

Screening of Phosphoinositide-3 Kinase Inhibitors from *Argemone mexicana* Leaves

Sowmya Priya Manoharan, Sangilimuthu Alagar Yadav*,
Gnanaselvan Suvathika, Priyadharshini Anandhan and
Balamurugan Pandiyan

Department of Biotechnology, Karpagam Academy of Higher Education,
Coimbatore, Tamil Nadu, India.

*Corresponding Author E-mail: smuthu.al@gmail.com

<https://dx.doi.org/10.13005/bpj/2926>

(Received: 19 September 2022; accepted: 22 February 2023)

Phosphoinositide 3 kinase belongs to the enzyme family which is responsible for the development of cellular trafficking and uncontrolled cellular division which in turn to cancer metastasis. Activation of the PI3k-Akt-mTOR pathway tends to promote oncogenesis in Lung Cancer and its mutation leads to resistance in EGFR tyrosine kinase inhibitors. Inhibition of the initial PI3k receptor through natural bioactive phytochemicals from *Argemone mexicana* that may prevent the side effects caused by the synthetic medication and improve the patient's life quality as natural medicine. According to the previous research we did the Bioactive phytochemical screening from the methanol extract (AME) and powder (AMP) of *A. mexicana* leaf by analysing the GC-MS (Agilent), UPLC-QTOF-ESI-MS (Waters India Pvt Limited). Analyzed phytochemicals from the studies were subjected to molecular docking analysis using SeeSAR 9.2 software against PI3K (PDP ID: 4FA6) receptor. GC-MS revealed 40 compounds including Cryptopine (17.5), beta-sitosterol (9.57), and Protopine (8.6) were found as major compounds. LC-MS analysis showed the presence of major compounds with 13 elements compared to the literature survey in both positive and negative ESI ranges. 29 compounds from the GC-MS and LC-MS were selected for analyzing the interaction between the cancer receptor-ligand complex according to the binding strategy. Phytoconstituents such as Galactitol, Succinic acid and N-feruloyltryamine from *A. mexicana* showed good binding affinity range and maximised hydrogen bonding that may assure possessing anticancer effect by controlling the PI3K pathway. This study would lead a major role in the preliminary screening of Drug Targets against the PI3K receptor.

Keywords: *Argemone mexicana*; GC-MS; Lung Cancer; Molecular docking; PI3Kreceptor; UPLC-Q-TOF-MS.

Phosphoinositide 3 kinase (PI3K) is also known as Phosphatidylinositol-3-kinase is a breakthrough receptor that responsible for the regulation of the pathway PI3K/Akt/mTOR (mechanistic target of rapamycin). Activation of PI3K involves numerous hallmarks of cancer

such as apoptosis inhibition, invasion of tissue at an increased level, sustained angiogenesis, insensitivity to anti-growth signals and acquiring growth signal anatomy¹. Impairment in the downstream signaling of the Phosphoinositide 3-kinase (PI3K/Akt/mTOR) pathway causes an

instant signal transduction cascade in the lung carcinoma cell growth. Activation of such PI3K pathway results in anti-apoptotic cancer cell proliferation and cell differentiation². Copansilib is such a pan inhibitor standard drug of PI3K α inhibiting the autophosphorylation in the tyrosine residues of the receptor. This is also effective against PI3K β form by inducing apoptosis in tumor cells as well as primary inhibition in malignant B cell proliferation. Although side effects symptoms also persist in Copansilib usage as the drug for the malignant types³.

Cells are the structural and functional unit of a healthy being. Several mutations or genetic impairment develops the proliferation of abnormal aberrated cells that leads to cancer prognosis. There are more than 200 types of cancer effects unisexually both men and women worldwide. Each cancer type differs in its location and exhibits diverse causes⁴. Among the other types of cancer, Non-Small Cell Lung Cancer (NSCLC) remains the second most predominant case and the first in deaths worldwide. NSCLC is caused mainly due to direct and passive smoking, disordered unhealthy diet, pollution, radioactive and stress at last. These external, as well as internal effects, cause changes in the PI3K/Akt/mTOR pathway which are responsible for the abnormal growth of squamous cell carcinoma (NSLC)¹.

Medicinal plants remain in debate for cancer treatment and thus they have been used since the ancient period as traditional medications against several ailments. Phytocompounds such as withaferin, vinblastine, Camptothecin, Taxol and diosgenin have anticancer effects as already reported⁵. Still, most of the anticancer potential of phytomedicine and medicinal plants remains unexplored. *Argemone mexicana* also known as the Mexican poppy belongs to the Papaveraceae family and grows up to 1.2 meters alongside agricultural and wasteland weed areas. This plant is found widely in Bangladesh and all parts of India. *A. mexicana* has potential medicinal properties such as anti-oxidant, analgesic, anti-malarial, antispasmodic, chemo sterilant, wound healing, nematocidal and remedy for snake poisoning. In Ayurveda and Yunani, the plant parts were used as purgative and sedative with skin disease treatment properties⁶. Phytochemicals such as Phenolics, Alkaloids, Tannins, Carotenoids,

Flavonoids, Terpenoids, Saponins, and Pectin were reported from *A. mexicana*⁷. In silico screening of medicinally important phytocompounds of this plant against cancer, receptor remains less explored.

Artificial Intelligence (AI) plays important role in discovering the lead molecules by molecular interaction with specific receptors through Machine Learning (ML). This trending method adds more advantages to the primary selection of the drug targets and receptors of a particular disease that would help at the cellular level⁸. Preliminary screening of the drugs which hits the molecular pathways should be focussed celestially to develop a medication for the predominant disease. The detailed study of functional and structural prospects of drug properties is so important in the drug development field that is carried out by pharmaceutical as well as research industries⁹. Investigation of the essential phytocompounds mainly secondary metabolites can be acquired in mass with the help of analytical instruments. Automated screening of volatile and non-polar phytocompounds such as alkaloids, lipids, fatty acids and volatile essential oil is majorly achieved by Gas Chromatography-Mass Spectrum Analysis (GC-MS). Phenolic phytoconstituents are detected with the help of LC-QTOF- ESI-MS in terms of positive and negative ionization. In this study, we attempted the integrated approach with both the volatile and phenolic phytocompound screening to fulfill the research gap in the binding mechanism against the PI3K receptor which is responsible for lung cancer proliferation.

MATERIALS AND METHODS

Plant collection

Fresh and healthy plant of the *A. mexicana* was collected from Rangareddy District, Telangana state and authenticated in Botanical Survey of India, Coimbatore (vide No: BSI/SRC/5/23/2013-14/Tech./1855). Separated leaves were washed under tap water to free them from sand and dust. This is made to shade dry for a continuous week. Thus, dried leaves were course powdered in an electric grinder and weighed. This was stored in an air-tight container at room temperature for further studies.

Sample Preparation

For GC-MS analysis, Soxhlet extraction was performed with 50 ± 0.8 g, of course, powdered *A. mexicana* Leaves. Analytical grade reagent of Carbinol was purchased from Himedia and used as the solvent for extraction. Up to 8 suction were undergone to obtain the extract yield from *A. mexicana* (AME) in 24 hrs continuous extraction¹⁰. Preparing the plant sample is a bit tedious process. The dried coarse powder of *A. mexicana* Leaves is mixed with a 1:2 proportion of carbinol and made centrifuged for 5 mins at 3000rpm. Thus, the supernatant was carefully removed without disturbing the pellet surface and sterile-filtered in a 0.22 μ m PAL syringe filter. Followed by the process of sonication and degassing until 5 mins thoroughly in ultra sonicator - *A. mexicana* Powder (AMP). Respective extracts (AME & AMP) were taken for GC-MS and LC-MS studies.

Gas Chromatography-Mass Spectrum Analysis

AME has been checked for the Bioactive volatile phytoconstituents utilizing Agilent technologies GC Systems with GC7890A/ MS-59795C model (Agilent Technologies, Santa Clara, CA, USA). Capillary column - DB5MS was allocated with a length of 30 meters and diameter of 0.25mm with a film thickness of about 0.25mm for the screening of phytochemicals. High electron energy of 70eV was used to perform the spectroscopic detection of the electron ionization system with a mass scan range of 45-380 m/z. The initial temperature range was set to 30°C- 350°C and maintained at 10°C for 4min as standard. Pure helium gas of 99.995% was used and the flow rate derives as 1ml/min. The fragmentation pattern of *A. mexicana* Leaf extract for the Retention time of 25mins has been determined using the stored phytocompound in the NIST Library (National Institute of Science and Technology). Thus, retrieved data are separated and noted down for the Tentative name, structure, Molecular weight, Molecular Formula and Retention time¹¹.

Conditions and Sampling in UPLC-ESI-Q-TOF-MS analysis

Tentative identification of phenolic compounds present in the AMP was performed using Waters ACQUITY UPLC-ESI-Q-TOF-MS (Waters, USA). C18 Reverse Phase column has been used for the chromatographic separation and maintained the temperature of 40°C. The gradient

solvent A contains 100% Milliq water (HPLC Grade) and 0.1% of Formic acid whereas solvent B consisted of 100% of acetonitrile. The flow rate of the solvent was set at 1ml/ min with a 5ml injection volume. Sample AMP ran for optimizing the gradient elution program at initially 5% solvent B with a 6-minute duration. Followed by the gradient uptake of 10% (7mins), 15% (7 mins) and 20% (10 mins) of solvent B. Electron spray Ionization (ESI) Interference was performed to analyze the AMP's Mass by Mass Spectrometer. Fragmented MS spectra obtained from the UPLC-MS are compared to the literature records to characterize the positive and negative modes¹².

Receptor and Ligand Preparation for *In Silico* studies

Identified tentative Bioactive compounds from AME & AMP from the analyzed GC-MS and LC-MS chromatogram was subjected to the ligand preparation for computational molecular interaction with Phosphoinositide 3-kinase (PI3K) receptors. All the respective ligands (53 Å molecules) were fetched from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in 3D SDF format. Copansilib, a standard drug treated against the PI3K receptor was also downloaded in SDF format. The receptor responsible for the PI3K/ Akt/mTOR pathway is PI3K was downloaded from the Protein Data Bank as PDB Files in the name of PDB ID:4JPS (<https://www.rcsb.org/>). This also checked for the binding site in the UniProt database to confer the amino acid residues¹³. The process of dehydration and elimination of external ligands is done with Discovery Studio Client Software 4.5 and saved as a PDB file.

Molecular Docking

SeeSAR 9.2 commercial molecular docking software is used for structural interaction between the Phytomolecules from AME & AMP and PI3K receptor. The resolution of the receptor 4JPS (PDB DOI: 10.2210/pdb4JPS/pdb) is found to be 2.20 Å with a sequence length of 1074. The systematic interaction between the ligands and receptor along with the standard was subjected to the identification of Hydrogen Dehydration Calculation (Hyde), Ligand Efficiency (LE) and Estimated Binding affinity in the Docking mode. The binding poses of the interaction are confirmed for the top 5 poses. A binding score of best ligand-receptor hits is determined using the

command prompt in the build software FlexX 4.1. Hydrogen bond (H Bonds) interaction promotes cellular function and increases the binding energy of bonded receptors and Ligands as the actual binding site of the receptor was highly preferred. The final selected Receptor-Ligand complex was analysed using DS Visualizer 4.5 software¹⁴.

RESULTS

Extraction of AME and AMP

50g of coarse powdered *A. mexicana* Leaf undergoing hot Soxhlet extraction yielded 9.8 ± 0.20 mg of AME extract taken for GC-MS analysis. 2g of coarsely powdered *A. mexicana* subjected to sonication and degassing yielded 0.85 ± 0.18 ml of AMP extracts taken for LC-MS analysis.

Analysis of Volatile phytochemicals from AME

Qualitative estimation of AME in the determination of volatile medicinally important bioactive compounds was carried out with GC-MS analysis. Every phytochemicals has been identified using the highest similarity search hits with NIST Library having patterns of more than 2,00,000 by the spectrum interpretation. They were focused to attain the Molecular Weight (MW), Molecular Formula (MF) and percentage

of peak area (Concentration) according to the Retention time. About 34 Bioactive tentative phytochemicals were identified from GC-MS analysis of AME shown in Table 1. The highest abundant peaks identified from the GC-MS analysis are Cryptopine (17.5), beta-sitosterol (9.57), Protopine (8.6), Butane 2,2,—thiobis (5.52) beta-amyrin (4.88), Phytol(4.72), triethylphosphine (4.69), 1,2-Benzenedicarboxylic acid diisooctyl ester (4.43) and 2,4,6-Cycloheptatrien-1-one, 3,5-b is-trimethylsilyl- (3.9). The least compounds found in AME are 1-Ethyl-1-Ethyl-2-pyrrolidinecarboxylic acid, methyl ester (0.49), 3H-Benz[e]indene, 2-methyl- (0.52), Benzofuran, 2, 3-dihydro-4-tert-Butoxystyrene (0.54). Many of the phytochemicals were reported in previous studies but the study of GC-MS analysis demonstrated 40 bioactive phytochemicals. The total TIC chromatogram is shown in Figure 1.

Non-volatile phytochemicals from AMP

Quadrupole Time Of Flight (Q-TOF) plays important role in the finding of the mass by spectral data with a limited absorbance range directly proportionate to the retention time in LC-MS analysis. According to the literature, phenolic compounds are most responsible for the anticancer potential and lie in the mass range of 200-500 m/z ¹⁵.

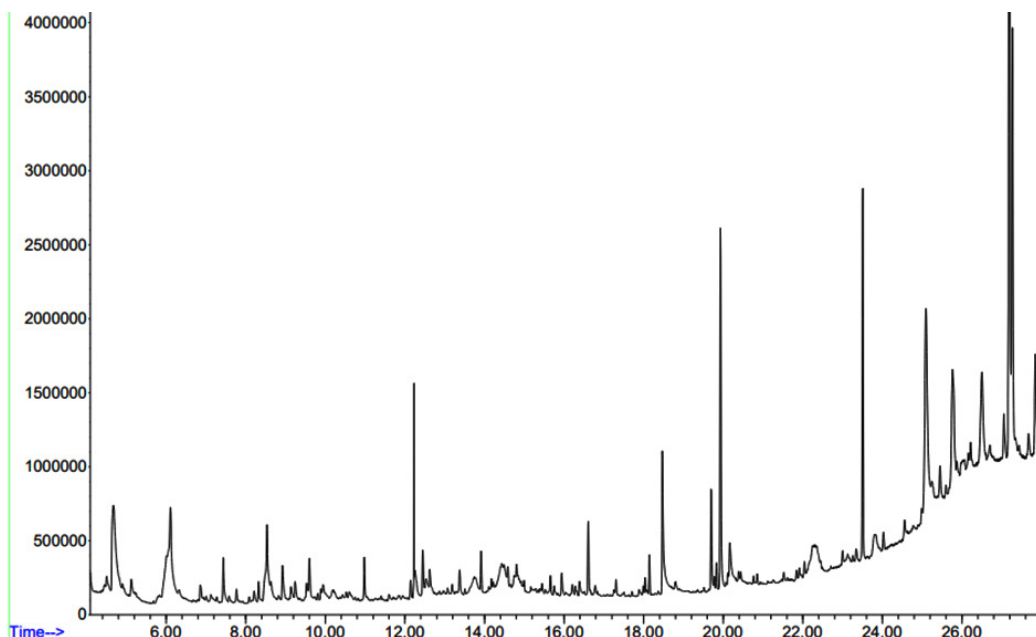


Fig. 1. TIC chromatogram of AME from GC-MS analysis

Non-volatile phytoconstituents of AMP have been screened by utilizing UPLC-QTOF-ESI-MS which was done with the positive and negative modes. The Electron Spray Ionization (ESI) denotes the Charge of the mass ESI (M+H⁺/M-H⁻). Totally 13 tentative compounds have been identified concerning the indexed literature¹⁶⁻²¹. All the mass fragments showed at the highest 100 % were taken into the count and denoted in Table 2. Six tentative

phytoconstituents identified from the negative and seven from the positive mode were identified. The Total Ion Chromatogram obtained from AMP is shown in Figure 2a and 2b.

Molecular interaction of phytoconstituents from AME and AMP with PI3K receptor

Each identified tentative phytoconstituent from GC-MS and LC-MS analysis was allowed to interact in *In silico* with the 4FA6 (PI3K)

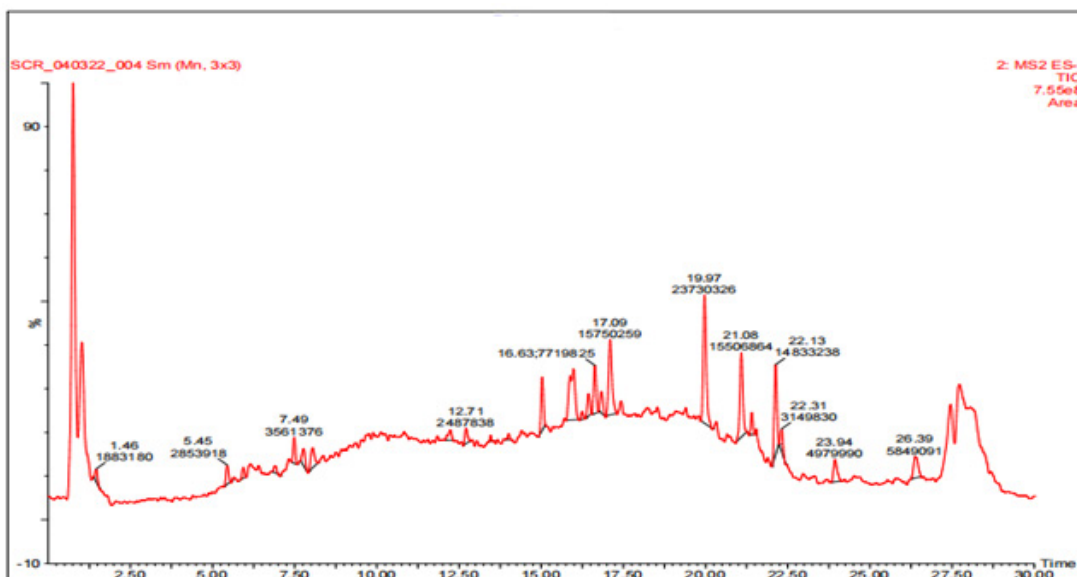


Fig. 2a. ES negative mode TIC chromatogram from AMP

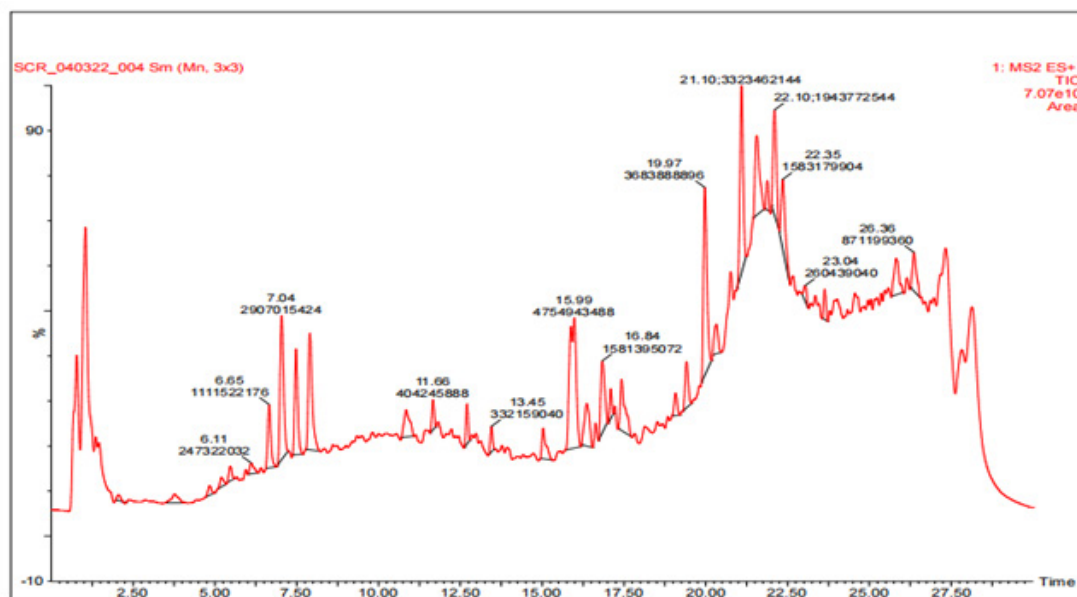


Fig. 2b. ES positive mode TIC chromatogram from AMP

receptor of human origin. Table 1 & 2 shows the Doc score value between the ligand and receptor. The highest negative score shows their stronger interaction bonding which has been highlighted in the table. Thus, the selected high Doc score ligands have been narrowed down for analyzing their interaction sites. Total 47 ligands (34 from GC-MS, 13 from LC-MS and a standard

Copansilib). Ligands such as 6-methyluracil, 1-Ethyl-1-Ethyl-2-pyrrolidinecarboxylic acid, methyl ester, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-cyclotrisiloxane, hexamethyl-, beta-, sitosterol, 4a,7,7,10a-Tetramethyl-dodecahydro -benzo[f]chromen-3-one, beta.-Amyrin, Methyltris(trimethylsiloxy)silane and Cryptopine from the GC- MS analysis doesn't provide the

Table 1. GC-MS analysed volatile phytochemicals from AME

S. No	Identified compound	Retention Time	Area%	Molecular Weight	Molecular Formula	Doc score interacted to 4FA6
1.	2-Cyclopentene-1,4-dione	4.505	0.66	96.084	C ₅ H ₄ O ₂	-6.170
2.	Triethylphosphine	4.676	4.69	118.13	C ₆ H ₁₅ P	3.070
3.	2-Propenoic acid	5.123	1.35	128.13	C ₆ H ₈ O ₃	-5.560
4.	Butane,2,2—thiobis	6.107	5.52	146.30	C ₈ H ₁₈ S	2.560
5.	Propionic acid, mercapto	6.324	0.63	241.3	C ₁₀ H ₁₁ NO ₂ S ₂	-10.560
6.	6-methyluracil	7.434	0.85	126.112	C ₅ H ₆ N ₂ O ₂	-
7.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.533	2.99	144.12	C ₆ H ₈ O ₄	-10.380
8.	Pyrrolidine, N-(4-methyl-3-pentenyl)-	8.922	0.76	153.26	C ₁₀ H ₁₉ N	-4.010
9.	2,3- Dihydrobenzofuran	9.603	0.62	120.15	C ₈ H ₈ O	-11.080
10.	Propane,1,1,3,3-tetraethoxy	9.941	0.8	220.31	C ₁₁ H ₂₄ O ₄	2.230
11.	2-Methoxy-4-Vinylphenol	10.982	0.77	150.17	C ₉ H ₁₀ O ₂	2.230
12.	Thiazolidine-2,5-dione	12.229	1.53	117.13	C ₃ H ₃ NO ₂ S	-8.240
13.	2-(4-hydroxyphenyl)ethanol	12.447	0.7	138.16	C ₈ H ₁₀ O ₂	-8.610
14.	Benzene, 1-hexyl-4-nitro	12.624	0.69	275.27	C ₁₃ H ₁₆ F ₃ NO ₂	-6.920
15.	1-Ethyl-1-Ethyl-2-pyrrolidinecarboxylic acid, methyl ester	13.374	0.49	171.24	C ₉ H ₁₇ NO ₂	-
16.	Galactitol	13.746	1.29	182.17	C ₈ H ₁₄ O ₆	-18.800
17.	Benzofuran, 2,3-dihydro-4-tert-Butoxystyrene	13.912	0.54	134.13	C ₈ H ₆ O ₂	-11.080
18.	3H-Benz[e]indene, 2-methyl-	14.181	0.52	180.24	C ₁₄ H ₁₂	-13.520
19.	3-Deoxy-d-mannonic lactone	14.432	1.189	162.14	C ₆ H ₁₀ O ₅	-10.380
20.	3-Hydroxy-beta-damascone	14.804	1.04	208.30	C ₁₃ H ₂₀ O ₂	-0.340
21.	5,5,8a-Trimethyldecalin-1-one	16.613	1.05	194.31	C ₁₃ H ₂₂ O	2.500
22.	n-Hexadecanoic acid	18.4732	3.43	256.42	C ₁₆ H ₃₂ O ₂	4.090
23.	1-octadecene	19.697	1.151	252.5	C ₁₈ H ₃₆	13.00
24.	Phytol	19.931	4.72	296.5	C ₂₀ H ₄₀ O	2.180
25.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	20.166	1.85	278.42	C ₁₈ H ₃₀ O ₂	-
26.	Benzo[h]quinoline, 2,4-dimethyl-	22.294	3.52	207.27	C ₁₅ H ₁₃ N	-18.280
27.	1,2-Benzenedicarboxylic acid diisooctyl ester	23.508	4.43	823.7	C ₃₈ H ₅₆ O ₈ S ₂ Sn	-2.810
28.	Acetamide,N-[4-(trimethyl)-phenyl]-	24.56	0.89	204.34	C ₁₁ H ₁₇ NO	-7.600
29.	beta,-sitosterol	25.098	9.57	414.7	C ₂₉ H ₅₀ O	-
30.	4a,7,7,10a-Tetramethyl-dodecahydro -benzo[f]chromen-3-one	25.453	0.64	264.4	C ₁₇ H ₂₈ O ₂	-
31.	.beta.-Amyrin	25.762	4.88	426.7	C ₃₀ H ₅₀ O	-
32.	Cryptopine	27.193	17.5	369.4	C ₂₁ H ₂₃ NO ₅	-
33.	Protopine	27.267	8.16	353.4	C ₂₀ H ₁₉ NO ₅	-14.940
34.	1H-Indole,1-methyl-2-phenyl-	27.673	0.66	207.27	C ₁₅ H ₁₃ N	-16.150

Table 2. LC-MS analysed phenolic phytoconstituents against AMP

S. no	Tentative compound identified	Molecular weight	Molecular formula	Retention time	Observed QTOF/MS (ESI-)m/z	Observed QTOF/MS (ESI+)m/z	Referred (m/z) (ESI+/ESI-)	Doc score interacted to 4FA6	Reference
1.	Succinic acid	118.09	C ₄ H ₆ O ₄	26.39	117.49	-	117	-10.240	[15]
2.	N-feruloyltryamine	313.3	C ₁₈ H ₁₉ O ₄	3.75	-	314.19	314	-17.410	[15]
3.	3-Hydroxyphloretin	452.4	C ₂₁ H ₂₄ O ₁₁	5.45	451.29	-	451.1246	-13.200	[16]
4.	2'-O-glucoside	448.4	C ₂₁ H ₂₀ O ₁₁	6.65	447.16	-	447.0936	-	[17]
5.	Leachianol F	472.5	C ₂₈ H ₃₂ O ₇	17.09	471.65	-	471.1450	-12.820	[17]
6.	Catechin	290.27	C ₁₅ H ₁₄ O ₆	13.45	-	291.18	291.058	-18.870	[17]
7.	Genkwanin	284.26	C ₁₆ H ₁₂ O ₅	23.94	283.77	-	283.06	-19.810	[18]
8.	Liquiritigenin	256.25	C ₁₅ H ₁₂ O ₄	22.13	255.59	-	255.068	-18.010	[19]
9.	Caffeic acid	180.16	C ₉ H ₈ O ₄	11.66	-	181.43	181	-45.820	[20]
10.	Berberrubine	357.8	C ₁₉ H ₁₆ CINO ₄	7.93	-	336.22	336.12	-18.810	[21]
11.	Curcumin	368.4	C ₂₁ H ₂₀ O ₆	7.04	-	369.86	369.13	-15.150	[21]
12.	Protopine	353.4	C ₂₀ H ₁₉ NO ₅	7.50	-	354.12	354.13	-14.940	[21]
13.	Linarin	592.5	C ₂₈ H ₃₂ O ₁₄	21.10	-	593.21	593.18	-	[21]

Table 3. Selected Ligands with best hits

S. no.	Name of the Ligand	Source	Estimated affinity	Log P.	No. H Bonds	Residue interacted	Distance in Å
1.	2-Cyclopentene-1,4-dione	GC-MS	mM	0.08	1	TYR A: 867	2.76
2.	2-Propenoic acid	GC-MS	>mM	-1.08	1	TYR A: 867	2.60
3.	Propionic acid, mercapto	GC-MS	µM	1.26	3	TYR A: 867 ASP A: 964	2.88, 3.062.82
4.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	GC-MS	µM	-0.26	2	GLU A: 880 VAL A: 882	2.702.78
5.	Pyrrolidine, N-(4-methyl-3-pentenyl)-	GC-MS	µM	1.02	-	-	-
6.	2,3-Dihydrobenzofuran	GC-MS	µM	1.62	1	VAL A: 882	2.85
7.	Thiazolidine-2,5-dione	GC-MS	µM	-0.03	1	VAL A: 882	2.84
8.	2-(4-hydroxyphenyl) ethanol	GC-MS	µM	0.93	3	ASP A: 964 TYR A: 867 VAL A: 882	2.812.962.77
9.	Benzene, 1-hexyl-4-nitro	GC-MS	nM	4.74	1	VAL A: 882	2.80
10.	Galactitol	GC-MS	µM	-3.59	7	ALA A: 885 VAL A: 882 GLU A: 880	
11.	2,70,2.86,2.642.78,2.893.09,2.66 Benzofuran, 2,3-dihydro-4-tert-Butoxystyrene	GC-MS	µM	2.14	1	VAL A: 882	2.85
12.	3H-Benz[e]indene, 2-methyl-	GC-MS	µM	3.80	-	-	-
13.	Benzo[h]quimoline, 2,4-dimethyl-	GC-MS	µM	4.00	-	-	-
14.	1,2-Benzenedicarboxylic acid diisooctyl ester	GC-MS	µM	-0.38	1	VAL A: 882	2.90
15.	Acetamide, N-[4-(trimethyl)phenyl]-	GC-MS	µM	2.19	2	ASP A: 964 TYR A: 867	2.802.70
16.	2,4,6-Cycloheptatrien-1-one, 3,5-b is-trimethylsilyl-	GC-MS	µM	2.14	2	ASP A: 964 TYR A: 867	3.182.92
17.	Protopine	GC-MS	µM	1.14	2	VAL A: 882 SER A: 806	3.293.42
18.	1H-Indole, 1-methyl-2-phenyl-	GC-MS	µM	3.85	-	-	-
19.	Succinic acid	LCMS	µM	-2.73	5	CYS A: 869 ASP A: 841 TYR A: 867 ASP A: 964	3.482.912.82, 2.832.91
20.	3-Hydroxyphloretin 2'-O-glucoside	LCMS	µM	-0.50	3	GLU A: 880 ASP A: 964 LYS A: 890	2.722.733.22
21.	Leachianol F	LCMS	µM	4.67	2	LYS A: 890 ASP A: 950	2.622.41
22.	Genkwanin	LCMS	nM	2.88	4	VAL A: 882 ASP A: 964	2.94, 2.54, 3.053.28
23.	Liquiritigenin	LCMS	µM	2.80	4	VAL A: 882 ASP A: 964 TYR A: 867	2.65, 3.012.86, 2.93
24.	Caffeic acid	LCMS	µM	1.20	3	TYR A: 867 VAL A: 882 MET A: 953	2.732.90, 2.96
25.	N-feruloyltryamine	LCMS	nM	2.48	6	TYR A: 867 ASP A: 964 VAL A: 882 GLU A: 880	3.142.862.84, 3.30, 3.172.65
26.	Berberubine	LCMS	µM	2.96	1	TYR A: 867	2.70
27.	Curcumin	LCMS	pM	3.37	4	TYR A: 867 VAL A: 882 GLU A: 880	2.712.83, 3.202.69
28.	Protopine	LCMS	µM	1.14	2	SER A: 806 VAL A: 882	2.903.39
29.	Catechin	LCMS	µM	1.55	3	TYR A: 867 VAL A: 882	2.713.20, 2.83

Doc score by SeeSAR 9.2 software that may be due to the ligands weight, length or ungenerated poses respectively. From the LC-MS analysis, Iso-orientin and Linarin didn't show the docked poses and binding score. Table 3 shows the estimated affinity range within the Picomolar (pM) to millimolar (mM), Log. P value, Number of Hydrogen Bonds, Binding residues and distance of interaction in Angstroms. The highlighted three residues, SERA: 806, LYS A: 890 and ASPA: 950 are the unmarked binding site that interacted with the amino acid residue of the PI3K receptor. 29 selected ligands from both GC-MS and LC-MS are denoted in Table 3. Most of the ligands bonded in the exact binding site (residue) of the receptor.

Copansilib revealed the binding site value as 6.780 within the log. P value of -0.73 in iM affinity range with binding site residue as given in figure 3.

DISCUSSION

Overactive PI 3-kinase (PI3K) in cancer and immunological dysregulation has prompted substantial research into PI3K inhibitors for therapeutic use having several categories. Early investigations have shown that the pan-PI3K inhibitors (LY294002) might reverse cancer cell resistance to a wide range of treatments, including chemotherapy, radiation, and targeted therapies²². Epigenetic modulators have been found to manage

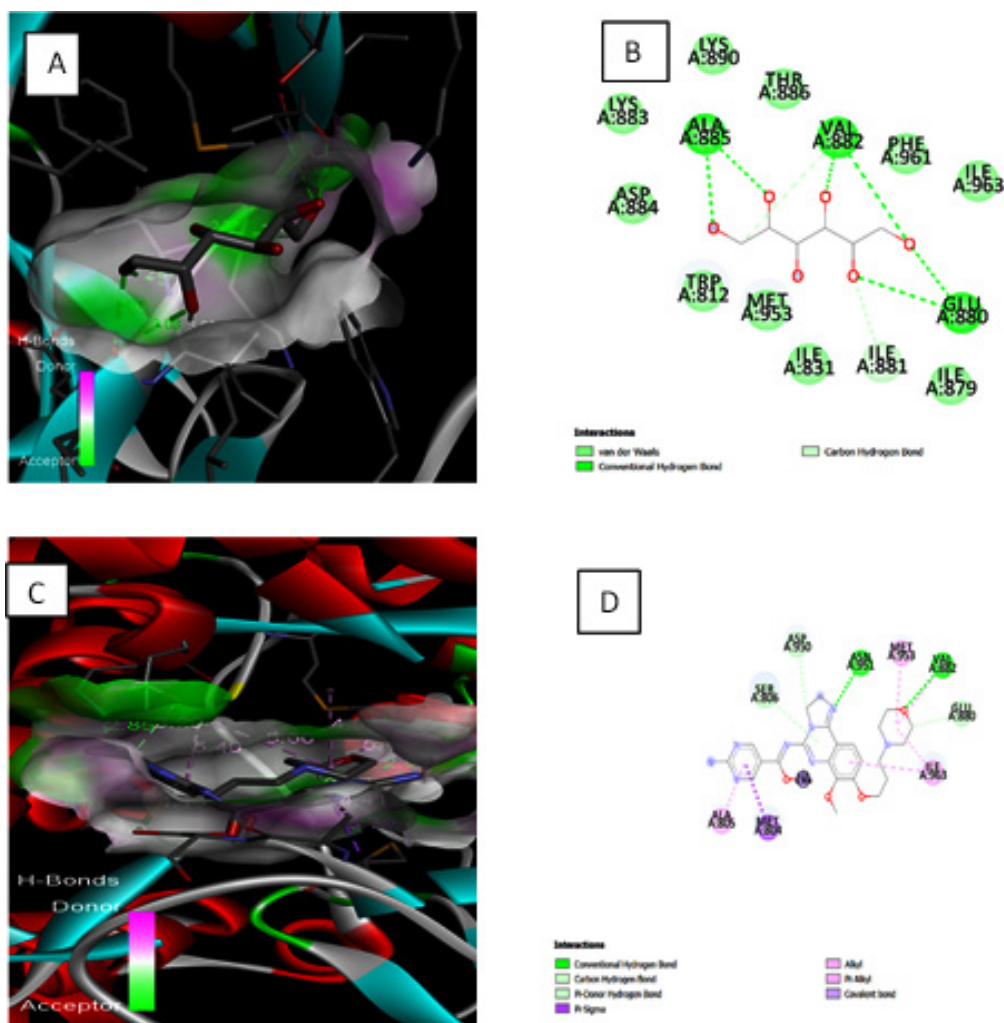


Fig. 3. A) Galactitol 3D in hydro pocket with receptor B) Galactitol 2D showing binding residue C) Copansilib 3D in hydro pocket D) Copansilib 2D showing binding residue.

the epigenome by participating in the PI3K/AKT pathway contributing to oncogenicity in certain malignancies, especially in Non-small Cell Lung Cancer which has a higher projectile prognosis²³. Induction of apoptosis in lung cancer and prevention of metastasis could be healthily induced with the help of bioactive phytoconstituents from medicinal plants²⁴. *A. mexicana* has been proven for anticancer activity against several cell lines²⁵. The study of *In silico* anticancer modeling from the bioactive volatile and phenolic constituents against the PI3K receptor, responsible for lung cancer metastasis has been researched less. Regarding previous studies, we attempted the identification of phytoconstituents of the extracts AME and AMP have been done through GC-MS and LC-MS analysis. GC-MS analysis of AME revealed the presence of cryptopine with the highest peak percentile of 17.5, which is found mostly in the species of Indian Opium and Protopine responsible for latex formation¹⁰. The presence of phytol shows that *A. mexicana* has potential antioxidant and anticancer activity. Beta-sitosterol and Beta-amyrin are reported to be non-toxic to normal cells (Table 1)²⁶. UPLC-Q-TOF-ESI-MS analysis of AMP (Table 2) revealed 13 major phenolic components reported to possess anti-cancer activity in several studies. Genkwanin is the Flavonoid found in AMP at the retention time of 23.959 in the negative mode also found in antioxidant-rich rosemary oil^{18,4}. Virtual screening *in silico* is a good choice before the preliminary research of *in vitro* or *in vivo* to inhibit cancer proliferation¹⁵. Out of 47 ligands found from analysing the GC-MS and LC-MS spectrum peaks, 29 were filtered out from the ligand-receptor complex analysis with good binding affinity parameters. 4FA6 receptors possess 20 different binding sites analysed from the Uniprot database compared with binding sites of ligand residues. All ligands showed the exact binding nature except Leachinol F. Galactitol has the highest binding affinity range (iM) having 7 hydrogen bonds with a log. P value of -3.59 in the binding site of ALA A:885 (2.70, 2.86, 2.64), VAL A:882 (2.78,2.89), GLU A:880 (3.09,2.66). Next lies N-feruloyltryamine with 6 H Bonds and 4 H bonds shown in Genkwanin, Liquiritigenin, and Curcumin with the highest log p-value with exact binding residue. Ligands such as Propionic

acid, mercapto, 2-(4-hydroxyphenyl) ethanol, 3-Hydroxyphloretin 2'-O-glucoside, Caffeic acid, and Catechin have 3 H Bonds with the PI3K receptor. This may inhibit the capacity of metastatic proliferation in PI3K/Akt/mTOR pathways by controlling the 4FA6 (PI3K) protein found in phytocompounds found from *A. mexicana* Leaves.

CONCLUSION

Different extracts such as AME and AMP from the leaves of *A. mexicana* were checked for bioactive phytoconstituent screening and carried through molecular docking to reveal the anticancer potential at *In silico* level. We have screened the impact of phytochemicals through GC-MS analysis with 34 different phytoconstituents. LCMS revealed 13 phenolic and flavonoid phytocompounds which is none other reported in any of the research studies in the *A. mexicana* plant. Totally 47 compounds including the standard drug were targeted against the PI3K receptor and narrowed down to 29 ligands by analyzing the binding score parameter. Phytoconstituents such as Galactitol (GC-MS), Succinic acid (LC-MS) and N-feruloyltryamine (LC-MS) resulted in good binding affinity and best hydrogen bonding with the exact binding site of the 4FA6 (PI3K) receptor paving to analyze the anticancer target against the Phosphoinositide kinase pathway by inducing apoptosis and controlling the cell growth. This would lead to greater alternatives in the drug discovery from *A. mexicana*.

ACKNOWLEDGMENT

All the authors contributed their sincere effort in designing, analyzing and reporting on this project. The authors acknowledge sincere gratitude to the Karpagam Academy of Higher Education for providing the network and molecular modeling facility for implementing this research manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Funding Sources

The authors declare the funding sources from DST-FIST (SR/FST/LS-1/2018/187) for providing the infrastructure facilities and commercial software for this research work.

REFERENCES

1. Tan AC. Targeting the PI3K/AKT/mtor pathway in non small cell lung cancer (NSCLC). *Thoracic Cancer*. 2020;11(3):511-518. doi:10.1111/1759-7714.13328.
2. Govindammal M, Prasath M, Kamaraj S, Muthu S, Selvapandiyam M. Exploring the molecular structure, vibrational spectroscopic, Quantum Chemical Calculation and molecular docking studies of curcumin: A potential PI3K/AKT uptake inhibitor. *Heliyon*. 2021;7(4). doi:10.1016/j.heliyon.2021.e06646.
3. Le T, David J, Bryan LJ. Update on the role of Copanlisib in hematologic malignancies. *Therapeutic Advances in Hematology*. 2021;12:204062072110060. doi:10.1177/20406207211006027.
4. Ahmed B, Joseph A, Das S, et al. Structure-activity relationship insight of naturally occurring bioactive molecules and their derivatives against non-small cell lung cancer: A comprehensive review. *Current Medicinal Chemistry*. 2022;29(39):6030-6062. doi:10.2174/0929867329666220509112423
5. Mallavarpu Ambrose J, Veeraraghavan VP, Kullappan M, et al. Molecular modeling studies of the effects of withaferin A and its derivatives against oncoproteins associated with breast cancer stem cell activity. *Process Biochemistry*. 2021;111:186-199. doi:10.1016/j.procbio.2021.09.007
6. Khan AM, Bhadauria S. Analysis of medicinally important phytochemicals from Argemone Mexicana. *Journal of King Saud University - Science*. 2019;31(4):1020-1026. doi:10.1016/j.jksus.2018.05.009
7. Dénou A, Ahmed A, Dafam DG, et al. Pharmacognostic, physicochemical and phytochemical investigations on aerial parts of Argemone mexicana L. *Research Journal of Pharmacognosy*. 2020;7(3):15-24. doi:10.22127/rjp.2020.220380.1559
8. Paul D, Sanap G, Shenoy S, Kalyane D, Kalia K, Tekade RK. Artificial Intelligence in drug discovery and development. *Drug Discovery Today*. 2021;26(1):80-93. doi:10.1016/j.drudis.2020.10.010
9. Talevi A. Multi-target pharmacology: Possibilities and limitations of the "Skeleton key approach" from a medicinal chemist perspective. *Frontiers in Pharmacology*. 2015;6. doi:10.3389/fphar.2015.00205
10. Paul D, Sanap G, Shenoy S, Kalyane D, Kalia K, Tekade RK. Artificial Intelligence in drug discovery and development. *Drug Discovery Today*. 2021;26(1):80-93. doi:10.1016/j.drudis.2020.10.010
11. Kiran Chiliveriyaravi, Alagar Sangilimuthu, Manoharan Sowmyapriya. Volatile phytoconstituent profile of Argemone Mexicana L. Leaves and pharmacological importance. *International Journal of Pharma and Bio Sciences*. 2017;8(3). doi:10.22376/ijpbs.2017.8.3.b523-528
12. Sunisha K, Dhivya R. GC-MS analysis and antioxidant activity of organic extracts of Psidium cattleianum leaves. *International Journal of Scientific Research in Biological Sciences*. 2020;7(2):128-133. doi: https://doi.org/10.26438/ijrsbs/v7i2.128133
13. Ananth DA, Rameshkumar A, Jeyadevi R, et al. Amelioratory effect of flavonoids rich Pergularia daemia extract against CFA induced arthritic rats. *Biomedicine & Pharmacotherapy*. 2016;80:244-252. doi:10.1016/j.biopha.2016.03.019
14. Halder D, Das S, R. A, R. S. J. Molecular docking and dynamics based approach for the identification of kinase inhibitors targeting PI3K α against non-small cell lung cancer: A computational study. *RSC Advances*. 2022;12(33):21452-21467. doi:10.1039/d2ra03451d
15. Bhardwaj VK, Singh R, Sharma J, Das P, Purohit R. Structural based study to identify new potential inhibitors for dual specificity tyrosine-phosphorylation- regulated kinase. *Computer Methods and Programs in Biomedicine*. 2020;194:105494. doi:10.1016/j.cmpb.2020.105494
16. Hao Y, Huo J, Wang T, Sun G, Wang W. Chemical profiling of Coptis Rootlet and screening of its bioactive compounds in inhibiting staphylococcus aureus by UPLC-Q-TOF/MS. *Journal of Pharmaceutical and Biomedical Analysis*. 2020;180:113089. doi:10.1016/j.jpba.2019.113089
17. Al-Yousef HM, Hassan WH, Abdelaziz S, Amina M, Adel R, El-Sayed MA. UPLC-ESI-MS/MS profile and antioxidant, cytotoxic, antidiabetic, and antiobesity activities of the aqueous extracts of three different *hibiscus* species. *Journal of Chemistry*. 2020;2020:1-17. doi:10.1155/2020/6749176
18. Tang J, Dunshea FR, Suleria HA. LC-ESI-QTOF/MS characterization of phenolic compounds from medicinal plants (hops and juniper berries) and their antioxidant activity. *Foods*. 2019;9(1):7. doi:10.3390/foods9010007
19. Avula B, Bae J-Y, Zhao J, et al. Quantitative determination and characterization of polyphenols from *cissus quadrangularis* L. and dietary supplements using UHPLC-PDA-MS, LC-

- QToF and HPTLC. *Journal of Pharmaceutical and Biomedical Analysis*. 2021;199:114036. doi:10.1016/j.jpba.2021.114036
20. Velamuri R, Sharma Y, Fagan J, Schaefer J. Application of UHPLC-ESI-QTOF-MS in phytochemical profiling of sage (*salvia officinalis*) and Rosemary (*rosmarinus officinalis*). *Planta Medica International Open*. 2020;07(04). doi:10.1055/a-1272-2903
21. Zhou J, Cai H, Tu S, et al. Identification and analysis of compound profiles of Sinisan based on 'individual herb, herb-pair, herbal formula' before and after processing using UHPLC-Q-TOF/MS coupled with multiple statistical strategy. *Molecules*. 2018;23(12):3128. doi:10.3390/molecules23123128
22. Kumar BR. Application of HPLC and ESI-MS techniques in the analysis of phenolic acids and flavonoids from green leafy vegetables (glvs). *Journal of Pharmaceutical Analysis*. 2017;7(6):349-364. doi:10.1016/j.jpha.2017.06.005
23. Vanhaesebroeck B, Perry MW, Brown JR, André F, Okkenhaug K. PI3K inhibitors are finally coming of age. *Nature Reviews Drug Discovery*. 2021;20(10):741-769. doi:10.1038/s41573-021-00209-1
24. Noorolyai, S. *et al.* (2019) "The relation between PI3K/akt signalling pathway and cancer," *Gene*, 698, pp. 120–128. Available at: <https://doi.org/10.1016/j.gene.2019.02.076>.
25. Hosseini A, Ghorbani A. Cancer therapy with phytochemicals: evidence from clinical studies. *Avicenna journal of phytomedicine*, 2015; 5(2):84.
26. Gali K, Ramakrishnan G, Kothai R, Jaykar B. In vitro anticancer activity of methanolic extract of leaves of *Argemone Mexicana* Linn. *International Journal of Pharmtech Research*, 2011; 3(3):1329-33.
27. Aati HY, Anwar M, Al-Qahtani J, et al. Phytochemical profiling, in vitro biological activities, and in-silico studies of *Ficus vasta* forssk.: An unexplored plant. *Antibiotics*. 2022;11(9):1155. doi:10.3390/antibiotics11091155.