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In Silico Study of Compounds from Nanoherbal Jopan (*Clibadium surinamense* L.) Leaves as Inhibitors AKT1 Interaction

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Abstract

Breast cancer is the most prevalent cause of cancer-related deaths worldwide, and mortality rates are on the increase. This study aims to evaluate the potential of bioactive compounds from *Clibadium surinamense* L. leaves as inhibitors of AKT1 protein interactions, which play a crucial role in tumor growth mechanisms. Leaves of *Clibadium surinamense* L., obtained from Padangsidimpuan, North Sumatra, were extracted using methanol at a ratio of 1:20 for 48 h. The resulting extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the bioactive compounds. Drug likeness was then assessed according to Lipinski's rules, and molecular docking analysis was performed using Autodock Vina. GC-MS results identified 11 bioactive compounds, including 2-Cyclopenten-1-one and Hexadecanoic acid, methyl ester. Of the 8 compounds evaluated, 6 met the criteria for drug candidates. Molecular docking analysis revealed significant interactions between the bioactive compounds and the AKT1 protein. These findings suggest that bioactive compounds from *Clibadium surinamense* L. have potential as AKT1 interaction inhibitors, which could enhance cancer treatment mechanisms. This research paves the way for further studies on the therapeutic applications of these compounds in breast cancer treatment.

Keywords: AKT1 inhibitors, *Clibadium surinamense* L., Breast cancer, Bioactive compounds, Nanoherbal, Molecular docking, Drug-likeness, GCMS

Introduction

Breast cancer is the second most lethal form of cancer globally in 2022, resulting in an estimated 666,000 fatalities, which constitute 6.9 % of all cancer-related deaths. This cancer is the most frequently diagnosed in women and is a prominent cause of cancer-related deaths worldwide, with an incidence in 157 countries and a mortality rate of 112 countries. Around the world, breast cancer is responsible for nearly 1 in 6 cancer-related fatalities and nearly 1/4 of all cancer cases in women [1]. The mechanism of cancer tumor growth involves complex interactions between cancer cells and the surrounding environmental cells, including immune cells, macrophages and other components in the tumor microenvironment, which can influence proliferation, metastasis, and response to cancer therapy [2–4].

The PI3K/Akt/mTOR pathway plays a critical role in breast cancer development by supporting cell survival, growth and proliferation through a series of signaling events that activate key associated proteins [5,6]. Dysregulation in this pathway can lead to the disruption of normal cell function, contributing to breast cancer development and progression progression [7]. AKT is a critical component of the intracellular PI3K/Akt/mTOR pathway, frequently activated in breast cancer, contributing to tumor growth and resistance to therapy [8]. AKT has 3 isoforms: AKT1, AKT2 and AKT3. AKT1 focuses on cell proliferation and inhibition of the apoptosis process, AKT2 plays a role in cytoskeleton dynamics that support invasion and metastasis, while the role of AKT3 in the context of breast cancer is still under investigation [9,10].

PI3K/Akt pathway activity is activated when ligands bind to tyrosine kinase receptors such as EGFR and HER2, which triggers PI3K phosphorylation and activation of the AKT1 protein, which then activates a

signaling cascade that affects the expression of genes related to breast cancer cell growth and proliferation [11]. Synthetic AKT inhibitors, such as capivasertib, are effective in directly inhibiting AKT1 activity, preventing the signaling cascade and related gene expression [12,13]. Meanwhile, natural inhibitors, such as compounds from herbal plants, can affect this pathway in various ways, including inhibiting PI3K or AKT1 activity, reducing signaling pathway activation, or modulating related proteins to control cancer cell growth and proliferation [14].

Nanomedicine models have become the focus of attention in modern medicine due to their ability to enhance the delivery and efficacy of therapeutic agents [15]. The development of nanoherbal formulations aims to strengthen the effectiveness of herbal medicine by improving the physicochemical characteristics of active ingredients, such as increasing bioavailability, stability and delivery of active ingredients to specific targets in the body [16]. Nanoparticle technology is used to reduce the particle size of herbal ingredients, which increases solubility and absorption in the body. The advantages of nanoherbal include increased penetration of active ingredients through cell membranes, prolonging the half-life of active ingredients in the body, and reducing side effects by minimizing the dose required for the desired therapeutic effect [16,17]. The development of nanoherbal formulations aims to increase the bioavailability and effectiveness of these compounds, especially in targeting AKT1, a crucial protein in breast cancer therapy, as is the case with bioactive compounds from *Clibadium surinamense* L.

Natural compound inhibitors of AKT1 disrupt the phosphorylation of the AKT1 protein, essential for activating pro-survival signaling pathways and the proliferation of cancer cells, including breast cancer cells. The development and discovery of new drugs are greatly influenced by the presence of volatile compounds and phytochemicals in herbal plants, which play an essential role in herbal-based therapy as an effective and valuable treatment for cancer [18,19]. *Clibadium surinamense* L., or Jopan, has been used in traditional medicine and is known for its bioactive compounds. Previous studies have shown that essential oils from *Clibadium surinamense* L. leaves have significant cytotoxic activity against T47D breast cancer cells and HeLa cervical cancer cells, with an LC50 value of 0.9261 µg/mL against *Artemia salina* shrimp larvae and an IC50 value of 12.72 µg/mL against T47D cancer cells and 30.14 µg/mL against HeLa cancer cells [20]. However, research on the mechanisms and contributions of this plant in cancer treatment is still minimal. This study explores the *in-silico* potential of bioactive compounds from Nanoherbal *Clibadium surinamense* L. in inhibiting AKT1 isoforms, with a focus on molecular docking experiments to assess their binding interactions and effectiveness.

Materials and methods

Plant collection and nanoherbal preparation

The leaves of *Clibadium surinamense* L. were obtained from Padangsidimpuan, North Sumatra, amounting to 2 kg. Plant identification was conducted by the Herbarium of Andalas University (ANDA) with determination number 101/K-ID/ANDA/I/2024. *Clibadium surinamense* L. leaf powder underwent nanoparticle processing using a Planetary Ball Mill (PT. NanoTech Indonesia) with a grinding principle involving the movement of balls. The balls hit and grind the material, producing high energy that breaks the particles into nano sizes. The grinding process was performed at an optimal speed (300 - 350 rpm) for 1 - 4 h.

Nanoherbal extraction and GC-MS analysis

Nanoherbal *Clibadium surinamense* L. was extracted using methanol with a ratio of 1:20, namely 10 g of nano herbal *Clibadium surinamense* L. dissolved in 200 mL of methanol. The extraction process lasted for 48 h, with stirring twice a day to ensure its efficiency and effectiveness. After extraction, the methanol was filtered and evaporated using a rotary evaporator until a dry extract was obtained. This methanol extract was then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) with the Bruker SCION SQ 450 instrument. GC-MS analysis was performed using a DB-1 capillary column (0.25 µm film × 0.25 mm inner diameter × 30 m length) in electron ionization mode at 70 eV, with the injector temperature set at 230 °C and the detector temperature at 280 °C. The oven temperature program started at 80 °C for 5 min, then was ramped to 200 °C at a rate of 5 °C/min, and further increased to 280 °C at

the same rate for 16 min. Compound identification was achieved by comparing the obtained mass spectra with the NIST and WILEY libraries [21].

Protein-Ligan preparation

The bioactive compounds from Nanoherbal *Clibadium surinamense* L. used in this study are: Methanamine (PubChem CID: 6329), 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one (PubChem CID: 539293), 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl) (PubChem CID: 11083), 2-Undecanone 2,4-dinitrophenylhydrazone (PubChem CID: 5717665), 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene (PubChem CID: 5368073), 6-Tridecene (PubChem CID: 5364429), Hexadecanoic acid, methyl ester (PubChem CID: 8181), 2-Cyclopentene-1-undecanoicacid (PubChem CID: 110680), Cyclopentaneundecanoic acid, methyl ester (PubChem CID: 535041). **Figure 1** illustrates the 2D architectures of the bioactive chemical molecules derived from *Clibadium surinamense* L. Furthermore, the compound capivasertib (PubChem CID: 25227436) was employed as a reference control chemical to evaluate the inhibitory properties of the bioactive compounds obtained from Nanoherbal *Clibadium surinamense* L. The 3D structures of the active ingredients found in Nanoherbal *Clibadium surinamense* L. and the control were acquired from the appropriate chemical databases on PubChem (https://pubchem.ncbi.nlm.nih.gov). Subsequently, these structures were optimized for energy and transformed into the .pdb file format using Open Babel in PyRx software [22].

The potential of bioactive compounds from Nanoherbal *Clibadium surinamense* L. as AKT1 inhibitors is assessed through molecular docking. AKT1, a serine/threonine kinase, functions as a critical regulator in the PI3K/AKT/mTOR signaling pathway, playing an essential role in cell growth, survival and proliferation, particularly in breast cancer, where its abnormal activation, often due to mutations or amplifications, is associated with poor prognosis and tumorigenesis, making it a significant therapeutic target with various AKT inhibitors being developed and tested, especially in combination with treatments such as hormonal therapy, to enhance efficacy in hormone receptor-positive breast cancer [8,23,24]. The AKT1 protein is obtained from the RSCB protein databank (PDB ID: 6HHF; https://www.rcsb.org/structure/6HHF) and is docked against each of the bioactive compounds from Nanoherbal *Clibadium surinamense* L. and capivasertib using Autodock Vina in the PyRx software and pyMol [25,26].

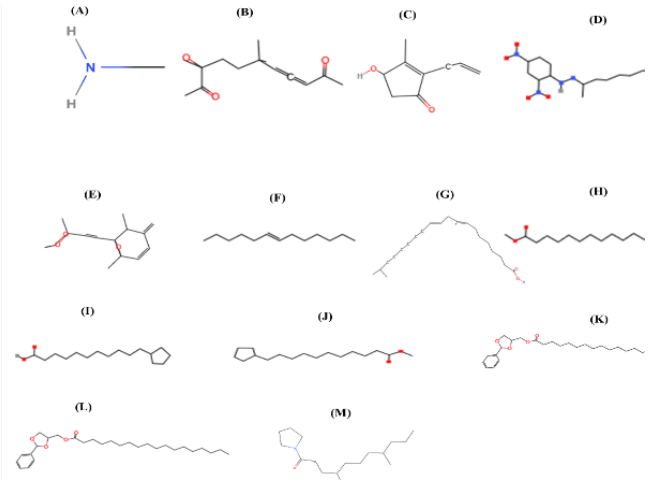

Figure 1 2D structures of the compounds from nanoherbal *Clibadium surinamense* L. (A) Methanamine; (B) 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one; (C) 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-; (D) 2-Undecanone 2,4-dinitrophenylhydrazone; (E) 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene; (F) 6-Tridecene; (G) 9,19-Dimethyl-eicosa-8, 11-dienoic acid; (H) Hexadecanoic acid, methyl ester; (I) 2-Cyclopentene-1-undecanoicacid; (J) Cyclopentane undecanoic acid, methyl ester; (K) Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-; (L) 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1 [(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-; (M) 2,4,8-Trimethylundecanoly Pyrrolidine.

Biological activities prediction using the PASS online

The prediction of biological activity of the nanoherbal compound *Clibadium surinamense* L. was conducted using the Prediction of Activity Spectra for Substances (PASS) server via the Way2drug server (http://way2drug.com/PassOnline/). The prediction process involved obtaining the SMILES structure from PubChem (http://pubchem.ncbi.nlm.nih.gov) and submitting it to the Way2drug server. The following were the criteria for the PASS Online test's result (Pa value): A Pa value > 0.7 indicates a high probability of biological activity, while a Pa value $0.5 \leq Pa \leq 0.7$ indicates low biological activity, and a Pa value < 0.5 indicates deficient activity [27].

Prediction drug-likeness

The compounds' drug-likeness was assessed using Lipinski's rule of 5. SwissADME (http://www.swissadme.ch) was employed to evaluate the drug-likeness of all compounds. This resource comprises parameters such as the molecule's number of hydrogen bond acceptors and donors, molecular weight and bioavailability. Lipinski's rule of 5 facilitates the determination of whether a compound can be orally assimilated [28].

Molecular docking analysis

The study employed Autodock Vina within PyRx 8.0.0 to perform docking analysis, treating the protein as the macromolecule and examining bioactive compounds and capivasertib as ligands [29,30]. Docking utilized grid parameters centered at (1.0192×1.9062×3.6164) with dimensions (38.2447×23.4834×22.5798 $Å^3$). Visualization of the docking outcomes was accomplished using Discovery Studio 2024 software.

Results and discussion

Gas chromatograpy-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) is an advanced analytical technique that combines gas chromatography's separation capabilities with mass spectrometry's mass analysis features. This technique enables the identification and quantification of volatile and semi-volatile compounds in complex mixtures, with applications in environmental assessment, food safety, clinical diagnostics, pharmaceutical research, and fuel and petroleomics analysis [31,32]. Raw data from GC-MS and individual chromatograms were processed using Origin software. GC-MS test results showed that there are 11 bioactive compounds present in Nanoherbal *Clibadium surinamense* L., namely: Metahnamine, 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one; 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-; 2-Undecanone 2,4-dinitrophenylhydrazone; 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene; 6-Tridecene; 19,19-Dimethyl-eicosa-8, 11-dienoic acid; Hexadecanoic acid, methyl ester; 2-Cyclopentene-1-undecanoicacid; Cyclopentaneundecanoic acid, methyl ester; Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-; and 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1 [(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-. The retention times of these 15 compounds can be seen in **Table 1**, and the chromatogram is presented in **Figure 2**. Based on screening in PubChem, 9 compounds have 3D structures. So, for further docking analysis, there will be 10 compounds that will be used in the investigation of potential Akt1 inhibitors using molecular docking.

The results of the GC-MS analysis detected various compounds with different retention times and concentrations (**Figure 2**; **Table 1**). Methanol is the main solvent component, with a retention time of 1.540 min and the highest concentration of 22.389 %. It is an organic substance frequently used in the chemical industry as raw materials for various applications, including pharmaceuticals [33]. Methanamine appears at 2.843 min with a concentration of 5.044 %. Other compounds with high concentrations include Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- with a concentration of 6.031 % and 2-Cyclopenten-1-one,4-hydroxy-3-methyl-2-(2-propenyl) - with a concentration of 2.747 %. Compounds such as 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene and 6-Tridecene had long retention times (29.095 and 30.915 min) and relatively high concentrations of 0.705 and 0.614 %, respectively, indicating the possibility of significant biological activity. Another compound, such as 19,19-Dimethyl-eicosa-8,11-dienoic acid, showed a good concentration of 0.635 % with a long retention time, indicating the potential for significant biological activity. Compounds with deficient

concentrations included 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one (0.022 %) and 2-Undecanone 2,4-dinitrophenylhydrazone (0.167 %).

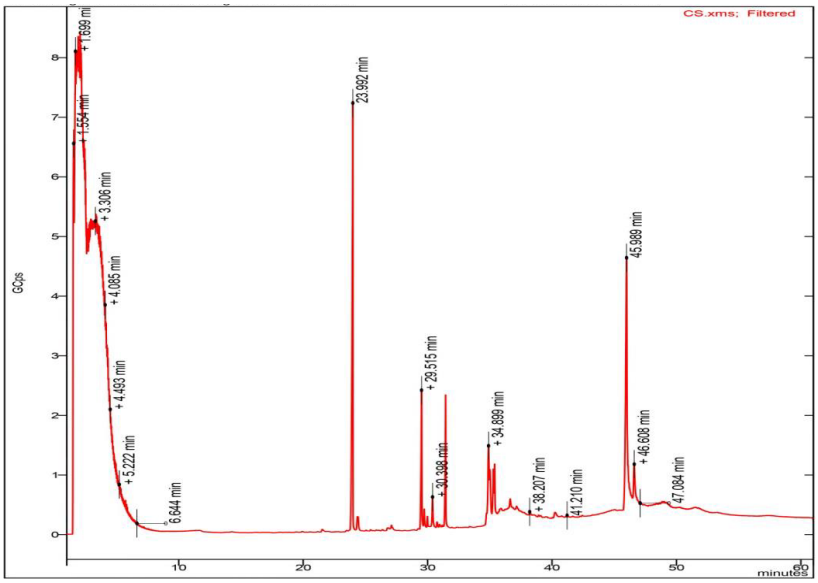

Figure 2 GC-MS Chromatogram of nanoherbal *Clibadium surinamense* L.

Table 1 Identified compounds of nanoherbal *Clibadium surinamense* L. by GC-MS.

No	Compound name	Mol. formula	Mass (g/mol)	R. time (min)	Concen-tration (%)	Smiles	PubChem CID
1	Methanol	CH_4O	32	1,540	22.389	CO	887
2	Methanamine	CH_5O	31	2.843	5.044	CN	6329
3	8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one	$C_{14}H_{20}O_3$	236	20.571	0.022	CC(=O)C=C=CC(C)(C)CCC1(CO1)C(=O)C	539293
4	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	$C_9H_{12}O_2$	152.19	23.482	2.747	CC1=C(C(=O)CC1O)CC=C	11083
5	2-Undecanone 2,4-dinitrophenylhydrazone	$C_{17}H_{26}N_4O_4$	350.4	25.188	0.167	CCCCCCCCCC(=NNC1=C(C=C(C=C1)[N+](=O)[O-])[N+](=O)[O-])C	5717665
6	1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene	$C_{16}H_{22}O_3$	262.34	29.095	0.705	CC1(C(=C)C=CC2(C1(O2)C=CC3(OCCO3)C)C)C	5368073
7	6-Tridecene	$C_{13}H_{26}$	182.35	30.915	0.614	CCCCCCC=CCCCCC	138758
8	19,19-Dimethyl-eicosa-8, 11-dienoic acid	$C_{22}H_{40}O_2$	336.6	31.152	0.635	CC(C)(C)CCCCCCC=CCC=CCCCCCCC(=O)O	5364968
9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	31.737	0.082	CCCCCCCCCCCCCCCC(=O)OC	8181
10	Methanamine	CH_5O	34.626	2.011	2.011	CN	6329
11	2-Cyclopentene-1-undecanoicacid	$C_{16}H_{28}O_2$	252.39	36.241	3.617	C1CC(C=C1)CCCCCCCCCCC(=O)O	110680
12	Cyclopentane undecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268.4	38.946	1.574	COC(=O)CCCCCCCCCCCC1CCCC1	535041
13	2,2',5,5'-Tetraethyl-β-bithienyl	$C_{16}H_{22}S_2$	278	42.630	0.968	-	-
14	Octadecanoic acid, (2-	$C_{28}H_{46}O_4$	446.7	45.577	6.031	CCCCCCCCCCCCCCC	569173

No	Compound name	Mol. formula	Mass (g/mol)	R. time (min)	Concen-tration (%)	Smiles	PubChem CID
	phenyl-1,3-dioxolan-4-yl)methyl ester, cis-					CCC(=O)OCC1COC(O1)C2=CC=CC=C2	
15	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1 [(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436.6	48.302	2.884	CCC=CCC=CCC=CCC CCCCCC(=O)OC(COC(=O)C)COC(=O)C	21159744
16	2,4,8-Trimethylundecanoly Pyrrolidine	$C_{18}H_{35}NO$	281.484	49.645	2.509	C(N1CCCC1)(C(CC(CCC(CCC)C)C)C)=O	-

Prediction of compound biological activities

The PASS online server predicts the biological activity of compounds related to various mechanisms of action and possible therapeutic effects before conducting further experimental studies. In addition to its activity against AKT1, PASS was also used to predict breast cancer healing pathways by identifying compounds that affect breast cancer-related signaling pathways. This assessment provides a basis for designing subsequent studies to explore and optimize the potential of Nanohebal *Clibadiium surinamense* L. The positive control passonline results of the AKT1 inhibitor (Capivasertib) showed very low probability of activity especially for the targets Protein Kinase B Inhibitor (Pa = 0.473), Protein Kinase B alpha Inhibitor (Pa = 0.303), Protein Kinase B beta Inhibitor (Pa = 0.291), and CDK9/cyclin T1 Inhibitor (Pa = 0.345). These low values from the passonline may seem contradictory to the known efficacy of Capivasertib as a Protein Kinase B (AKT) inhibitor. This discrepancy in the PassOnline test arises because PassOnline is likely limited by its reliance on a limited database that may not include recent or specific data on compounds already known as commercial drugs. Therefore, while the PASSOnline score provides a proper initial assessment, it must be interpreted with caution and complemented with experimental data to evaluate the therapeutic potential of compounds such as Capivasertib accurately [34,35].

Numerous bioactive compounds from Nanoherbal *Clibadium surinamense* L. exhibit substantial potential for cancer treatment, as indicated by the web-based PASS analysis results (**Table 2**). 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl] is one of the most notable compounds.-4-methylene-7-oxa-bicyclo[4.1.0] hept-2-ene, which has a very high probability of activity (Pa) for antineoplastic effects at 0.935. This compound also exhibits the capacity to stimulate Caspase 8 and inhibit MMP9 and JAK2 expression, albeit with lower Pa values. These activities indicate that the compound has the potential to impede the growth of tumors and the spread of metastases. The inhibition of MMP9 is especially significant because it is a critical component of extracellular matrix degradation, a critical step in the progression of cancer metastasis. Furthermore, 6-Tridecene demonstrates substantial anticancer activity due to its ability to inhibit BRAF expression, which is essential for the regulation of cancer cell proliferation, with a Pa of 0.862. The suppression of tumor cell proliferation can be achieved by inhibiting BRAF, a protein kinase that is implicated in the RAS/MAPK signaling pathway. This compound's therapeutic value is further enhanced by its anti-inflammatory properties and the inhibition of MMP9 and TNF expression, among other activities. Anti-inflammatory effects are essential in cancer therapy, as chronic inflammation is recognized to contribute to the progression of cancer and the microenvironment of the tumor.

In contrast, some compounds exhibit reduced activity against the treatment of cancer. For example, 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one demonstrates very low probabilities of activity in inhibiting MMP9, JAK2 and BRAF with Pa values of 0.331, 0.326 and 0.194, correspondingly. Besides, it has minimal activity as a macrophage colony-stimulating factor agonist, Pa = 0.596, influencing macrophage differentiation and proliferation. Similarly, 2-undecanoate 2,4-dinitrophenylhydrazone lowered the activity as a Mcl-1 antagonist and CDK9/cyclin T1 inhibitor, in which the relative Pa values are 0.632 and 0.525, respectively. Inhibition of the anti-apoptotic protein Mcl-1 induces apoptosis in cancer cells. In addition, there is a methanamine compound in the

nanoherbal *Clibadium surinamense* L., which has polar properties and a simple carbon structure, namely only 1 carbon atom. Compounds such as methanamine, with very small structures and less than 3 carbon atoms, cannot be tested in Way2Drug because the system does not support the analysis of compounds with too small molecular sizes [36]. The inability to process these compounds in Way2Drug is due to the limitations in detecting and analyzing compounds with carbon structures that are too simple, which hinders the accurate evaluation of their biological activity. Notwithstanding their biological activities, these compounds could be less potent for cancer treatment than others. The findings prove great potential, but additional selection and optimization are required concerning Nanoherbal *Clibadium surinamense* L derived bioactive compounds to achieve a maximal therapeutic effect.

Table 2 Prediction biological activity compounds of Nanoherbal *Clibadium surinamens*e L. on pass onlint test.

No	Compound name	Biological activity	Pa	Pi	Criteria
Positive control					
1.	Capivasertib	Protein kinase B inhibitor	0.473	0.003	Extremely low
		Protein kinase B alpha inhibitor	0.303	0.004	Extremely low
		Protein kinase B beta inhibitor	0.291	0.003	Extremely low
		CDK9/cyclin T1 inhibitor	0.345	0.081	extremely low
Bioactive components of the nanoherbal *Clibadium surinamense* L.					
2.	8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one	Macrophage colony stimulating factor agonist	0.596	0.033	Low
		MMP9 expression inhibitor	0.331	0.081	Extremely low
		JAK2 expression inhibitor	0.326	0.121	Extremely low
		BRAF expression inhibitor	0.194	0.041	Extremely low
3.	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	Caspase 3 stimulant	0.761	0.008	High
		Antineoplastic	0.724	0.020	High
		MMP9 expression inhibitor	0.619	0.014	Low
		JAK2 expression inhibitor	0.572	0.037	Low
		Antiinflammatory	0.529	0.049	Low
		Caspase 8 stimulant	0.509	0.016	Low
		Antineoplastic (breast cancer)	0.175	0.112	Extremely low
		Antineoplastic (carcinoma)	0.115	0.067	Extremely low
4.	2-Undecanone 2,4-dinitrophenylhydrazone	Preneoplastic conditions treatment	0.876	0.011	High
		Superoxide dismutase inhibitor	0.681	0.015	Low
		Mcl-1 antagonist	0.632	0.005	Low
		CDK9/cyclin T1 inhibitor	0.525	0.011	Low
		Macrophage colony stimulating factor agonist	0.453	0.087	Low
5.	1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene	Antineoplastic	0.935	0.004	High
		MMP9 expression inhibitor	0.491	0.030	Low
		Caspase 8 stimulant	0.426	0.039	Low
		JAK2 expression inhibitor	0.407	0.083	Low
		Antiinflammatory	0.239	0.226	Extremely low
6.	6-Tridecene	BRAF expression inhibitor	0.862	0.001	High
		MMP9 expression inhibitor	0.663	0.009	Low
		TNF expression inhibito	0.654	0.009	Low
		Antiinflammatory	0.622	0.027	Low

No	Compound name	Biological activity	Pa	Pi	Criteria
		Caspase 8 stimulant	0.584	0.008	Low
		Apoptosis antagonist	0.357	0.012	Extremely low
7.	Hexadecanoic acid, methyl ester	Macrophage colony stimulating factor agonist (M-CSF agonist)	0.794	0.005	High
		Superoxide dismutase (SOD) inhibitor	0.738	0.010	High
		Beta-adrenergic receptor kinase inhibitor	0.746	0.018	High
		Cytoprotectant	0.701	0.005	High
		BRAF expression inhibitor	0.697	0.003	Low
		Caspase 8 stimulant	0.609	0.006	Low
		JAK2 expression inhibitor	0.548	0.042	Low
		MMP9 expression inhibitor	0.532	0.024	Low
		Antiinflammatory	0.510	0.054	Low
		Apoptosis agonist	0.473	0.046	Extremely low
		Immunostimulant	0.430	0.048	Extremely low
8.	2-Cyclopentene-1-undecanoicacid	Macrophage colony stimulating factor agonist	0.737	0.009	High
		Prostaglandin-E2 9-reductase inhibitor	0.736	0.013	High
		Oxidoreductase inhibitor	0.705	0.015	High
		Cytoprotectant	0.674	0.009	Low
		Antihypoxic	0.444	0.057	Extremely low
		BRAF expression inhibitor	0.442	0.010	Extremely low
9.	Cyclopentaneundecanoic acid, methyl ester	Cytoprotectant	0.714	0.004	High
		Oxidoreductase inhibitor	0.655	0.023	Low
		Macrophage colony stimulating factor agonist	0.628	0.026	Low
		Prostaglandin-E2 9-reductase inhibitor	0.535	0.034	Low
		Caspase 8 stimulant	0.509	0.016	Low
		JAK2 expression inhibitor	0.413	0.081	Extremely low

Prediction of drug-likeness

Based on the evaluation of physicochemical properties according to Lipinski's Rule of 5 for the bioactive compounds extracted from *Clibadium surinamense* L (**Table 3**), the analysis reveals varied adherence to drug-likeness criteria. Compounds such as Methanamine, 8-(2-Acetyloxiran-2-yl)-6,6-dimethyloc ta-3,4-dien-2-one, 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-, 2-Undecanone 2,4-dinitrophe nylhydrazone, 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dio xolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0] he pt-2-ene, 2-Cyclopentene-1-undecanoic acid and Cyclopentaneundecanoic acid, methyl ester conform to Lipinski's rules, with MlogP values below the 4.15 threshold and hydrogen bond counts within acceptable limits, suggesting good potential for oral bioavailability. However, 6-Tridecene and Hexadecanoic acid, methyl ester do not meet the criteria due to their elevated MlogP values of 5.52 and 4.44, respectively, which exceed the maximum allowable limit. These deviations indicate a need for further optimization of these compounds to enhance their pharmacokinetic properties and overall drug-likeness. High MLogP values in a compound can lead to excessive lipophilicity, resulting in challenges related to drug absorption, distribution, metabolism and excretion in the body [37].

Table 3 Physicochemical properties of compounds from Nanoherbal *Clibadium surinamense* L. based on Lipinski's rule of 5 test.

No	Compound name	Lipinski				Violasi
		MW	MlogP ≤ 4.15	NorO ≤ 10	NHorOH ≤ 5	
Positive control						
1.	Capivasertib	428.92	1.41	5	4	yes (0)
Bioactive components of the nanoherbal *Clibadium surinamense* L.						
2.	Methanamine	31.06	−0.43	1	1	yes (0)
3.	8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one	236.31	1.31	3	0	yes (0)
4.	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	152.19	0.87	2	1	yes (0)
5.	2-Undecanone 2,4-dinitrophenylhydrazone	350.41	2.39	5	1	yes (0)
6.	1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0] hept-2-ene	262.34	4.08	3	0	yes (0)
7.	6-Tridecene(CAS)	182.35	5.52	0	0	yes (1)
8.	Hexadecanoic acid, methyl ester	270.45	4.44	2	0	yes (1)
9.	2-Cyclopentene-1-undecanoicacid	252.39	3.69	2	1	yes (0)
10.	Cyclopentaneundecanoic acid, methyl ester	268.43	4.04	2	0	yes (0)

They used Lipinski's rule to look at the physicochemical properties of Nanoherbal *Clibadium surinamense* L. compounds to see how good they might be as drug candidates showed that 6 of the 8 compounds met the criteria for being drug-like compounds. This suggests potential suitability for therapeutic applications, particularly in cancer treatment. This is significant because compounds that adhere to Lipinski's criteria typically exhibit optimal ADME (absorption, distribution, metabolism and excretion) profiles, which are crucial in developing practical and safe medicines [28,38].

Molecular interactions of bioactive compounds Nanoherbal *Clibadium surinamense* L. with AKT1

Based on the results of molecular docking modelling analysis, a comparison between the control compound Capivasertib and various bioactive compounds from Nanoherbal *Clibadium surinamense* L. showed significant variations in the type of interaction and their binding energy to protein targets. Capivasertib, as a control compound, showed the highest binding energy among all the compounds tested, namely −6.6 kcal/mol. The main interactions involved are hydrogen bonds with residues ARG328 and ASP325, as well as hydrophobic interactions with residues ILE36, TYR38, ALA329, PRO388 and LYS389. This interaction supports its strong affinity for the target, indicating that Capivasertib functions effectively as an inhibitor of the target protein (**Table 4**; **Figures 3** and **4**).

Table 4 Residue and biding energy of ligand and AKT1 interaction.

No	Ligand	Interaction type				Binding Energy (Kcl/mol)
		Hydrogen bond		Hydrophobic	Electrostatis	
		Hydrogen bond	Carbon hydrogen bond			
Positive control						
1	Capivasertib (control)	ARG328	ASP325, ASP387	ILE36, TYR38, ALA329, PRO388, LYS389	ASP325	−6.6
Bioactive components of the nanoherbal *Clibadium surinamense* L.						
2	Methanamine	LEU275, TYR315	ARG273	-		−1.8
3	8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one	TYR38, ARG328, ALA329	-	-	-	−4.7
4	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	-	-	LEU275, ALA317, LEU316, VAL320, VAL330	-	−5.9
5	2-Undecanone 2,4-dinitrophenylhydrazone	ARG328, ALA329	-	PRO318, LYS386, PRO388	GLU319, ASP325	−6.6
6	1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene	TYR18	-	ILE84, ARG273		−6.2
7	6-Tridecene	-	-	TYR18, ILE84	-	−3.8
8	Hexadecanoic acid, methyl ester	ASN54	ASP274	TYR18, ILE84, CYS296	-	−4.4
9	2-Cyclopentene-1-undecanoicacid	ARG328	GLY394	PRO51, LEU5, ILE36	-	−4.4
10	Cyclopentaneundecanoic acid, methyl ester	-	TYR326	ILE36, LEU52, PHE55, ARG328, ALA329	-	−5

Bioactive compounds from nanoherbal *Clibadium surinamense* L. have been proven to show various potentials through molecular docking. For example, 2-Undecanone 2,4-dinitrophenylhydrazone has the same binding energy as Capivasertib, namely −6.6 kcal/mol, and shows interactions with ARG328 and ALA329 via hydrogen bonds and PRO318, LYS386 and PRO388 via hydrophobic interactions. This compound also interacts with GLU319 and ASP325, increasing their potential biological effects. On the other hand, 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one shows a lower binding energy (−4.7 kcal/mol) and only interacts with residues TYR38, ARG328 and ALA329 via hydrogen bonds, without hydrophobic or electrostatic interactions. This

suggests that this compound may have a lower affinity for the target compared to Capivasertib.

Methanamine shows a deficient binding energy (−1.8 kcal/mol) and only interacts with residues LEU275 and TYR315 through hydrophobic interactions and ARG273 through electrostatic interactions. This indicates that Ethanamine has a much lower affinity for the target compared to the compounds present in Nanoherbal *Clibadium surinamense* L, especially 6-Tridecene, which also shows a low binding energy (−3.8 kcal/mol) and only interacts hydrophobically with residues TYR18 and ILE84. Both compounds demonstrate significantly lower affinity for the target than Capivasertib, exhibiting better binding potential and interactions.

Hexadecanoic acid, methyl ester also shows low binding energy (−4.4 kcal/mol), with hydrogen interactions at residue ASN54 and hydrophobic interactions at residues TYR18, ILE84 and CYS296. The compound 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)- shows a fairly good binding energy (−5.9 kcal/mol) with the main hydrophobic interactions at residues LEU275, ALA317, LEU316, VAL320 and VAL330. Finally, Cyclopentaneundecanoic acid, methyl ester and 2-Cyclopentene-1-undecanoicacid have binding energies of −5 and −4.4 kcal/mol, respectively, with varying hydrophobic interactions with residues such as TYR326, ILE36, LEU52, PHE55 and ARG328. Overall, this analysis suggests that compounds with lower binding energies tend to have less strong interactions with targets. In contrast, compounds with higher binding energies show potentially better affinity and stronger biological effects, similar to Capivasertib as a control.

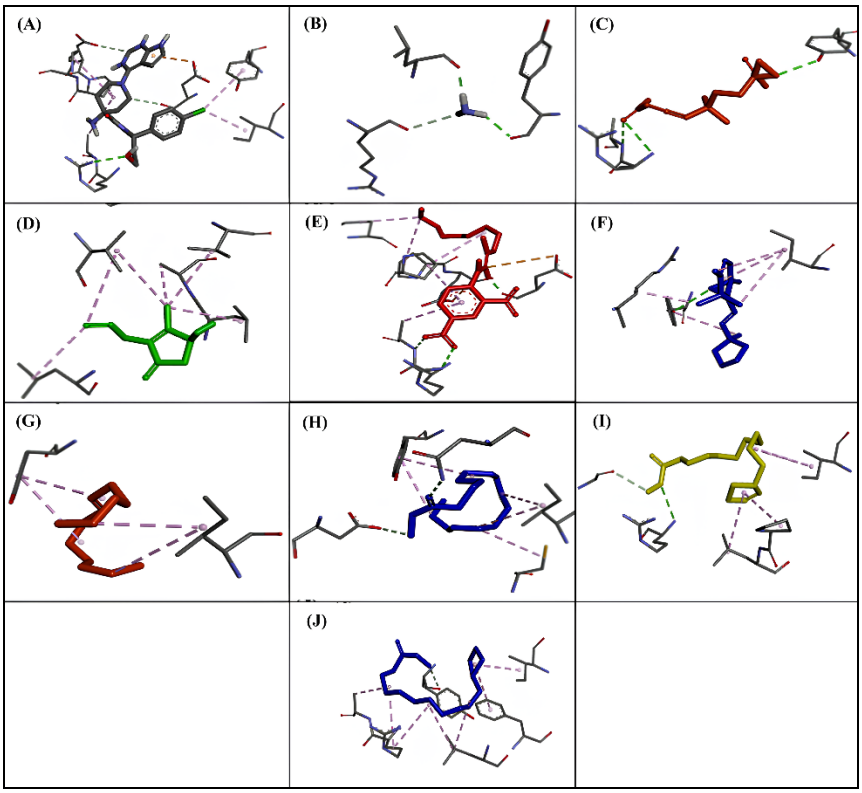

Figure 3 3D interactions of Capivasertib and bioactive compounds from *Clibadium surinamense* L. against the AKT1 protein. (A) Capivasertib; (B) Methanamine; (C) 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one; (D) 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-; (E) 2-Undecanone 2,4-dinitrophenylhydrazone; (F) 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene; (G) 6-Tridecene; (H)Hexadecanoic acid, methyl ester; (I) 2-Cyclopentene-1-undecanoicacid; (J) Cyclopentaneundecanoic acid, methyl ester.

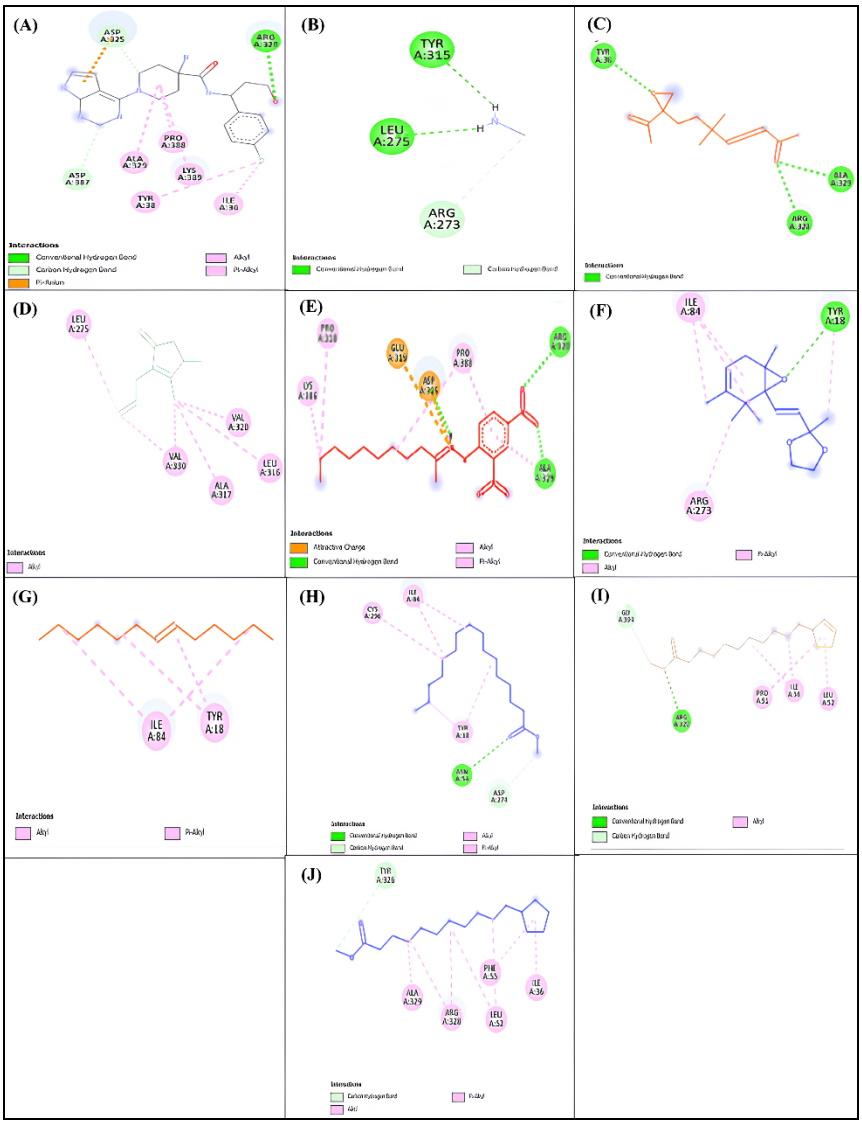

Figure 4 2D interactions of Capivasertib and bioactive compounds from *Clibadium surinamense* L. against the AKT1 protein. (A) Capivasertib; (B) Methanamine; (C) 8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one; (D) 2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-; (E) 2-Undecanone 2,4-dinitrophenylhydrazone; (F) 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxa-bicyclo[4.1.0]hept-2-ene; (G) 6-Tridecene; (H)Hexadecanoic acid, methyl ester; (I) 2-Cyclopentene-1-undecanoicacid; (J) Cyclopentaneundecanoic acid, methyl ester.

Akt1 (protein kinase B) is a key element in cell signaling pathways that regulate cell survival, proliferation and metabolism, which is frequently dysregulated in various types of cancer, including breast cancer [39]. Inhibitors targeting Akt1 have become a major focus in cancer therapy because the Akt1 signaling pathway plays a role in tumor growth and resistance to apoptosis. Akt plays a crucial role in the PI3K/Akt/mTOR signaling pathway, which is essential for controlling important biological functions like cell survival and proliferation [37]. Capivasertib, known as an Akt1 inhibitor, showed strong binding and inhibitory potential, making it an effective control in molecular docking studies [12,13,40]. Its interaction with key residues such as ARG328, ASP325, ILE36, TYR38, ALA329 and PRO388 indicates its ability to effectively inhibit Akt1, interfering with cancer cell survival and proliferation pathways.

This study highlights the potential of *Clibadium surinamense* L. nanoherbal formulations as promising agents in breast cancer treatment. The bioactive components of *Clibadium surinamense* L. nanoherbals,

particularly those interacting with key residues similar to Capivasertib, exhibit significant potential as anticancer agents with analogous mechanisms of action. Notably, bioactive compounds such as 2-Undecanone 2,4-dinitrophenylhydrazone and 1,5,5-Trimethyl-6-[2-(2-methyl-[1,3]dioxolan-2-yl)-vinyl]-4-methylene-7-oxabicyclo[4.1.0]hept-2-ene from *Clibadium surinamense* L. nanoherbals show high potential as inhibitors of AKT1 protein activity, which is implicated in breast cancer development. However, these *in silico* predictions require further validation through *in vitro* and *in vivo* studies to confirm that *Clibadium surinamense* L. nanoherbal formulations can be effectively utilized as natural inhibitors of AKT1 in breast cancer therapy. This represents a potential breakthrough in the field of natural product-based cancer treatment.

Conclusions

The conclusion of this study revealed that *in silico* investigation of nanoherbal compounds from *Clibadium surinamense* L. leaves provides valuable insights into their potential as AKT1 inhibitors in cancer treatment. This study successfully identified specific compounds that meet the druggable criteria and showed promising interactions with AKT1, indicating their potential use in therapeutic applications, especially for cancer treatment. These findings emphasize the importance of exploring natural resources in the search for new drug candidates and the significance of molecular docking analysis in drug discovery research. However, it should be emphasized that reliance on *in silico* methods, although valuable, needs to reflect the complexity of biological systems fully, and these results require further validation. Future research should focus on *in vitro* studies to verify the biological activity of these compounds and explore their mechanisms of action in cancer cells. The results of this study provide a basis for further exploration of compounds from *Clibadium surinamense* L. as anticancer agents while highlighting the potential for developing effective and safe drugs based on natural products. Integration of experimental validation, mainly through *in vitro* and *in vivo* studies, will be critical to advancing these findings toward clinical application, and future research in this area promises to advance cancer treatment strategies and improve outcomes for patients battling cancer.

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References

[1] F Bray, M Laversanne, H Sung, J Ferlay, RL Siegel, I Soerjomataram and A Jemal. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2024; **74(3)**, 229-263.

[2] SK Kim and SW Cho. The evasion mechanisms of cancer immunity and drug intervention in the tumor microenvironment. *Frontiers in Pharmacology* 2022; **13**, 868695.

[3] R Tang, J Xu, B Zhang, J Liu, C Liang, J Hua, Q Meng, X Yu and S Shi. Ferroptosis, necroptosis, and pyroptosis in anticancer immunity. *Journal of Hematology & Oncology* 2020; **13**, 110.

[4] MR Paul, TC Pan, DK Pant, NNC Shih, Y Chen, KL Harvey, A Solomon, D Lieberman, JJD Morrissette, D Soucier-Ernst, NG Goodman, SW Stavropoulos, KN Maxwell, C Clark, GK Belka, M Feldman, A DeMichele and LA Chodosh. Genomic landscape of metastatic breast cancer identifies preferentially dysregulated pathways and targets. *The Journal of Clinical Investigation* 2020; **130(8)**, 4252-4265.

[5] MM Kenna, S McGarrigle and GP Pidgeon. The next generation of PI3K-Akt-MTOR pathway inhibitors in breast cancer cohorts. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 2018; **1870(2)**, 185-197.

[6] MK Fath, RA Masouleh, N Afifi, S Loghmani, P Tamimi, A Fazeli, SA Mousavian, MM Falsafi and G Barati. PI3K/AKT/MTOR signaling pathway modulation by circular RNAs in breast

cancer progression. *Pathology - Research and Practice* 2023; **241**, 154279.

[7] MA Ortega, O Fraile-Martínez, Á Asúnsolo, J Buján, N García-Honduvilla and S Coca. Signal transduction pathways in breast cancer: The important role of PI3K/Akt/MTOR. *Journal of Oncology* 2020; **2020**, 9258396.

[8] N Hinz and M Jücker. Distinct functions of AKT isoforms in breast cancer: A comprehensive review. *Cell Communication and Signaling* 2019; **17**, 154.

[9] M Song, AM Bode, Z Dong and MH Lee. AKt as a therapeutic target for cancer. *Cancer Research* 2019; **79(6)**, 1019-1031.

[10] A Basu and CB Lambring. Akt isoforms: A family affair in breast cancer. *Cancers* 2021; **13**, 3445.

[11] D Miricescu, A Totan, II Stanescu-Spinu, SC Badoiu, C Stefani and M Greabu. PI3K/AKT/MTOR signaling pathway in breast cancer: From molecular landscape to clinical aspects. *International Journal of Molecular Sciences* 2021; **22(1)**, 173.

[12] A Andrikopoulou, S Chatzinikolaou, E Panourgias, M Kaparelou, M Liontos, MA Dimopoulos and F Zagouri. The emerging role of capivasertib in breast cancer. *Breast* 2022; **63**, 157-167.

[13] LM Smyth, K Tamura, M Oliveira, E Ciruelos, IA Mayer, MP Sablin, L Biganzoli, H Ambrose, J Ashton, A Barnicle, D Cashell, C Corcoran, EC de Bruin, A Foxley, J Hauser, JPO Lindemann, R Maudsley, R McEwen, M Moschetta, …, DM Hyman. Capivasertib, an AKT kinase inhibitor, as monotherapy or in combination with fulvestrant in patients with AKT1E17K-mutant, ER-positive metastatic breast cancer. *Clinical Cancer Research* 2020; **26(15)**, 3947-3957.

[14] Y Yuan, H Long, Z Zhou, Y Fu and B Jiang. PI3K-AKT-targeting breast cancer treatments: Natural products and synthetic compounds. *Biomolecules* 2023; **13(1)**, 93.

[15] MK Dewi, AY Chaerunisaa, M Muhaimin and IM Joni. Improved activity of herbal medicines through nanotechnology. *Nanomaterials* 2022; **12(22)**, 4073.

[16] Y Guo, Y Zhao, X Zhao, S Song and B Qian. Exploring the anticancer effects of tin oxide nanoparticles synthesized by pulsed laser ablation technique against breast cancer cell line through downregulation of PI3K/AKT/MTOR signaling pathway. *Arabian Journal of Chemistry* 2021; **14(7)**, 103212.

[17] G Baskar, T Palaniyandi, M Ravi, S Viswanathan, MRA Wahab, H Surendran, M Govindaraj, A Sugumaran, MH Almutairi and BO Almutairi. Biosynthesis of iron oxide nanoparticles from red seaweed hypnea valentiae and evaluation of their antioxidant and antitumor potential via the AKT/PI3K pathway. *Process Biochemistry* 2024; **141**, 155-169.

[18] M Ali, S Ud, D Wani, SN Manjula, K Mruthunjaya, T Dey, M Iqbal and J Singh. Recent advance of herbal medicines in cancer - a molecular approach. *Heliyon* 2023; **9(2)**, e13684.

[19] DA Mcgrowder, FG Miller, CR Nwokocha, MS Anderson, C Wilson-clarke, K Vaz, L Anderson-jackson and J Brown. Medicinal herbs used in traditional management of breast cancer: Mechanisms of action. *Medicines* 2020; **7(8)**, 47.

[20] RV Ulia, Suryati and A Santoni. Cytotoxic potential of essential oil isolated from Semambu (*Clibadium Surinamese* L) leaves against T47D breast and HeLa cervical cancer cells. *Molekul* 2023; **18(2)**, 289-299.

[21] MK Swamy, UR Sinniah and MS Akhtar. *In vitro* pharmacological activities and GC-Ms analysis of different solvent extracts of *Lantana camara* leaves collected from tropical region of Malaysia. *Evidence-Based Complementary and Alternative Medicine* 2015; **2015**, 506413.

[22] S Dallakyan and AJ Olson. *Small-molecule library screening by docking with PyRx*. *In*: J Hempel, C Williams and C Hong (Eds.). Chemical biology. Methods in molecular biology. Vol 1263. Humana Press, New York, p. 243-250.

[23] Y Peng, Y Wang, C Zhou, W Mei and C Zeng. PI3K/Akt/MTOR pathway and its role in cancer therapeutics: Are we making headway? *Frontiers in Oncology* 2022; **12**, 819128.

[24] H Hua, H Zhang, J Chen, J Wang, J Liu and Y Jiang. Targeting Akt in cancer for precision therapy. *Journal of Hematology & Oncology* 2021; **14**, 128.

[25] D Das, M Nanda, P Banjare and S Lanjhiyana. Exploration of multitargeted antialzheimer's activity of safflower leaves phytoconstituents: *In silico* molecular docking approach. *European Journal of Medicinal Chemistry Reports* 2024; **10**, 100119.

[26] S Rosignoli and A Paiardini. DockingPie: A consensus docking plugin for PyMOL. *Bioinformatics* 2022; **38(17)**, 4233-4234.

[27] P Santoso, S Ilyas, YH Midoen, R Maliza and DF Belahusna. Predictive bioactivity of compounds from *Vitis gracilis* leaf extract to counteract doxorubicin-induced cardiotoxicity via sirtuin 1 and adenosine monophosphate-activated protein kinase: An *in-silico* study. *Journal of Applied Pharmaceutical Science* 2024; **14(4)**, 99-114.

[28] A Daina, O Michielin and V Zoete. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports* 2017; **7**, 42717.

[29] A Allouche. Software news and update autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 2010; **31(2)**, 174-182.

[30] M Azmal, MS Hossen, MNH Shohan, R Taqui, A Malik and A Ghosh. A computational approach to identify phytochemicals as potential inhibitor of acetylcholinesterase: Molecular docking, ADME profiling and molecular dynamics simulations. *PLoS One* 2024; **19(6)**, e0304490.

[31] JF Ayala-Cabrera, L Montero, SW Meckelmann, F Uteschil and OJ Schmitz. Review on atmospheric pressure ionization sources for gas chromatography-mass spectrometry. Part II: Current applications. *Analytica Chimica Acta* 2023; **1238**, 340379.

[32] C Abadie, J Lalande and G Tcherkez. Exact mass GC-MS analysis: Protocol, database, advantages and application to plant metabolic profiling. *Plant, Cell & Environment* 2022; **45**, 3171-3183.

[33] Y Marcus. Extraction by subcritical and supercriticalwater, methanol, ethanol and their mixtures. *Separations* 2018; **5(1)**, 4.

[34] DA Filimonov, AA Lagunin, TA Gloriozova, AV Rudik, DS Druzhilovskii, PV Pogodin and VV Poroikov. Prediction of the biological activity spectra of organic compounds using the pass online web resource. *Chemistry of Heterocyclic Compounds* 2014; **50**, 444-457.

[35] P Vijaya and G Sundaraselvan. Synthesis, characterization, PASS analysis and ADMET properties of oxazolone ring containing hydrazone derivatives. *Materials Today: Proceedings* 2019; **48**, 502-507.

[36] SM Ivanov, AA Lagunin, DA Filimonov and VV Poroikov. Relationships between the structure and severe drug-induced liver injury for low, medium, and high doses of drugs. *Chemical Research in Toxicology* 2022; **35(3)**, 402-411.

[37] A Tsantili-kakoulidou and VJ Demopoulos. Drug-like properties and fraction lipophilicity index as a combined metric. *ADMET DMPK* 2021; **9(3)**, 177-190.

[38] GR Bickerton, GV Paolini, J Besnard, S Muresan and AL Hopkins. Quantifying the chemical beauty of drugs. *Nature Chemistry* 2012; **4**, 90-98.

[39] AL Whitworth, NH Mann and AWD Larkum. Inhibition assays for AKT1, ΔPH-AKT1, AKT2, ΔPH-AKT2 and AKT3 activity, AKT1 activation, and binding kinetics. *Ultrasound in Obstetrics & Gynecology* 2006; **50**, 776-780.

[40] Z Qureshi, F Altaf, M Khanzada, Z Zaheer, E Fatima and M Bakhtiar. Capivasertib in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer. *Current Problems in Cancer* 2024; **51**, 101114.