from SDF to PDBQT format using Open Babel v3.1.1. For each EGFR structure, the grid box was individually defined to encompass the entire protein, thereby enabling a blind docking approach. Docking was performed with an exhaustiveness value of 8 to ensure adequate conformational sampling. The docked protein–ligand complexes were subsequently analyzed for binding affinity and molecular interactions using Discovery Studio Visualizer.

RESULT

GC-MS analysis of the ethanol extract of *Plumeria obtusa* showed the presence of multiple bioactive phytocompounds. These included various classes, such as fatty acid esters, sterols, diterpenes, tocopherol, triterpenoids, and nucleosides. A total of 42 distinct peaks were observed based on retention times and mass spectral matching with reference libraries (Table 1). The chromatographic profile spanned from 14.384 to 36.876 min, indicating the presence of constituents with wide chromatographic properties (Figure 2). The relative peak-area distribution revealed the major constituent detected was Lup-20(29)-en-ol acetate, which comprised 30.57% of the total relative peak area. This was followed by 24-Norursa-3,12-diene (13.04%), 9,19-Cyclolanost-24-en-3-ol (7.42%), Phytol (4.50%), and Stigmast-5-en-3-ol (4.40%). Other significant compounds contributing to the extract's bioactivity were Olean-12-en-3-ol acetate (3.17%), Vitamin E (2.51%), Neophytadiene (2.02 percent and 0.66 percent on two chromatographic peaks) and Ergost-5-en-3-ol (1.62%). The minor peaks of several diterpenes, sterol esters, and fatty acid esters were observed, depicting the complex phytochemical properties. The GC-MS profile therefore provided a broad preliminary characterization of the phytochemical composition of the ethanolic extract and allowed identification of its constituents for future computational prioritization. The compounds detected from the extract were based on mass-spectral library matching and without reported confirmation using authentic standards; therefore, the identified compounds are tentatively identified compounds. This distinction is relevant when considering the GC-MS profile and subsequent *in silico* analysis.

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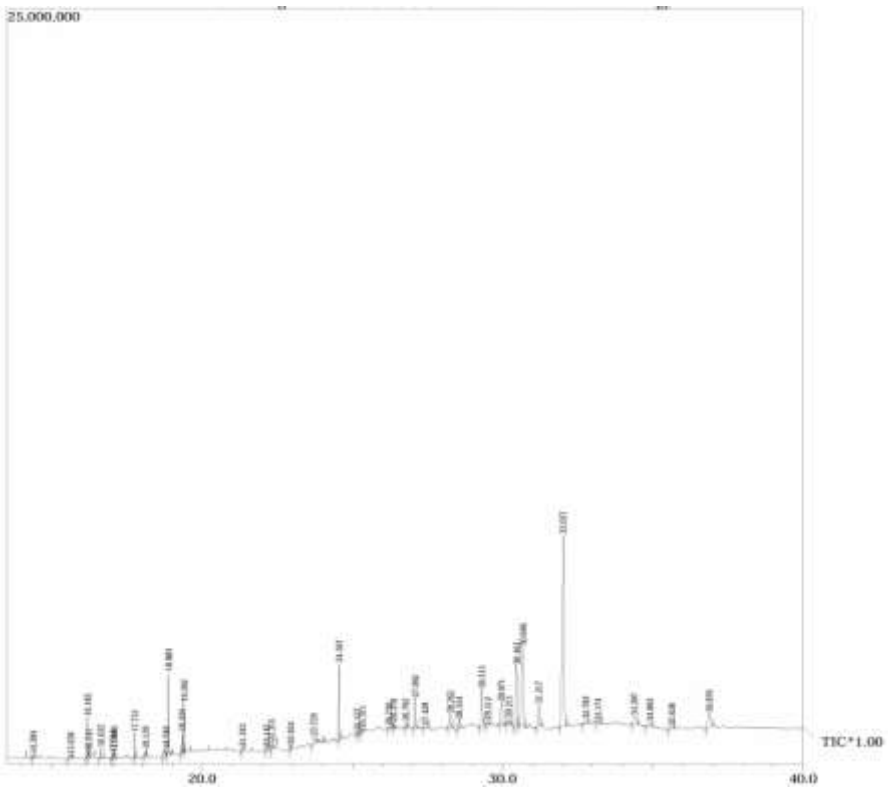


Figure 2 represents the chromatogram of mass spectral peaks of 42 compounds.

Table 1. Represent the retention time (R.Time) and relative peak area (%) of bioactive compounds observed in the ethanol extract of *Plumeria obtusa*

Peak#	R.Time	Area	Area%	Name
1	14.384	280767	0.28	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1
2	15.636	555760	0.56	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl-
3	16.183	2015171	2.02	Neophytadiene
4	16.241	234127	0.23	1-Dodecanol, 3,7,11-trimethyl-
5	16.632	655365	0.66	Neophytadiene
6	17.014	278320	0.28	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1
7	17.080	442569	0.44	HEXADECANOIC ACID, METHYL ESTER
8	17.751	1272698	1.27	HEXADECANOIC ACID, ETHYL ESTER
9	18.129	805031	0.81	2-Methoxy-5-phenylbenzoic acid
10	18.783	829132	0.83	6-Octadecenoic acid, methyl ester, (Z)-
11	18.883	4502914	4.50	Phytol
12	19.334	1017087	1.02	Linoleic acid ethyl ester
13	19.392	2511354	2.51	cis,cis,cis-7,10,13-Hexadecatrienal
14	21.343	253086	0.25	Eicosanoic acid, ethyl ester

15	22.145	305408	0.31	Decane, 1,9-bis[(trimethylsilyl)oxy]-
16	22.315	488251	0.49	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este
17	22.934	248781	0.25	Docosanoic acid, ethyl ester
18	23.729	1229779	1.23	2-Methylhexacosane
19	24.567	3994411	4.00	Squalene
20	25.157	255646	0.26	Pentatriacontane
21	25.311	294242	0.29	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, ace
22	26.230	250463	0.25	Octadecanal, 2-bromo-
23	26.378	460064	0.46	.gamma.-Tocopherol
24	26.792	568884	0.57	Octacosanol
25	27.092	2509525	2.51	Vitamin E
26	27.428	382424	0.38	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,
27	28.262	1623843	1.62	Ergost-5-en-3-ol, (3.beta.)-
28	28.554	940593	0.94	Stigmasterol
29	29.315	4394506	4.40	STIGMAST-5-EN-3-OL, (3.BETA.)-
30	29.512	706152	0.71	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-
31	29.971	3063703	3.06	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6
32	30.211	775205	0.78	Lup-20(29)-en-3-one
33	30.462	7415973	7.42	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
34	30.666	13033473	13.04	24-Norursa-3,12-diene
35	31.217	3163934	3.17	Olean-12-en-3-ol, acetate, (3.beta.)-
36	32.027	30554581	30.57	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
37	32.783	797849	0.80	Phytyl stearate
38	33.174	253631	0.25	Olean-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)
39	34.397	1719432	1.72	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3.beta.)-
40	34.893	999115	1.00	Ursolic aldehyde
41	35.638	559829	0.56	Uvaol
42	36.876	3316441	3.32	Uvaol
		99959519	100.00	

Screening of Phytocompounds present in the extract of *Plumeria obtusa* by Swiss ADME

The compounds were then further screened for drug-likeness and predicted ADME properties by using SwissADME to further prioritize the tentatively identified constituents (Table 2). The screening dataset consisted of 39 compound entries and included evaluated molecular weight, number of hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), predicted gastrointestinal (GI) absorption, Abbott bioavailability score, Lipinski's rule of five, and PAINS alerts. The screening revealed considerable variation in predicted ADME profiles of the evaluated constituents, especially in terms of GI absorption. Both high and low GI absorption classifications were observed for screened compounds. The Abbott bioavailability score was

predominantly 0.55, whereas a score of 0.85 was observed for 2-Methoxy-5-phenylbenzoic acid. Based on these findings, 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- (PubChem ID: 5363777) and 2-methoxy-5-phenylbenzoic acid (PubChem ID: 17834343) were selected for comparative molecular docking with EGFR.

Table 2 showing the screening passed compounds with no breaching of Lipinski's rule of Five, presenting their pharmacokinetic analysis predicted by Swiss ADME

S.No.	Compound Name	PubChem ID	Mol.Wt (g/mol)	HBD	HBA	Bioavailability	G.I. absorption
1	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-	5363777	226.31	2	3	0.55	high
2	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl-	107166	236.39	0	1	0.55	high
3	NEOPHYTADIENE	10446	278.5	0	0	0.55	low
4	1-Dodecanol, 3,7,11-trimethyl-	138824	228.41	1	1	0.55	high
5	METHYL HEXADECANOATE	8181	270.5	0	2	0.55	high
6	HEXADECANOIC ACID, ETHYL ESTER	12366	284.5	0	2	0.55	high
7	2-Methoxy-5-phenylbenzoic acid	17834343	228.24	1	3	0.85	high
8	6-Octadecenoic acid, methyl ester, (Z)-	5362717	296.5	0	2	0.55	high
9	Phytol	5280435	296.5	1	1	0.55	low
10	Linoleic acid ethyl ester	5282184	308.5				
11	cis,cis,cis-7,10,13-Hexadecatrienal	5367366	234.38	0	1	0.55	high
12	Eicosanoic acid, ethyl ester	29009	340.6	0	2	0.55	low
13	Decane, 1,9-bis[(trimethylsilyl)oxy]-	575943	318.64	0	2	0.55	high
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	123409	330.5	2	4	0.55	high
15	Docosanoic acid, ethyl ester	22199	368.6	0	2	0.55	low
16	2-Methylhexacosane	150931	380.7	0	0	0.55	low
17	Squalene	638072	410.7				

18	Pentatriacontane	12413	492.9	0	0	0.55	low
19	3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraene-1-OL trimethylsilyl ether	101588531	362.7	12	0	0.55	low
20	Octadecanal, 2-bromo-	537255	347.4	0	1	0.55	low
21	.gamma.-Tocopherol	92729	416.7	1	2	0.55	low
22	Octacosanol	68406	410.8	1	1	0.55	low
23	Vitamin E	14985	430.7	1	2	0.55	low
24	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	522326	222.37	1	1	0.55	high
25	Ergost-5-en-3-ol, (3.beta.)-	173183	400.7	1	1	0.55	low
26	Stigmasterol	5280794	412.7	1	1	0.55	low
27	STIGMAST-5-EN-3-OL, (3.BETA.)-	222284	414.7	1	1	0.55	low
28	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	6443745	426.7	1	1	0.55	low
29	OLEAN-12-EN-3-ONE	6454747	424.7	0	1	0.55	low
30	Lup-20(29)-en-3-one	323075	424.7	0	1	0.55	low
31	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	92110	426.7	1	1	0.55	low
32	24-Norursa-3,12-diene	91735342	394.7	0	0	0.55	low
33	Olean-12-en-3-ol, acetate, (3.beta.)-	92156	468.8	0	2	0.55	low
34	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	92157	468.8	0	2	0.55	low
35	Phytyl stearate	14486562	563	0	2	0.17	low
36	Olean-12-en-28-oic acid	54671898	502.7	4	6	0.56	high
37	Urs-12-en-28-oic acid	1.63E+08	470.7	2	4	0.85	high
38	Ursolic aldehyde	14423521	440.7	1	2	0.55	low
39	Uvaol	92802	442.7	2	2	0.55	low

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***In Silico* molecular docking with EGFR**

Comparative molecular docking was carried out against five experimentally determined EGFR kinase-domain structures (PDB IDs: 1XKK, 2ITX, 2GS6,3VJO, and 4I23) to investigate the possible interaction of the selected phytocompounds with EGFR. In all five receptor conformations, 2-Methoxy-5-phenylbenzoic acid (PubChem ID 17834343) consistently demonstrated favourable binding affinity with docking scores ranging from −6.2 to −7.1 kcal/mol (Figure 3). Despite conformational variations among the EGFR crystal structures, comparable docking scores suggested a consistent binding mode. With residues like Ala722 and Arg841 in the 1XKK structure, Ala698 and Arg831 in 3VJO, and Asn700 and Arg831 in 4I23, the ligand established traditional hydrogen bonds. Ala698 and Arg831 in 3VJO, Asn700 and Arg831 in 4I23, and two conventional hydrogen bonds with Met769 in the 2GS6 structure. In the 2GS6 complex, additional π-sulfur interactions with Met742, π-sigma interactions with Val702, alkyl/π-alkyl interactions involving Lys721, Leu694, and Leu820, together with multiple van der Waals contacts with Gln767, Thr766, Leu768, Pro770, Gly772, and Leu764, further supported the predicted binding orientation of the ligand. Furthermore, the complexes were further stabilised by π-anion, π-alkyl, hydrophobic, and van der Waals interactions, including residues such as Val726, Cys797, Leu844, Glu697, and Leu861. Residues like Val726, Cys797, and Leu844, which are linked to the binding of ATP-competitive EGFR inhibitors, are involved, indicating that this chemical is in a biologically significant area of the kinase active site. These results suggest that among the two assessed EGFR-binding phytocompounds, 2-Methoxy-5-phenylbenzoic acid is the more promising.

On the other hand, docking scores for 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- (PubChem ID 5363777) ranged from −5.5 to −6.7 kcal/mol, suggesting a lower anticipated binding affinity for EGFR. Although the ligand formed conventional hydrogen bonds with Thr830 and Glu738 in the 2GS6 structure together with several hydrophobic interactions, the presence of an unfavourable acceptor–acceptor interaction with Gln767 may reduce the stability of the predicted protein–ligand complex. In contrast few conventional hydrogen bonds, the interaction profile was dominated by π–π stacking, carbon-hydrogen bonds, and hydrophobic contacts. Moreover, unfavourable donor–donor and acceptor–acceptor interactions were found in specific receptor conformations, which could impair the overall stability of the protein–ligand complex. The compound's interaction network was smaller than that of 2-Methoxy-5-phenylbenzoic acid , which explains its lower docking scores and suggests a somewhat poorer affinity for the EGFR active site, even though it connected with residues implicated in kinase regulation.

Crucially, neither drug consistently interacted with other important EGFR residues like Lys745 and Thr790 that are frequently targeted by clinically licensed EGFR inhibitors, nor did they generate the distinctive hinge-region interaction with Met793. Therefore, even though both phytocompounds are expected to interact inside the EGFR kinase domain, their classification as strong ATP-competitive EGFR inhibitors is not supported by the docking data alone. In order to confirm its therapeutic potential, 2-Methoxy-5-phenylbenzoic acid should be the primary choice for more research using molecular dynamics simulations, MM/PBSA or MM/GBSA binding free-energy calculations, and experimental kinase inhibition experiments.

Table 3. Molecular docking scores (binding affinity, kcal/mol) of selected phytocompounds against four EGFR crystal structures (PDB IDs 1XKK, 2ITX, 2GS6, 3VJO, and 4I23).

Compound	PubChem ID	1XKK	2ITX	2GS6	3VJO	4I23
7-Oxabicyclo[4.1.0]heptan-3-ol,	5363777	-5.5	-5.5	-6.7	-6.0	-5.7
2-Methoxy-5-phenylbenzoic acid	17834343	-6.3	-6.3	-7.1	-6.2	-6.2

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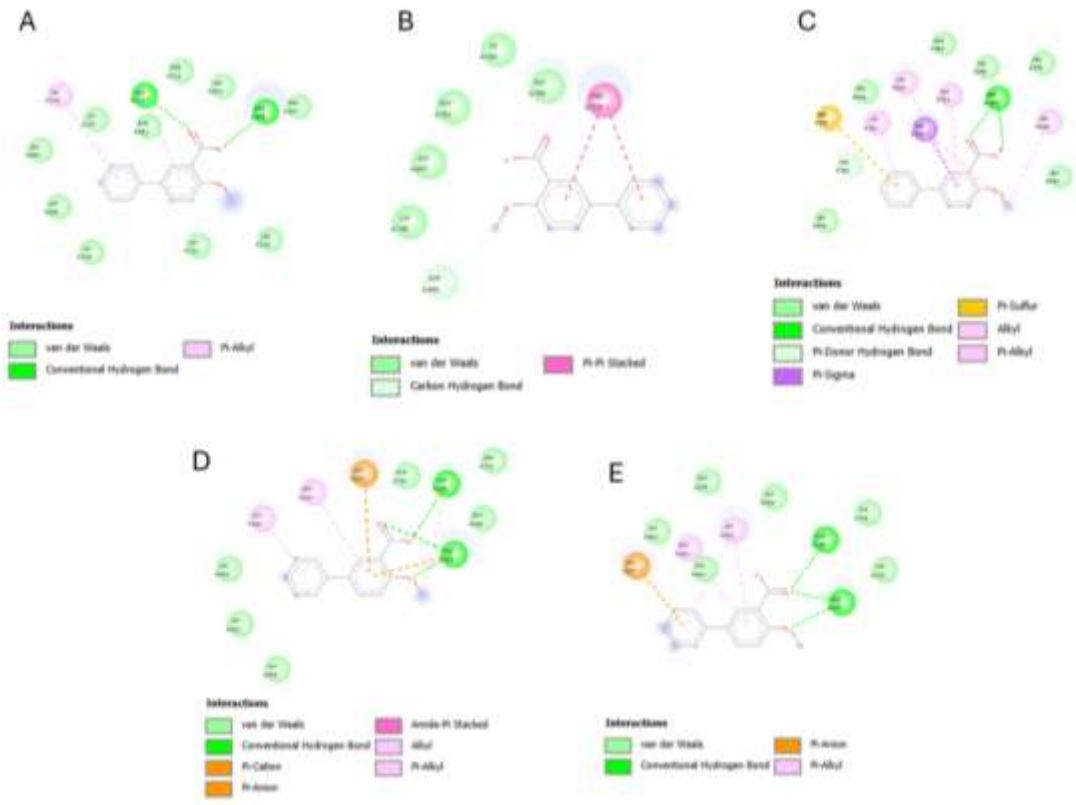


Figure 3. Two-dimensional protein-ligand interaction diagrams of the chosen phytocompounds docked with various EGFR kinase domain conformations. (A) 2-methoxy-5-phenylbenzoic acid with EGFR (PDB ID: 1XKK); (B) 2-methoxy-5-phenylbenzoic acid with EGFR (PDB ID: 2ITX); (C) 2-methoxy-5-phenylbenzoic acid with EGFR (PDB ID: 2GS6); (D) 2-methoxy-5-phenylbenzoic acid with EGFR (PDB ID: 3VJO); and (E) 2-methoxy-5-phenylbenzoic acid with EGFR (PDB ID: 4I23). Conventional hydrogen bonds, hydrophobic interactions, π-interactions, and van der Waals contacts that contribute to ligand stabilisation within the EGFR binding pocket are depicted in the diagrams.

DISCUSSION

EGFR is an established molecular target in NSCLC due to its ability to activate downstream signaling pathways such as the PI3K/AKT pathway and RAS/RAF/MEK/ERK pathways, which can stimulate tumor-cell proliferation, survival, migration, and progression. (16). EGFR tyrosine kinase inhibitors (TKIs), including gefitinib, erlotinib, afatinib and osimertinib, are clinically effective in patients with selected molecular subtypes; however, resistance and drug side effects are significant issues in EGFR-targeted therapy (17,18). Natural products are a chemically diverse group of molecules that can be screened for preliminary identification of compounds that can interact with therapeutically important molecular targets. Therefore, the present study explored the phytochemical profiling of the ethanolic extract of *Plumeria obtusa*, further drug-likeness and ADME screening, and then comparative molecular docking against the EGFR kinase domain.

The *P. obtusa* ethanolic extract had 39 phytoconstituents belonging to chemically varied classes, including terpenoids, sterols, fatty acid esters, tocopherols, triterpenoids, and aromatic chemicals. The predominant detected constituent, based on relative peak area, was lup-20(29)-en-3-ol acetate, followed by 24-Norursa-3,12-diene, 9,19-Cyclolanost-24-en-3-ol, Phytol and Stigmast-5-en-3-ol. This diversity yielded a broad chemical pool from which compounds could be considered for subsequent computational prioritization. SwissADME evaluation revealed that the chosen phytocompounds had favourable pharmacokinetic and physicochemical characteristics. Several compounds exhibited high gastrointestinal absorption, while others demonstrated low predicted absorption. The two selected lead compounds fulfilled the screening criteria (19). They also displayed no PAINS alarms. While 7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl- showed a bioavailability score of 0.55, which is still within the acceptable range for drug candidates, 2-Methoxy-5-phenylbenzoic acid

showed a bioavailability score of 0.85, indicating superior predicted oral bioavailability when compared to the majority of other screened compounds. Therefore, the Swiss ADME results were used as a primary lead for further prioritization rather than a definitive suitable drug candidate (20,21).

Following initial screening, binding energies ranging from −6.2 to −7.1 kcal/mol were observed for 2-Methoxy-5-phenylbenzoic acid, which demonstrated the best docking profile among the investigated phytochemicals against five EGFR crystal structures (PDB IDs: 1XKK, 2ITX, 2GS6, 3VJO, and 4I23). Despite structural differences in the EGFR kinase domain, the ligand appears to establish a consistent binding orientation based on its binding affinity across several receptor conformations. In addition to hydrophobic, π-anion, π-alkyl, and van der Waals interactions, including Val726, Cys797, Leu844, Glu697, and Leu861, protein–ligand interaction studies revealed the formation of typical hydrogen bonds with residues such as Ala722, Ala698, Asn700, Arg831, and Arg841. Crucially, residues like Val726, Cys797, and Leu844 are often engaged in ligand identification by ATP-competitive EGFR inhibitors, suggesting that the compound resides in an area of the ATP-binding pocket that is biologically significant.

On the other hand, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-7-Oxabicyclo[4.1.0]heptan-3-ol showed relatively lower binding energies (−5.5 to −6.0 kcal/mol) and less stabilising interactions with EGFR. Hydrophobic contacts and π–π interactions dominated the interaction profile, while there were sporadic unfavourable donor–donor and acceptor–acceptor interactions that would have decreased the overall stability of the complex. Despite having a discernible affinity for the kinase domain, this compound's interaction network was significantly smaller than that of 2-Methoxy-5-phenylbenzoic acid, which accounts for its comparatively lower docking scores.

The conventional hinge-region hydrogen bond with Met793, which is thought to be a signature interaction for clinically authorised EGFR tyrosine kinase inhibitors like gefitinib, erlotinib, afatinib, and Osimertinib, was not consistently produced by either molecule despite these promising results. Similarly, there were inconsistent observations of interactions with catalytic residues such as Lys745 and the gatekeeper residue Thr790. These findings imply that while both phytochemicals are capable of occupying the EGFR binding pocket, their manner of binding is different from that of known ATP-competitive inhibitors. Therefore, rather than definite kinase inhibition, the current docking results should be viewed as evidence of possible EGFR binding.

The current study shows how phytochemical profiling, molecular docking, and ADMET prediction can be used to quickly identify interesting lead compounds from therapeutic plants. The importance of *in silico* screening as a first step in the development of anticancer drugs has been demonstrated by the successful identification of natural EGFR inhibitors from a number of medicinal plants using similar computational methods. However, receptor flexibility, solvent effects, and binding kinetics cannot be fully taken into account by molecular docking, which is merely a static prediction of protein–ligand interactions. To verify the biological activity of these phytochemicals, additional computational analyses such as molecular dynamics (MD) simulations, MM/PBSA or MM/GBSA free-energy calculations, in vitro EGFR kinase inhibition assays, A549 cell viability studies, and apoptosis analyses are needed.

The most promising phytochemical candidate from *Plumeria obtusa* for EGFR-targeted therapy, according to the current research, is 2-Methoxy-5-phenylbenzoic acid. Its excellent pharmacokinetic profile, absence of PAINS alerts, consistent binding affinity across numerous EGFR conformations, and stable interaction network promote further preclinical exploration as a possible lead chemical for the development of new therapies against NSCLC (22,23).

Conflict of Interest: All the authors have no conflict of interest

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