International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 10, Issue 6, pp: 244-252, 2025
https://doi.org/10.55126/ijzab.2025.v10.i06.027 **http://www.ijzab.com**

Research Article

EXPLORING ANTIBACTERIAL COMPOUNDS IN *KALANCHOE PINNATA* (LAM.) PERS.: GC-MS ANALYSIS AND MOLECULAR DOCKING EVALUATION

[1*]Parvathy Menon PR, [1]Nirmala Devi N, [1]Sidharth K, [1]Reshma BP and [2]R Ragunathan

[1*]Department of Biochemistry, Sree Narayana Guru College, KG Chavadi, Coimbatore, Tamil Nadu-641105, India
[2]Centre for Bioscience and Nano science Research (CBNR), Tamil Nadu-641021, India

Article History: Received 1st September 2025; Accepted 26th October 2025; Published 1st November 2025

ABSTRACT

Kalanchoe pinnata (Lam.) Pers. (Miracle Leaf) in tropical and subtropical regions is widely recognized and utilized as an herbal medicine. The Role as a traditional medicine is rooted evidently in therapeutic properties for wound healing and its various bioactive compounds. This study was aimed to investigate the ethanolic extract of the *Kalanchoe pinnata (Lam.) Pers* leaves, Fresh Ethanolic extract of the leaves were prepared and GC-MS analysis were carried out to find the phytochemicals. The GC-MS analysis revealed 32 compounds some of them being Diethyl Phthalate, Squalene, Phytol, Dibutyl phthalate, Delta Tocopherol. The bioactive compounds revealed from the plants showed the anti-bacterial potentials that contributes to the role in wound care and its applications from pharmaceuticals. The Molecular docking was done with the bioactive compounds like squalene, Dibutyl Phthalate, Phytol, Diethyl Phthalate against the protein C-Met and *GOT1 with using the drug G*emcitabine that has potential activity against the pancreatic activity. The Results suggested that Squalene and the drug show to well interaction with protein having a binding affinity energy ranging from -7 and -7.7Kcal/mol. Based on the affinity, the C-Met and GOT1 can be used against cancer therapy using gemcitabine.

Keywords: GC-MS, Squalene, C-Met, GOT1, Gemcitabine, *Kalanchoe pinnata.*

INTRODUCTION

Natural Products from the plants in form of pure or as standardized, provide vast window for new drug leads because of their unparalled chemical diversity. The synthetic drugs due to concerns of potential toxicity, cross-resistance and their rising cost there is rising interest in the plant-based alternatives. Herbal and botanical preparation for medicinal uses contain different types of bioactive compounds (Sasidharan *et al*., 2011).Modern preferences are towards the natural drugs especially from the plants due to their abundance and less side effects compared to synthetic drugs that have wide toxicity and the side effects to the host .Novel active compounds of the plant origin that has the therapeutic properties are found to be effective and has minimal impacts (Gaddaguti *et al*., 2012) One Example of demonstrating this potential therapeutic activities is from *Kalanchoe pinnata(Lam.) Pers. Kalanchoe pinnata(Lam.) Pers.* is a plant that is used as a medicinal herb in all tropical regions of India, Africa, Asia, Australia, China, and Madagascar (Afzal *et al*., 2012) The leaves of the plant are found to be rich in alkaloids, triterpenes, glycosides, flavonoids, steroids, lipids and other bioactive compounds present in the ethanolic extract of the leaf were identified by the Gas chromatography (GC-MS) Similarly it contain a set of chemicals called as bufadienolides . Bryotoxins and related bufadienolides can be seen as multifunctional bioactive compounds with promising applications in medicine and agriculture (Pattewar, 2012). The recent year's antibiotic resistance in pathogenic micro-organisms has become an increasingly important public health problem worldwide (Abedini, 2013). This study aims to evaluate the anti-bacterial properties of the ethanolic extract of the leaves of *Kalanchoe pinnata.*

Pancreatic cancer is a deadly disease, and finding effective ways to treat it is still a major challenge. Pancreatic cancer is very aggressive and is usually found

*Corresponding Author: Parvathy Menon PR, Research Scholar, Department of Biochemistry, Sree Narayana Guru College, KG Chavadi, Coimbatore, Tamil Nadu-641105, India Email: parvathypayimenon@gmail.com.

only after it has already spread. It has the poorest outlook of all cancers, with only about 7% of patients living five years after diagnosis, mainly because it doesn't respond well to chemotherapy. Part of the reason pancreatic cancer resists chemotherapy is because of changes in mucins sticky proteins that form a dense barrier around the cancer cells. This barrier makes it hard for drugs to reach and attack their targets (Kalr & Campbell, 2007) (Nath *et al*., 2013). The innovative treatment for this disease is being continuously developed, where natural compounds have been considered as an alternative source to inhibit cancer cell growth by targeting its growth receptors. Numerous cancers related proteins like glutamate oxaloacetate transaminase 1 (GOT1), tyrosine-protein kinase Met(c-Met), peroxisome proliferator-activated receptor (PPAR) γ are used as therapeutic targets by suppressing the cell proliferation process (Aier *et al*., 2020; Liu *et al*., 2020). Gemcitabine has been assigned as a standardized chemotherapeutic drug against pancreatic metastase (Burris *et al*., 1997). Natural compounds have increasingly become a focal point in anticancer drug development due to their potent medicinal properties, with several plant-derived substances showing promising potential in the treatment of pancreatic cancer (Pan *et al*., 2017; Bimonte *et al*., 2016). The search for the new target molecules from plants could be identified with the Insilco Studies. In silico studies using molecular docking are computer-based methods that help predict how well a compound (like a drug) can bind to a target (like a protein). They show the best way the compound fits into the target and estimate how strong and effective that interaction might be. In drug discovery, computer-based methods like molecular docking (MD), molecular dynamics, and simulations (MDS) are used to study how potential drugs interact with their targets at the molecular level. These techniques help scientists understand how drugs might work, how stable they are in the body, and how to design more effective treatment (Yamari *et al*., 2023). These methods are crucial for accurately measuring how well potential drug molecules (ligands) attach to target proteins. They provide trustworthy results and speed up the drug development process, helping to save both time and money (Abchir *et al*., 2023). This present study also investigates the molecular docking interaction mechanism of C- Met and GOT1 protein with the specific ligands from *Kalanchoe pinnata (Lam.) Pers*. plant.

MATERIALS AND METHODS

Collection of plant and identification

Kalanchoe pinnata (Lam.) Pers was used for the investigation that was obtained from the botanical garden of Centre for Bioscience and Nano science Research (CBNR) Echanari, Coimbatore and were authenticated by Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) Campus, and Coimbatore. (BSI/SRC/5/23/2023/Tech-475).

Preparation of sample

Fresh leaves of *Kalanchoe pinnata(Lam.) Pers* was collected, washed with running tap water, dried and chopped into small pieces by hand and put into a conical flask.100ml of ethanol was added and was macerated in order to maximize the extraction, the solution was filtrated through Whatmann filter paper and stored in a container.1.5ml of the ethanolic extract was pipetted into a Eppendorf tube and analysed for GC-MS.

GC-MS analysis

The bioactive compounds of the leaf extract of *Kalanchoe pinnata (Lam.) Pers* were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The samples were analysed using the GC-MS (detector CH-GCMSMS-02). A capillary column of HP 5mts (30 m × 0.25 mm × 0.25 mm) was used, with a constant flow of helium at 1.5 ml/min and a source temperature of 280 °C. The temperature of the oven was initially set at 80 °C for 2 minutes, then ramped three times: first at 15 °C/min to 200 °C, then at 4 °C/min to 240 °C, and lastly at 15 °C/min to 280 °C, which was held for 5 minutes *(Paudel, Chand, Pant, & Pant, 2018)* The spectrum obtained was analysed by National Institute of Standards and Technology Mass Spectral (NIST-MS) database.

Antibacterial assay

The antibacterial assay for the extracts of *Kalanchoe pinnata (Lam.) Pers* was done by agar diffusion method against the *Escherichia coli (E. coli), Bacillus cereus (B. cereus), Salmonella typhi (S. typhi),* and *Staphylococcus aureus* (*S. aureus*) are all disease-causing pathogens. The nutrient agar media and nutrient broth poured in autoclaved Petri plates under sterilized conditions. The bacterial inoculum was spread in uniform manner and wells of 6 mm diameter were bored which were used for the loading of the plant extract. Four wells were pricked in each Petri plate. Levofloxacin was used as positive control and DMSO was considered as negative control. After loading the wells, plates were wrapped with paraffin tape and placed in an incubator at 37 °C for 24 h in an inverted position. The zone of Inhibition around the well was measured with ruler in millimetres (Javed *et al*., 2020).

Molecular docking: Software and hardware

The RCSB Protein Data Bank (https://www.rcsb.org) and the PubChem database (https://pubchem.ncbi.nlm.nih.gov) were used in this investigation. Utilizing PyRx and AutoDock Vina 0.8 (Dallakyan and Olson, 2015) software or, alternatively, using the BIOVIA Discovery Studio (RRID:SCR_015651) protein visualizer (Pawar & Rohane, 2021).

Preparation of Protein

The crystal structures of cancer-related proteins, namely GOT1 (PDB-3II0), c MET (PDB ID- 6SD9) were sourced from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) to facilitate docking studies (Figure 1 and Figure 2).

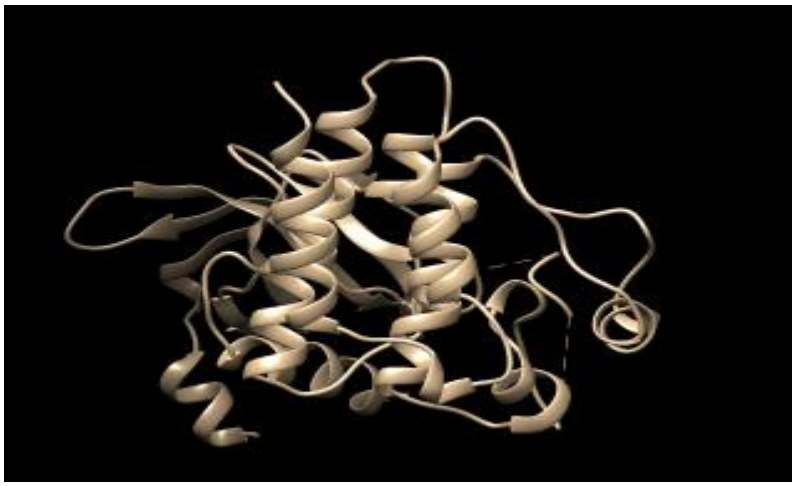

Figure 1. C MET PDB **ID:** 6SD9.

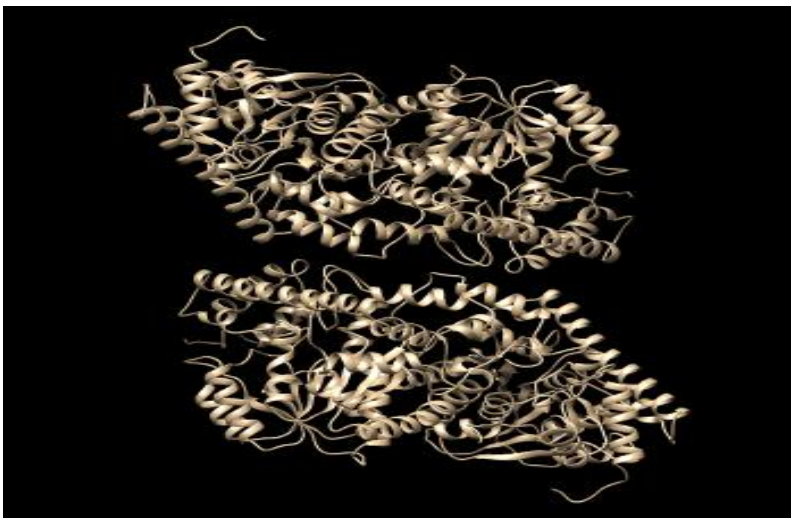

Figure 2. GOT1 **PDB ID:** 3II0.

Molecular Docking

Molecular docking studies were conducted utilizing the AutoDock Vina program, integrated within the PyRx software (version 0.8), to forecast the binding orientation and affinity of ligand molecules to a target protein. A grid box was established around the target protein to facilitate the docking calculations. The binding energies obtained from the molecular docking were represented in kilocalories per mole (kcal/mol). The energies presented offer an estimation of the binding strength between the ligand and the target protein. The 3D structure of the cancer receptor was obtained from the Protein Data Bank, while the ligand was sourced from PubChem and converted into PDBQT files suitable for the docking process. Following the molecular docking, the results were visualized and analyzed with BIOVIA Discovery Studio Visualizer, which facilitates the examination and comprehension of the binding interactions established between the protein and the ligand, including a standard drug utilized in the study.

RESULT AND DISCUSSION

GC-MS chromatogram of the ethanolic extracts showed 32 peaks which indicated the phytoconstituents. In comparison with the mass spectra of the constituents available in NIST library, one major peak of the spectra revealed Diethyl Phthalate Figure 3. Other Peaks revealed naphthalene, Dibuty Phthalate, Phthalic acid, di(2-propylpentyl) ester Table 1 and their biological properties were identified from NCBI.

Figure 3. GC-MS chromatogram of *Kalanchoe pinnata (Lam.)* Pers extract.

Table 1. Details of the bioactive compounds identified from the GC-MS analysis of *Kalanchoe pinnata*(Lam.) Pers.

Component RT	Component name	Molecular formula	Biological property
12.4764	Naphthalene	C10H8	Pesticide, carcinogenic
21.5865	Diethyl Phthalate	C12H14O4	Anti-cancer,anti-microbial
22.1374	Diethyl Phthalate	C12H14O4	Anti-cancer
23.2988	Phthalic acid, ethyl isoporpyl ester	C13H16O4	phytoremediation
26.7729	Dibutyl phthalate	C16H22O4	Anti-cancer
32.8386	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	Anti-trypanosomal ,anti-oxidant
34.2690	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	C42H63O3P	Anti-enterocoocal,anti-oxidant
35.5718	Squalene	C30H50	Anti-tumor

Table 2. Zone of Inhibition of plant extracts against various strains.

	Zone of Inhibition			
Test Organisms	Aqueous	Ethanol	Disc	DMSO
E. coli	15	27	21	Nil
B. cereus	11	19	Nil	Nil
S. typhi	15	19	Nil	Nil
S. aureus	14	19	15	Nil

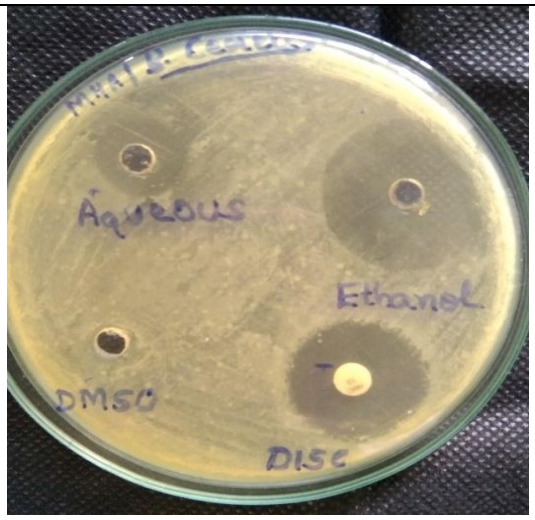

Figure 4*: B.cereus*

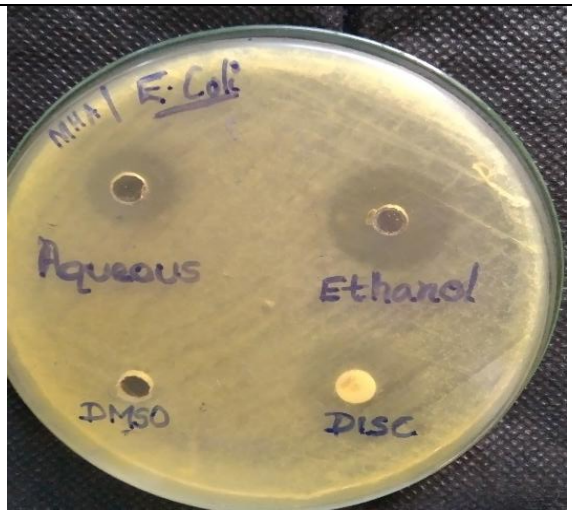

Figure 4a. *E.coli*

Figure 4b. *S.auereus*

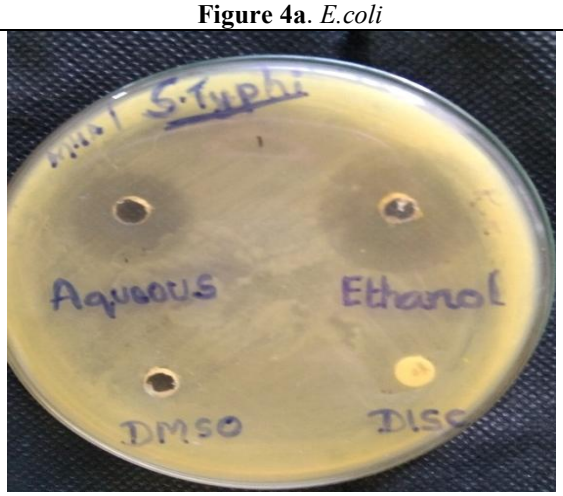

Figure 4c.*S.typhi*

Table 3. Docking Scores of Ligands against Proteins in comparison with standard Gemcitabine.

			PDB ID: 3II0			**PDB ID: 6SD9**		
Ligand Name	PUBCHEM NO	Ligand	Affinity (kcal/mol)			Affinity (kcal/mol)		
Gemcitabine	60750	D1	-6.6	-7	-7	-6.8	-6.4	-6.4
Diethyl Phthalate	6781	L1	-4.6	-6.1	-6.1	-4.8	-6.1	-6.1
Dibutyl phthalate	3026	L2	-7	-6.1	-6.1	-5.1	-6.5	-6.5
Phytol	5280435	L3	-3.8	-4.7	-4.7	-4.2	-3.5	-3.5
Squalene	638072	L4	-6.2	-7.7	-7.7	-5.5	-7	-7

Table 4. Grid Box Parameters for the Targeted Proteins.

PDB ID	Active Site Residues	Grid Box Parameter	
6SD9	PHE 1134,VAL 1220,LEU 1195,HIS 1202,MET 1131,ALA 1221, PHE 1200,GLU 1127,GLY 1224,ASP 1222,ILE 1084,MET 1211,VAL 1092,GLY 1163,PHE 1223,ALA 1108,ASP 1222,LEU 1157,LYS1110,PHE 1134,MET 1131,ALA 1221,GLU 1127,VAL1092,ALA 1108,LEU 1157,MET 1160,PRO 1158,LEU 1140	-9.67X14.202X-22.763	56X70X86
3II0	ALA 191,CYS 192,PRO 201,IIE 358,ARG 236,THR 202,ASP 237,ASP 200,SER 231,ALA 230,PHE 19,TYR 264,LYS 259, TRP 141,ASN 195, TYR 226,ASP 223, ALA 225, THR 110,ARG 267,SER 258,LYS 259,TYR 226, TRP 141, ARG 267	0.864 X 6.056 X -29.503	82 X 54 X 70

The anti-bacterial potent of the extract was assessed with four strains *Escherichia coli* (*E. coli*), *Bacillus cereus* (*B. cereus*), *Salmonella typhi* (*S. typhi*), and *Staphylococcus aureus* (*S. aureus*) against the aqueous and ethanol extract and the results of zone of inhibition were found (Figure 4 a, b, c) which was compared with the standard drug Amoxycillin. Out of which ethanol extract exhibited good activity against *Bacillus cereus* (Table 2). The computational tool like molecular docking were used employed to know the significance of the phytocomponents from the plant *Kalanchoe pinnata.* The compounds from the GC-MS profiling of the leaves of the plants were used as ligands and their interaction was studied against the human pancreatic cancer proteins using this tool. The four ligands like squalene, Dibutyl Phthalate, Phytol, Diethyl Phthalate were used for the pharmacokinetics analysis that were docked against the GOT-1 (Glutamic-Oxaloacetic Transaminase 1) and C-Met Protein, the docking scores of these compounds in comparison with the standard Gemcitabine is shown in the Table 3. A Grid box parameter was selected for the targeted proteins based on the binding sites as listed in the Table 4**.** The interaction between the ligand and protein have an impact on the affinity energy. Figure 5 and Figure 6 shows the hydrophobic interactions and the hydrogen bonds between the ligands and the proteins. The ligand Gemcitabine has produced the best affinity C-Met protein (Figure 5). The energy is from the hydrophobic interaction with the amino acid aspartate and the two hydrogen bonds with arginine as shown in the Table 5. The best efficiency was shown by squalene for GOT 1 protein and it is due to that they have the hydrophobic interactions at the active site of various residues as shown is the Figure 6 and Table 6. This data suggest that the compound derived from *Kalanchoe pinnata (Lam.) Pers* and the drug Gemcitabine has the good ability to inhibit the growth of pancreatic cancer cell process.

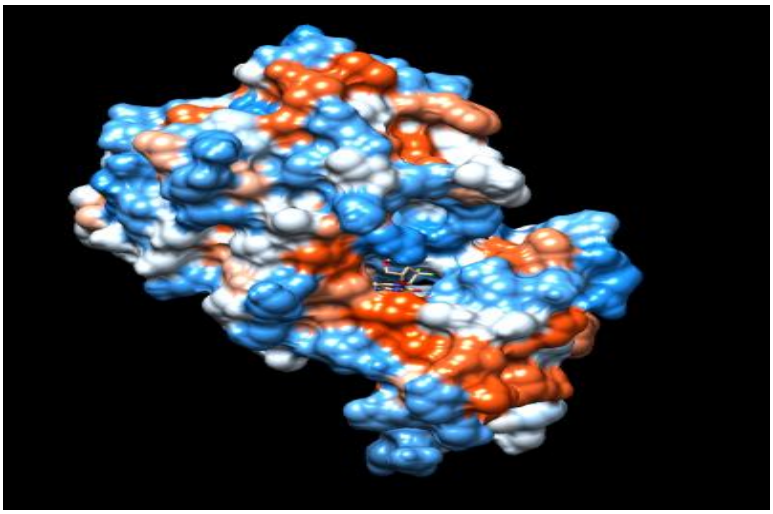

Figure 5. Interaction of 6SD9 with D1.

Table 5. Interaction of 6SD9 with D1.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	1222A	ASP	3.89	2615	1460

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	1203A	ARG	2.26	2.92	122.80	✓	✓	1276 [Ng+]	2606 [O3]
2	1203A	ARG	3.09	3.60	113.31	✓	✓	1277 [Ng+]	2606 [O3]

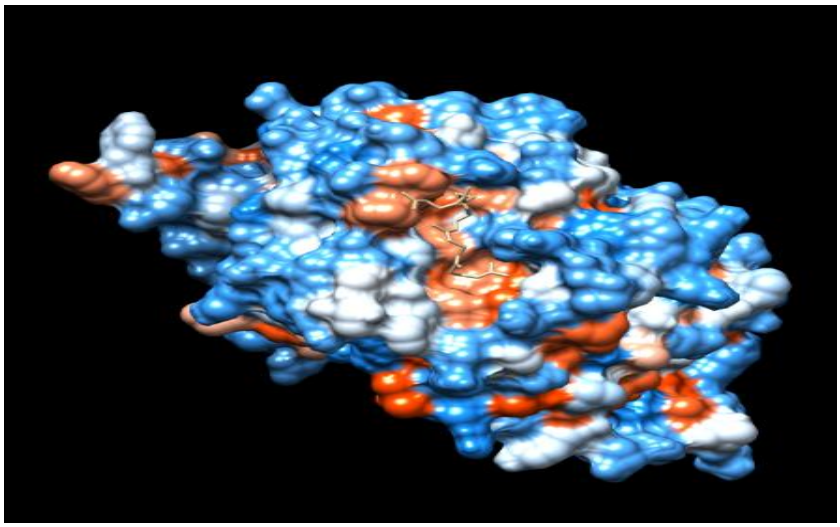

Figure 6. Interaction of 3II0 with L4.

Table 6. Interaction of 3II0 with L4 at various active site residues.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	119D	PHE	3.74	3988	1007
2	120D	LEU	3.97	3988	1018
3	124D	TYR	3.62	3972	1067
4	124D	TYR	3.53	3984	1065
5	184D	PHE	3.99	3989	1669
6	184D	PHE	3.49	3997	1671
7	184D	PHE	3.76	3990	1667
8	214D	LYS	3.95	3991	1943
9	217D	PHE	3.71	3993	1988
10	217D	PHE	3.76	3983	1986
11	217D	PHE	3.73	4001	1990
12	219D	PHE	3.53	3980	2007
13	249D	PHE	3.34	3991	2305
14	249D	PHE	3.70	3975	2303
15	252D	PHE	3.74	3994	2338
16	273D	VAL	3.61	3999	2545
17	284D	VAL	3.77	3999	2645

CONCLUSION

The findings of this study highlighted the biological activities of the *Kalanchoe pinnata* (Lam.) *Pers* leaf extract revealed the GC-MS analysis, anti-bacterial and *Insilico* studies'-MS showcased numerous compounds that has the therapeutic properties and suggest the support for the traditional medicines. *Kalanchoe pinnata* (Lam.) *Pers* is highly targeted plant for the microbial infections. This study recommends for the growing research towards the microbial agents and will be further investigated with advanced methods. The molecular docking studies suggested that squalene binds well with C-Met and GOT-1, the receptors for the pancreatic cancer with a great docking score, compared with gemcitabine. Further studies can be carried out to prove the mechanism of action that squalene shows against the receptors.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the Department of Biochemistry, Sree Narayana Guru College, KG Chavadi, Coimbatore, India for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Sasidharan, S. Y., Chen, D., Saravanan, K. M., Sundram, L., & Latha, Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines, 8*(1), 1–10.

Gaddaguti, V., Jwala Mounika, S., Sowjanya, K., Rao, T., Krishna Chakravarthy, M. S. R., & Rao, R. A. P. (2012). GC-MS analysis and *in silico* molecular docking studies of mosquito repellent compounds from *Hyptis suaveolens*. *International Journal of Bioassays, 1*(9), 36–41.

Afzal, M., Kazmi, I., Khan, R., Singh, R., Chauhan, M., & Bisht, T. (2012). *Bryophyllum pinnatum*: A review. *International Journal of Biological Sciences, 2*, 143–149.

Pattewar, S. V. (2012). *Kalanchoe pinnata*: Phytochemical and pharmacological profile. *International Journal of Pharmaceutical Sciences and Research, 3*(4), 993–1000.

Abedini, A. (2013). *Biological and phytochemical evaluation of natural substances from Hyptis atrorubens Poit. (Lamiaceae), selected by screening extracts from 42 plants* (Master's thesis). University of Law and Health—Lille II.

Kalra, A. V., & Campbell, R. B. (2007). Mucin impedes cytotoxic effect of 5-FU against growth of human pancreatic cancer cells: Overcoming cellular barriers for therapeutic gain. *British Journal of Cancer, 96*, 910–918.

Nath, S., Daneshvar, K., Roy, L. D., Grover, P., Kidiyoor, A., Mosley, L., *et al.* (2013). MUC1 induces drug resistance in pancreatic cancer cells via upregulation of multidrug resistance genes. *Oncogenesis, 2*, e51.

Aier, I., Semwal, R., Sharma, A., & Varadwaj, P. K. (2020). *In silico* identification of therapeutic compounds against microRNA targets in drug-resistant pancreatic ductal adenocarcinoma. *Journal of Biomolecular Structure and Dynamics*, 1–9.

Holt, M. C., Assar, Z., Beheshti Zavareh, R., *et al.* (2018). Biochemical characterization and structure-based mutational analysis provide insight into the binding and mechanism of action of novel aspartate aminotransferase inhibitors. *Biochemistry, 57*, 6604–6614.

Liu, H., Zhou, Q., Wei, W., *et al.* (2020). The potential drug for treatment in pancreatic adenocarcinoma: A bioinformatical study based on distinct drug databases. *Chinese Medicine, 15*, 26.

Burris, H. A. III, Moore, M. J., Andersen, J., Green, M. R., Rothenberg, M. L., Modiano, M. R., *et al.* (1997). Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: A randomized trial. *Journal of Clinical Oncology, 15*, 2403–2413.

Pan, P., Skaer, C., Yu, J., Zhao, H., Ren, H., Oshima, K., *et al.* (2017). Berries and other natural products in the pancreatic cancer chemoprevention in human clinical trials. *Journal of Berry Research, 7*, 147–161.

Bimonte, S., Barbieri, A., Leongito, M., Piccirillo, M., Giudice, A., Pivonello, C., *et al.* (2016). Curcumin anticancer studies in pancreatic cancer. *Nutrients, 8*, 433.

Yamari, I., Mouhib, A., Es-Sounni, B., Nejjari, R., Mazoir, N., Bakhouch, M., & Chtita, S. (2023). Oxidative functionalization of triterpenes isolated from *Euphorbia resinifera* latex: Semisynthesis, ADME-Tox, molecular docking, and molecular dynamics simulations. *Chemical Physics Impact, 7*, 100372.

Abchir, O., Daoui, O., Nour, H., Yamari, I., Elkhattabi, S., Errougui, A., & Chtita, S. (2023a). Cannabis constituents as potential candidates against diabetes mellitus disease using molecular docking, dynamics simulations and ADMET investigations. *Scientific African*, e01745.

Paudel, M. R., Chand, M. B., & Pant, B. (2018). Antioxidant and cytotoxic activities of *Dendrobium moniliforme* extracts and the detection of related compounds by GC-MS. *BMC Complementary and Alternative Medicine, 18*, 134. https://doi.org/10.1186/s12906-018-2197-6

Javed, B., Nawaz, K., & Munazir, M. (2020). Phytochemical analysis and antibacterial activity of tannins extracted from *Salix alba* L. against different Gram-positive and Gram-negative bacterial strains. *Iranian Journal of Science and Technology, Transaction A: Science, 44*, 1303–1314.

Pawar, S. S., & Rohane, S. H. (2021). Review on Discovery Studio: An important tool for molecular docking. *Asian Journal of Research in Chemistry, 14*(1), 86–88. https://doi.org/10.5958/0974-4150.2021.00014.6.