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Original Article

Anticancer activity of *Aucklandia costus* Falc. terpenes: Targeting MDM2 protein inhibition for therapeutic advancements

[Actividad anticancerígena de los terpenos de *Aucklandia costus* Falc.: Inhibición de la proteína MDM2 para avances terapéuticos]

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Abstract

Context: Protein 53 (p53) is a well-known tumor suppressor protein, while murine double minute 2 (MDM2) acts as a negative regulator of p53, leading to p53 inactivation and cancer development. *Aucklandia costus* Falc. or *Saussurea lappa* contains bioactive compounds, particularly terpenoids, known for their anticancer activity against various cancer cells. Targeting the p53-MDM2 protein interaction and inhibiting MDM2 are crucial strategies in cancer therapy.

Aims: To analyze the anticancer properties of *A. costus* terpenes against MDM2 protein.

Methods: The compounds costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin were docked with MDM2 (PDB ID: 4HG7) using AutoDockTools 1.5.6. Additionally, the physicochemical, pharmacokinetic, and toxicity properties were predicted using pkCSM.

Results: Lappadilactone exhibited the highest binding energy value, surpassing both the control and the native ligand. Following lappadilactone, cynaropicrin, costunolide, and dehydrocostus lactone displayed decreasing binding energies. When assessing ADMET properties with pkCSM, all compounds exhibited good permeability, suggesting their ability to penetrate intestinal cell membranes, and showed no signs of hepatotoxicity.

Conclusions: Lappadilactone emerges as a promising candidate with high intestinal absorption, distinctive distribution characteristics, and a lack of mutagenic or hepatotoxic effects.

Keywords: ADMET; *Aucklandia costus*; cancer; docking; MDM2; *Saussurea lappa*.

Resumen

Contexto: La proteína 53 (p53) es una conocida proteína supresora de tumores, mientras que la doble minúscula murina 2 (MDM2) actúa como regulador negativo de p53, lo que conduce a su inactivación y al desarrollo del cáncer. *Aucklandia costus* Falc. o *Saussurea lappa* contiene compuestos bioactivos, en particular terpenoides, conocidos por su actividad anticancerígena contra diversas células cancerosas. Dirigirse a la interacción proteína p53-MDM2 e inhibir MDM2 son estrategias cruciales en la terapia del cáncer.

Objetivos: Analizar las propiedades anticancerígenas de los terpenos de *A. costus* frente a la proteína MDM2.

Métodos: Los compuestos costunolide, dehydrocostus lactone, lappadilactone, y cynaropicrin fueron acoplados a MDM2 (PDB ID: 4HG7) usando AutoDockTools 1.5.6. Además, se analizaron las propiedades fisicoquímicas y moleculares de los terpenos. Además, se predijeron las propiedades fisicoquímicas, farmacocinéticas y de toxicidad utilizando pkCSM.

Resultados: Lappadilactona mostró el mayor valor de energía de unión, superando tanto al control como al ligando nativo. Tras la lappadilactona, la cinaropicrina, la costunolida y la lactona dehidrocostus mostraron energías de unión decrecientes. Al evaluar las propiedades ADMET con pkCSM, todos los compuestos mostraron una buena permeabilidad, lo que sugiere su capacidad para penetrar en las membranas celulares intestinales, y no mostraron signos de hepatotoxicidad.

Conclusiones: La lapadilactona emerge como un candidato prometedor con alta absorción intestinal, características de distribución distintivas y ausencia de efectos mutagénicos o hepatotóxicos.

Palabras Clave: ADMET; *Aucklandia costus*; cáncer; docking; MDM2; *Saussurea lappa*.

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INTRODUCTION

Cancer is a global health challenge that imposes significant physical, emotional, and financial burdens on individuals, families, communities, and healthcare systems (WHO, 2022). According to GLOBOCAN 2020, 19,292,789 new cancer cases were reported worldwide (Latha et al., 2016). Projections from the International Agency for Research on Cancer (IARC) indicate that by 2040, the number of new cancer cases could surge to 30.2 million, with an associated death rate of 16.3 million (IARC, 2020). The p53, known as a tumor suppressor, functions as a transcription factor that regulates the expression of various genes involved in apoptosis, cell cycle regulation, differentiation, and DNA repair. This pivotal role makes p53 a critical component of the cell's defense against cancer, as it participates in diverse signal transduction networks (Biswas et al., 2023). Inactivation of p53 and an increase in murine double minute 2 (MDM2) levels have been linked to cancer development. MDM2 plays a central role in inhibiting the tumor suppressor function of p53 by directly antagonizing it. Therefore, the inhibition of the p53-MDM2 interaction represents a promising approach to restoring p53's tumor suppressor function (Priatna et al., 2022; Zhao et al., 2015).

Contemporary medicine is increasingly exploring the potential of medicinal plants. *Aucklandia costus* Falc. or *Saussurea lappa* (Decne.) C.B. Clarke is a well-known medicinal plant and is commonly used in various medicinal systems throughout the world. This plant is famous due to its high medical importance and can be used to extract such bioactive compounds which can help scientists to discover new and potential drugs (Amara et al., 2017). Around 3000 species of *Saussurea* are known. *A. costus* is a perennial herb and has been traditionally used as an anticancer, antifungal, antidiabetic, anthelmintic, antitumor, antiulcer, antimicrobial, immunostimulant, anti-inflammatory, and antihepatotoxic without many adverse effects (Hassan and Masoodi, 2020; Zahara et al., 2014). Studies have demonstrated the anticancer activity of *A. costus* on various cancer cell types, including HepG2 (Alotaibi et al., 2021), breast cancer cells (MCF7), and human colon cancer (Patel et al., 2020). Consequently, *A. costus* has emerged as a leading candidate for the development of effective and safe anticancer drugs.

Phytochemical analyses have identified *A. costus* root as a rich source of bioactive compounds, such as sesquiterpenes, flavonoids, lignans, phytosterols alkaloids, terpenes anthraquinones, and flavonoids, among others (Hassan and Masoodi, 2020). Terpenoids, a prominent component of *S. lappa*, exhibit anticancer properties by suppressing early tumorigenesis through cell cycle arrest induction, inhibition of cancer cell differentiation, and activation of apoptosis (Kamran et al., 2022). Terpenes contained in *S. lappa,* like 3-trans-p-coumaroyl maslinic acid, silvestrol, and betulonic acid, can act as antagonists of p53-MDM2 interaction to reactivate p53 functioning (Muhseen and Li, 2020). Therefore, this research aimed to see whether other terpenes, which are the major constituents contained in *A. costus* extract, namely costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin, could also have inhibitor properties against MDM2.

To circumvent the time and cost constraints associated with conventional experimental approaches (BinShabaib et al., 2022), this research was conducted *in silico*. *In silico* predictions are used to assess pharmacokinetic properties and toxicity, providing insights into the drug candidate's absorption, distribution, metabolism, excretion, and toxicity profiles (Priatna et al., 2022). Researchers predict that the *in silico* analysis of *A. costus* terpenoids, specifically lappadilactone, will reveal its strong binding affinity to the MDM2 protein, thereby inhibiting the p53-MDM2 interaction. This interaction inhibition is expected to restore the tumor suppressor function of p53. Additionally, researchers anticipate that the pharmacokinetic and toxicity predictions for lappadilactone will demonstrate favorable properties, including good absorption, distribution, metabolism, excretion, and minimal toxicity. Consequently, lappadilactone may represent a promising candidate for the development of efficient and safe anticancer drugs.

MATERIAL AND METHODS

Tools

Molecular docking simulations and visualization of protein-ligand interactions were conducted using Autodock 4.2.6, Autodock Tools 1.5.6, Discovery Studio Visualizer 2019, and PyMol. The SwissADME website was utilized to assess the drug-likeness of compounds. The prediction of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties was performed using the pkCSM website, accessible at https://biosig.lab.uq.edu.au/pkcsm/.

Ligand and target protein

The ligands used in this study were terpenes compounds from *A. costus*, including costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin (Amara et al., 2017). Temozolomide was utilized as

the control drug (Biswas et al., 2023). The target protein selected for this study was MDM2.

The 3D structure of the ligands was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the SDF format (Arundina et al., 2023; Nugraha et al., 2023), while the target protein structure was retrieved from the Protein Data Bank (PDB) website (www.rcsb.org/) in PDB format (Priatna et al., 2022). Drug likeness analysis of the ligands extracted from *A. costus* was performed according to Lipinski's rules, utilizing the SwissADME website as a reference (Terefe and Ghosh, 2022).

Protein and ligan preparation

Protein structures, specifically MDM2, were prepared by the removal of water, solvent molecules, and native ligands. This process was executed using Discovery Studio Visualizer 2019, and the resultant structures were saved in PDB format. Subsequent preparation steps were carried out using Autodock Tools 1.5.6. These steps included the addition of hydrogen atoms and the assignment of Gasteiger charges. The prepared target protein was saved in pdbqt format. Ligand preparation involved several steps in enhancing Gasteiger charges, combining non-polar hydrogen atoms, and adjusting torsion angles. This process was conducted using Autodock 1.5.6. The prepared ligands were saved in pdbqt format (El Aissouq et al., 2021; Khoswanto and Siswandono, 2022).

Molecular docking simulation

Validation of the docking method is carried out by docking the native ligand that has been separated from the receptor back to the receptor using Autodock Tools 1.5.6. The docking method is said to be valid if the RMSD value is <2Å so that the docking method can be applied to proposed ligands (Pratama et al., 2021).

Ligand binding activity to specific target protein domains was determined through molecular docking simulations. Molecular docking experiments were conducted by employing Autodock 4.2.6 and Autodock Tools 1.5.6. A grid with dimensions of 40 × 40 × 40 was established, with grid center coordinates set at X=-23.945, Y=8.091, and Z=-13.667. The default distance of 0.375 Å was used in the docking calculations. Docking simulations were configured to use the Lamarckian Genetic Algorithm, employing a population size of 150 and a maximum number of evaluations set to 2,500,000. Analysis of the docking outcomes was conducted, focusing on key docking parameters such as binding energy values and inhibition constants. The binding energy generated by the ligand when binding to the target protein site plays a pivotal role in eliciting specific biological responses. A more negative binding score indicates a greater impact on the activity of the target protein (Priatna et al., 2022).

Molecular visualization

Protein-ligand interactions were visualized as hydrophobic, hydrogen, pi (π), and Van der Waals bonds formed within molecular complexes were identified in two dimensions (2D), Discovery Studio Visualizer 2019 was utilized. PyMol software was employed for three-dimensional (3D) visualization of molecular docking results. The three-dimensional (3D) structure of the ligand-protein molecule complex was illustrated in both figure and surface forms (Priatna et al., 2022).

ADMET prediction

ADMET predictions were made using the SMILES codes corresponding to each test ligand obtained from the PubChem database. The retrieved SMILES codes were then input into the web-based software known as pKCSM to perform ADMET predictions for each ligand. The primary objective of this testing was to predict key initial pharmacokinetic parameters, including absorption, distribution, and toxicity. These encompassed assessments of carcinogenic and mutagenic properties. Ligands that conformed to Lipinski's five rules exhibited favorable pharmacokinetic properties, and demonstrated non-toxic characteristics were considered more promising as potential drug candidates (Pires et al., 2015; Purwanto et al., 2021).

***In silico* data analysis**

In silico data analysis was carried out based on the value of the Lipinski rule, molecular docking results, and ADMET prediction. Phytochemical properties are measured according to Lipinski's rule where the chemical structure of compounds that have potential as drugs should not violate more than one of the following rules: molar mass <500 Da, logP <5, number of H-bond acceptors ≤10, number of H-bond donors ≤5, molar refractory between 40-130 (Ivanovic et al., 2020).

Analysis of data resulting from redocking of the native ligand against the receptor expressed in RMSD values where the docking method can be used if it has an RMSD value <2Å (Purwanto et al., 2021). After receptor validation achieves appropriate results, docking analysis of the test ligand is carried out based on docking parameters, including binding free energy values, where the lower the binding free energy value, the more stable the ligand-receptor bond is, so it is predicted to have higher biological activity. The bind-

ing free energy value is compared with that of the control ligand to determine whether the test ligand has activity against the receptor (temozolomide) (Biswas et al., 2023). From the ligand-receptor interaction at the binding site in the receptor, the interactions of the amino acid residues involved and the type of interaction of each test ligand and control ligand against RgpB were observed (PDB ID: 1CVR). The interacting amino acids are compared between the test ligand and the control ligand (Rizvi et al., 2013).

In ADMET analysis, 13 important ADMET properties were calculated. The intestinal absorption (IA) parameter predicts the percentage of drug absorbed through the human intestine. Absorbance less than 30% is considered bad (Flores-Holguín et al., 2021). Skin permeability indicates whether the drug can be administered through the skin or not. A compound is predicted to have low skin permeability with a log Kp value >-2.5 (Purwanto et al., 2021). The total dose of a drug requires a uniform volume in the blood plasma, which is called VDS. Compounds will be more distributed in tissues than in blood plasma for higher VDS. When the log VDSs value is <-0.15, the distribution volume is considered relatively low. Conversely, when the log VDSs value is >0.45, the volume of distribution is considered relatively high. For the permeability of the blood-brain barrier membrane, compounds with logBB >0.3 can pass through the blood-brain barrier easily, while compounds with logBB <-1 indicate that these compounds do not easily cross the blood-brain barrier (Han et al., 2019). Substrate microsomal enzymes that catalyze reactions involved in the metabolic activities of the drug were used to assess the metabolic activities of the selected compounds. Renal OCT2 substrates indicate that compounds can cause toxic effects when consumed together with renal OCT2 inhibitors (Anil et al., 2013). A positive result of Ames toxicity means that the compound is mutagenic and can act as a carcinogen (Honma, 2020).

RESULTS

Protein and ligand structure

Ligand samples were collected, consisting of costunolide (ID: 5281437), dehydrocostus lactone (ID: 73174), lappadilactone (ID: 11081540), cynaropicrin (ID: 119093), and temozolomide (ID: 5394). Information regarding their unique identifiers (ID), molecular formulas, canonical SMILES representations, and classification as potential drug candidates based on Lipinski's predictions was compiled and presented in Table 1.

The MDM2 protein utilized was sourced from the RCSB PDB. Information pertaining to the protein's identifier (ID), visualization method, resolution, number of atoms, molecular weight, chain designation, and sequence length was gathered and organized, as detailed in Table 2.

Molecular docking result

Validation of the docking method is carried out by redocking the native ligand back to its receptor using Autodock Tools 1.5.6. The RMSD value is <2Å, namely 0.71 Å, so the docking method can be used.

Four *A. costus* ligands showed several interactions with MDM2. Lappadilactone exhibited the highest binding energy of -9.13 Kcal/mol, signifying a strong binding affinity to the MDM2 protein. In contrast, temozolomide had a lower binding energy of -4.4 Kcal/mol, suggesting weaker binding.

Several ligands, including temozolomide, costunolide, and cynaropicrin, formed hydrogen bonds with the MDM2 protein. Notably, cynaropicrin formed only one hydrogen bond, indicating a relatively weaker interaction compared to the other ligands.

Lappadilactone, dehydrocostus lactone, and cynaropicrin exhibited substantial hydrophobic and Van der Waals interactions, with multiple amino acids involved. These interactions contribute to the stability of the ligand-protein complexes (Table 3). Overall, lappadilactone outperformed both control ligands in terms of binding energy, indicating its potential as a strong candidate for binding to the MDM2 protein.

Molecular interactions revealed that the ligands shared several interactions with the same amino acids as temozolomide and nutlin-3a (native ligands). This suggests the potential for overlap in the binding site, as depicted in Fig. 1. Detailed molecular interactions between the ligands and the MDM2 are visually represented in Fig. 2.

ADMET prediction

All tested ligands demonstrated significant intestinal absorption, with lappadilactone showing the highest absorption rate at 99.225%. Skin permeability, as indicated by log Kp values, varied among the compounds, with lappadilactone having the lowest permeability (-4.03) and costunolide the highest (-2.423). The volume of distribution (VDss) values indicated varying degrees of distribution within the human body. Notably, lappadilactone exhibited a unique negative VDss (-0.052), suggesting a different distribution pattern. Blood-brain barrier (BBB) permeability, measured by log BB values, varied among ligands (Table 4).

Table 1. The ligand structure of *Aucklandia costus* Falc.

Ligand	Ligand ID	Formula	MW (Dalton)	LogP	HBD	HBA	MR
Temozolimide	5394	$C_6H_6N_6O_2$	194.15	-0.92	1	5	44.40
Costunolide	5281437	$C_{15}H_{20}O_2$	232.32	2.97	0	2	69.85
Dehydrocostus lactone	73174	$C_{15}H_{18}O_2$	230.30	2.98	0	2	67.74
Lappadilactone	11081540	$C_{30}H_{38}O_6$	494.62	4.14	1	6	135.67
Cynaropicrin	119093	$C_{19}H_{22}O_6$	346.37	1.54	2	6	90.10

MW: molecular weight; HBD: number of hydrogen bond donor; HBA: number of hydrogen bond acceptor; MR: molecular replacement.

Table 2. The protein structure of MDM2.

Protein	PDB ID	Visualization	Resolution (A)	Atom (n)	MW (kDa)	Chain	Sequences length (MER)
MDM2	4HG7	X-RAY	1.60	932	11.76	A	97

MW: molecular weight.

Table 3. The molecular interaction of *Aucklandia costus Falc.* ligand and MDM2.

Ligands	Binding energy (Kcal/mol)	Ki (uM)	Type of bonds		
			Hydrogen	Hydrophobic	Van der walls
Temozolomide	-4.4	591.38	Leu54, Val93 (2)	Leu54, Leu57, Ile61, Phe86, Phe91, Ile99 (7)	Gly58, Leu82, Lys94, His96
Costunolide	-6.81	10.27	Lys51 (1)	Phe55, Leu54, His96 (5)	Ile19, Tyr100
Dehydrocostus lactone	-6.49	17.41	-	Leu54, Ile61, Val75, Phe91, Val93, Ile99 (9)	Phe55, Leu57, Gly58, Phe86
Lappadilactone	-9.13	203.60	-	Lys51, Phe55, Ile61, Val75, Val93 (7)	Leu54, Leu57, Gly58, Gln59, Met62, Tyr67, Phe91, Ile99, Ile103
Cynaropicrin	-7.10	6.24	Leu54 (1)	Leu54, His96, Ile99, Tyr100 (7)	Ile19, Gln24, Leu57, Gly58, Ile61, Phe86, Phe91, Val93

Ki: inhibition constant

Dehydrocostus lactone, lappadilactone, and cynaropicrin were substrates for CYP3A4, potentially influencing their metabolism. None of the compounds were substrates for CYP2D6, and none inhibited CYP2D6. Total clearance values varied significantly among the ligands, with costunolide having the highest clearance. Dehydrocostus lactone and lappadilactone were identified as substrates for renal OCT2. AMES toxicity was not observed for costunolide, dehydrocostus lactone, and lappadilactone, but cynaropicrin showed a positive result. None of the ligands exhibited hepatotoxicity (Table 4).

DISCUSSION

Cancer is a global leading cause of death, accounting for nearly 10 million fatalities in 2020 (Biswas et al., 2023). The protein known as p53, often referred to as the "Guardian of the Genome," holds significant importance as a tumor suppressor. However, cancer can develop when p53 malfunctions, undergoes alterations or mutations, or becomes inactivated. The inhibition of p53 frequently occurs due to the action of another protein called MDM2, which negatively regulates p53. MDM2's overexpression impairs the function of p53 by obstructing its transcriptional activity and causing the degradation of p53 protein. This process leads to the inactivation of p53 and contributes to the development of cancer. Consequently, targeting the interaction between p53 and MDM2, as well as inhibiting MDM2 itself, has emerged as a critical focal point in cancer therapy research to develop more precise and effective cancer treatments (Muhsen and Li, 2020; Nag et al., 2013; Zhu et al., 2022).

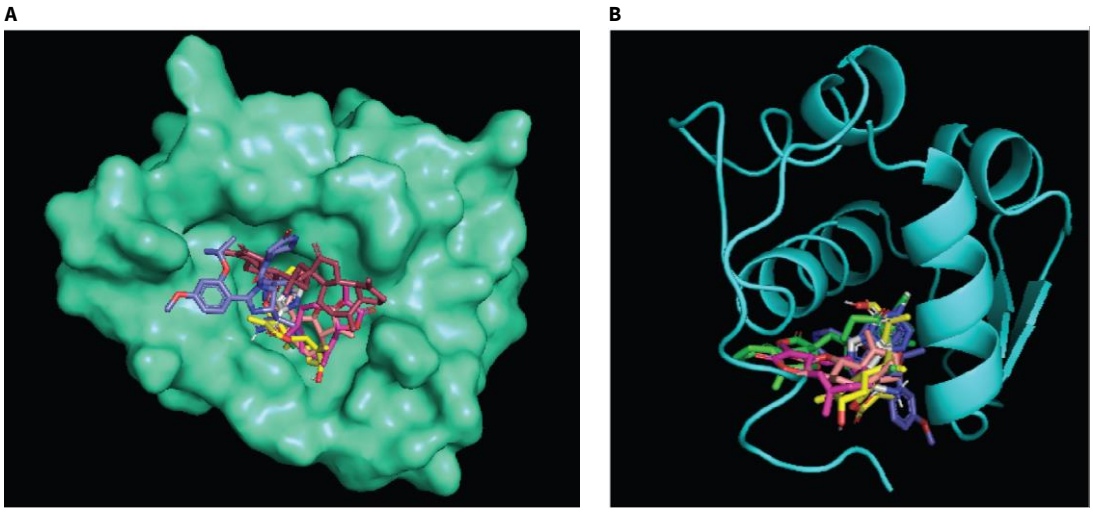

Figure 1. Bond poses of ligands and MDM2 target proteins and test compounds.
(A) Surface form and **(B)** Cartoon form.

Table 4. The ADMET analysis.

ADMET	Parameter	Compound			
		Costunolide	Dehydrocostus lactone	Lappadilactone	Cynaropicrin
Absorption	Intestinal absorption (%)	97.18	98.917	99.225	88.8
	Skin permeability (log Kp)	-2.423	-2.474	-4.03	-3.955
Distribution	VDss (human) (log L/kg)	0.31	0.347	-0.052	-0.249
	BBB permeability (log BB)	0.512	0.566	-0.309	-0.446
Metabolism	CYP3A4 substrate (Yes/No)	No	Yes	Yes	Yes
	CYP2D6 substrate (Yes/No)	No	No	No	No
	CYP2D6 inhibitor (Yes/No)	No	No	No	No
	CYP2C19 inhibitor (Yes/No)	No	Yes	No	No
	CYP2C9 inhibitor (Yes/No)	No	No	No	No
Excretion	Total Clearance (log ml/min/kg)	1.334	0.687	0.223	0.545
	Renal OCT2 substrate (Yes/No)	No	Yes	Yes	No
Toxicity	AMES toxicity (Yes/No)	No	No	No	Yes
	Hepatotoxicity	No	No	No	No

VDss: volume distribution; BBB: blood-brain barrier; CYP3A4: cytochrome enzyme 3A4; CYP2D6: cytochrome enzyme 2D6; CYP2C19: cytochrome enzyme 2C19; CYP2C9: cytochrome enzyme 2C9; OCT2: organic transporter 2.

Costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin are terpene compounds found in *A. costus,* serving as their primary chemical constituents. These compounds have demonstrated the potential to be developed into bioactive molecules (Hassan and Masoodi, 2020). Prior research has shown that various terpene compounds, including those from this group, can act as antagonists of the p53-MDM2 interaction. This antagonistic action results in the reactivation of p53's activity, ultimately safeguarding cells against carcinogenesis. Notably, costunolide has been identified for its ability to inhibit cell growth and induce apoptosis through the MDM2/P53 pathway, particularly in colon cancer, by targeting protein kinase B (AKT) (Huang et al., 2021). *In vitro* experiments show that costunolide and dehydrocostus lactone exhibit highly selective and marked cytotoxicity against breast cancer, leukemia, and so on (Li et al., 2020). Similarly, lappadilactone has exhibited robust cytotoxicity among *A. costus* fractions against various human cancer cell lines, including HepG2, OVCAR-3, and HeLa cells (Ansari et al., 2021). Cynaropicrin has been reported to inhibit the invasion, migration, and metastasis of leukemia can-

cer cells (Dhyani et al., 2022). Consequently, costunolide, lappadilactone, dehydrocostus lactone, and cynaropicrin represent promising candidates for cancer therapy due to their potential inhibitory effects on cancer development and progression.

The primary objective of molecular docking is to identify the most favorable arrangement between a ligand and target protein that yields the lowest binding free energy value (López-Camacho et al., 2016).

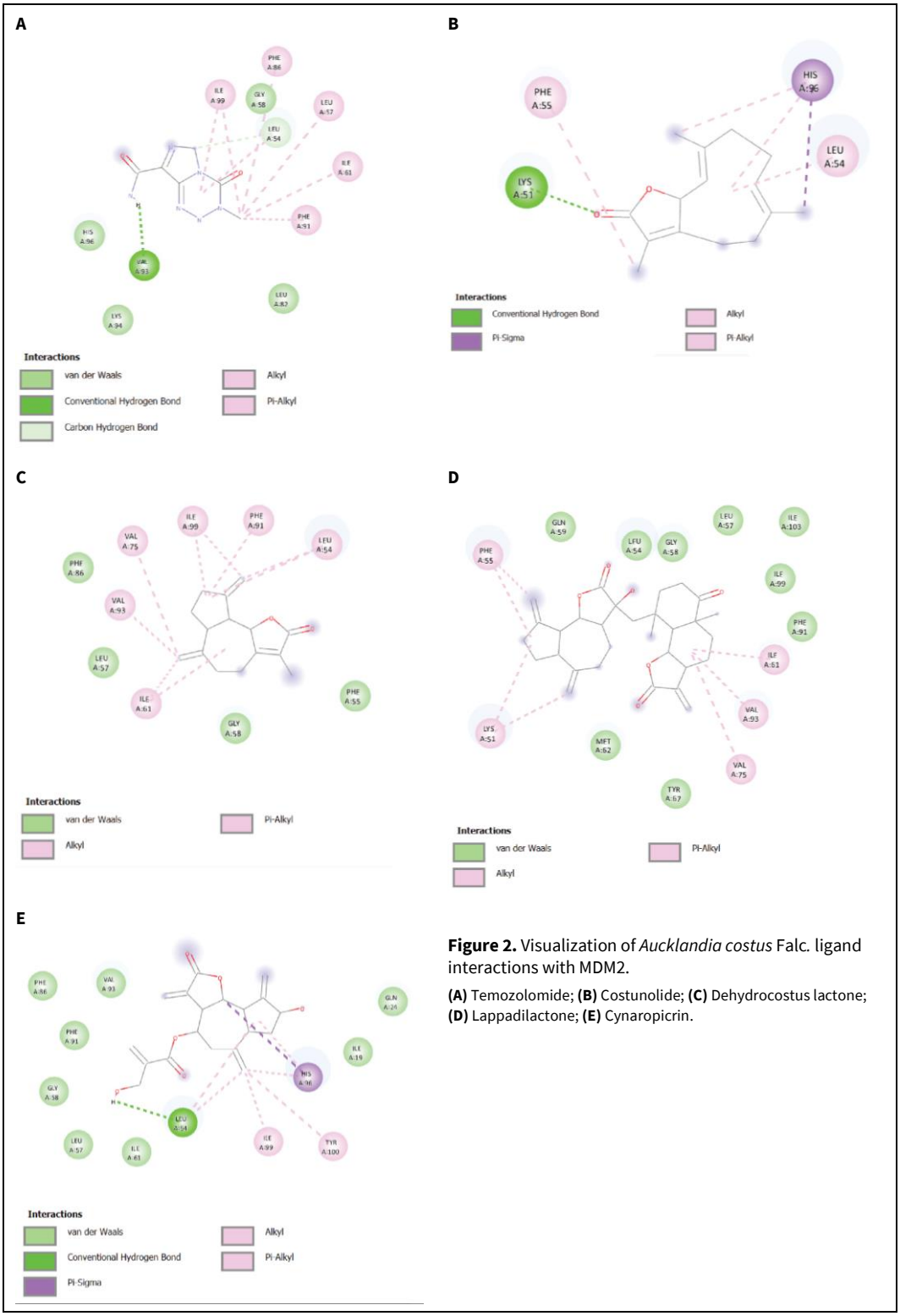

Figure 2. Visualization of *Aucklandia costus* Falc. ligand interactions with MDM2.

(A) Temozolomide; **(B)** Costunolide; **(C)** Dehydrocostus lactone; **(D)** Lappadilactone; **(E)** Cynaropicrin.

A higher degree of stability between the ligand and the target protein (receptor), signifying a stronger interaction (Mangande et al., 2020). Lappadilactone demonstrated the best binding energy, surpassing both the native and control ligands. This signifies that lappadilactone can establish a more robust and stable connection with the active site of the MDM2 protein compared to the native and control ligands. Other test compounds like costunolide, dehydrocostus lactone, and cynaropicrin also exhibited favorable binding energy values. Although these values were less negative than those of the native ligand, they were still more negative than the control ligand.

To function as an inhibitor, a compound or ligand must form a direct bond at the active site of the protein to deactivate it (Aja et al., 2021). This research targeted the MDM2 protein in the p53 site. The interactions were observed with fifteen amino acid residues that constitute structural components associated with the P53 site. These residues include Leu54, Leu57, Gly58, Ile61, Met62, Tyr67, Gln72, His73, Val75, Phe86, Phe91, Val93, His96, Ile99, and Tyr100. The docking results for temozolomide reveal interactions with residues such as Leu54, Leu57, Gly58, Ile61, Leu82, Phe86, Phe91, Val93, Lys94, His96, and Ile99. Conversely, lappadilactone, which exhibited the best binding energy, displayed interactions with residues including Lys51, Leu54, Phe55, Leu57, Gly58, Gln59, Ile61, Met62, Tyr67, Val75, Phe91, Val93, Ile99, and Ile103. These results indicate that lappadilactone can bind to a greater number of structural sites associated with the P53 site compared to temozolomide. Other test ligands, such as costunolide, dehydrocostus lactone, and cynaropicrin, also formed bonds with multiple structural sites linked to the p53 site, but lappadilactone demonstrated a stronger association with amino acids crucial for targeting MDM2. In summary, considering their similar binding energies, inhibition constants, and intermolecular interactions with MDM2's amino acid residues, costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin emerge as potent inhibitors.

The assessment of pharmacokinetic properties is crucial in evaluating the potential of ligands for therapeutic use. The comprehensive analysis of the pharmacokinetic profiles of the ligands of *A. costus* sheds light on their absorption, distribution, metabolism, and excretion characteristics. All tested ligands demonstrated noteworthy intestinal absorption, with lappadilactone exhibiting the highest absorption rate at an impressive 99.225%. This indicates that lappadilactone has a high likelihood of being effectively absorbed through the human intestine, a favorable characteristic for an orally administered drug. On the other hand, skin permeability, lappadilactone had the lowest permeability, suggesting that it may not readily penetrate the skin. In contrast, costunolide exhibited the highest skin permeability, implying its potential suitability for transdermal drug delivery. The volume of distribution (VDss) values offered insights into the distribution patterns of the ligands within the human body. It is worth noting that lappadilactone exhibited a negative VDss value, suggesting a distinctive distribution pattern compared to the other ligands. These variations in VDss values highlight the diversity in how each ligand may be distributed within different body tissues.

The blood-brain barrier (BBB) permeability provides essential information about a ligand's ability to pass through the BBB, which is crucial for drugs targeting the central nervous system (Han et al., 2019). The differences in log BB values among the ligands indicate variations in their potential to access the brain. In the metabolic and clearance aspect, dehydrocostus lactone, lappadilactone, and cynaropicrin were identified as substrates for CYP3A4, a key enzyme involved in drug metabolism. This suggests that these compounds may undergo metabolism mediated by CYP3A4 in the liver. None of the tested compounds were substrates for CYP2D6, nor did they inhibit CYP2D6 and CYP2C9, indicating a lack of interference with this particular metabolic pathway. For CYP2C19, only dehydrocostus lactone inhibit the enzyme, whereas the other compounds were not. On the other hand, the total clearance values exhibited significant variability among the ligands, with costunolide having the highest clearance rate. Total clearance reflects the rate at which a drug is eliminated from the body, and variations in clearance rates can influence a drug's duration of action and therapeutic effectiveness.

The important parameter for drug development is toxicity (Honma, 2020). None of the ligands displayed AMES toxicity, indicating a lack of mutagenic potential in bacterial cells. However, cynaropicrin yielded a positive result in the AMES toxicity test, suggesting potential mutagenic properties. Importantly, none of the ligands exhibited hepatotoxicity, indicating a favorable safety profile in terms of liver toxicity.

In conclusion, the comprehensive pharmacokinetic evaluation of the tested ligands provides valuable insights into their potential as drug candidates. Lappadilactone emerges as a promising candidate with high intestinal absorption, distinctive distribution characteristics, and a lack of mutagenic or hepatotoxic effects. However, further studies and *in vivo* assessments are warranted to validate these findings and ascertain the suitability of these ligands for therapeutic applications.

In summary, these findings showed valuable insights into the potential of *A. costus* ligands for cancer therapy as a basis for guiding future research efforts. Further research, including experimental validation and further preclinical and clinical investigations, are needed to confirm this condition.

CONCLUSION

Aucklandia costus terpenes, namely costunolide, dehydrocostus lactone, lappadilactone, and cynaropicrin emerge as potent inhibitors of MDM2. Therefore, they can be a focal point in future cancer therapy research. The comprehensive pharmacokinetic evaluation of the tested ligands provides valuable insights into their potential as drug candidates. Lappadilactone emerges as the most promising candidate for MDM2 inhibitors with high intestinal absorption, distinctive distribution characteristics, and a lack of mutagenic or hepatotoxic effects. However, further studies and *in vivo* assessments are warranted to validate these findings and ascertain the suitability of these ligands for therapeutic applications.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Budhy TI	Arundina I	Irmawati A	Sidarningsih	Surboyo MDC	Raharjo CO	Akbar CS	Rahmalia MQ
Concepts or ideas	x	x	x	x				
Design	x	x	x	x				
Definition of intellectual content	x	x	x	x				
Literature search						x	x	x
Experimental studies						x	x	x
Data acquisition	x	x	x	x		x	x	x
Data analysis					x			
Statistical analysis					x			
Manuscript preparation	x		x	x		x	x	x
Manuscript editing					x			
Manuscript review	x	x	x	x	x	x	x	x

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