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ANTICANCER ACTIVITIES OF BIOACTIVE COMPOUNDS FROM *Paracaudina australis*: AN IN-SILICO STUDY TARGETING AKT1 AND NOS2

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Abstract

Sea cucumber (*Paracaudina australis*) isolates have shown potential as agents with various bioactivities. Bioactivity refers to the ability of a substance to interact with biological tissues and elicit positive physiological responses. This study aimed to analyze the interaction between the target proteins RAC-alpha serine/threonine-protein kinase (AKT1) and Nitric Oxide Synthase 2 (NOS2) with compounds identified in *P. australis*. The methods employed included ligand preparation using the Open Babel 2.3.1 plug-in, bioactivity prediction via the PASS Online web server, ADMET analysis using Canonical SMILES, pathway prediction using the DAVID (Database for Annotation, Visualization, and Integrated Discovery) web server, prediction of active or functional sites in proteins using BIOVIA Discovery Studio software, molecular docking using AutoDock Vina integrated in PyRx 0.8, visualization with BIOVIA Discovery Studio 2019, and molecular dynamics simulation using the WebGro web server. The results revealed that the *P. australis* sea cucumber isolate contained 38 compounds predicted to exhibit bioactivities related to the target proteins, including anticancer, anti-proliferative, pro-apoptotic, anti-inflammatory, anti-type 2 diabetes, and anti-obesity effects. Based on five ADMET parameters, all compounds satisfied several pharmacokinetic and toxicity criteria. Protein interaction analysis indicated that AKT1 and NOS2 interacted with other proteins, such as MTCP1, TCL1A, and NOX1. Molecular docking results showed that the compounds Ergosta-7,22-dien-3-ol, (3β,5α,22E); Ergostan-3-ol; Ergost-7-en-3-ol, (3β,5α); and Stigmasta-7,16-dien-3-ol, (3β,5α) exhibited the most stable binding to AKT1. Additionally, the compounds Ergosta-7,22-dien-3-ol, (3β,5α,22E) and Ergosta-7,22-dien-3-ol, (3β,22E) demonstrated lower binding affinity values than the control NOS2 inhibitor, indicating strong potential as NOS2 inhibitors.

Keywords: bioactivity, docking, ligands, pharmaceuticals, sea cucumber

Aktivitas Antikanker Senyawa Bioaktif dari *Paracaudina australis*: Studi In Silico yang Menargetkan AKT1 dan NOS2

Abstract

Isolat teripang (*Paracaudina australis*) menunjukkan potensi sebagai agen dengan berbagai bioaktivitas. Bioaktivitas mengacu pada kemampuan suatu zat untuk berinteraksi dengan jaringan biologis dan menghasilkan respons fisiologis positif. Tujuan penelitian ini adalah menganalisis interaksi antara protein target RAC-alfa serin/treonin-protein kinase (AKT1) dan Nitric Oxide Synthase 2 (NOS2) dengan senyawa yang diidentifikasi dalam P. australis. Metode yang digunakan meliputi preparasi ligan menggunakan *plug-in* Open Babel 2.3.1; prediksi bioaktivitas melalui *server web* PASS Online; analisis ADMET menggunakan Canonical SMILES; prediksi jalur menggunakan *server web* DAVID (database for annotation, visualization,

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and integrated discovery); prediksi situs aktif atau fungsional dalam protein menggunakan perangkat lunak BIOVIA Discovery Studio; *docking* molekuler menggunakan AutoDock Vina yang terintegrasi dalam PyRx 0.8; visualisasi dengan BIOVIA Discovery Studio 2019; dan simulasi dinamika molekuler menggunakan *server web* WebGro. Hasil penelitian menunjukkan bahwa isolat teripang P. australis mengandung 38 senyawa yang diprediksi menunjukkan bioaktivitas yang berkaitan dengan protein target, termasuk efek antikanker, antiproliferatif, proapoptotik, antiinflamasi, antidiabetes tipe 2, dan antiobesitas. Berdasarkan lima parameter ADMET, semua senyawa memenuhi beberapa kriteria farmakokinetik dan toksisitas. Analisis interaksi protein menunjukkan bahwa AKT1 dan NOS2 berinteraksi dengan protein lain seperti MTCP1, TCL1A, dan NOX1. Hasil *docking* molekuler menunjukkan bahwa senyawa Ergosta-7,22-dien-3-ol, (3β,5α,22E); Ergostan-3-ol; Ergost-7-en-3-ol, (3β,5α); dan Stigmasta-7,16-dien-3-ol, (3β,5α) menunjukkan ikatan paling stabil dengan AKT1. Selain itu, senyawa Ergosta-7,22-dien-3-ol, (3β,5α,22E) dan Ergosta-7,22-dien-3-ol, (3β,22E) menunjukkan nilai afinitas pengikatan yang lebih rendah dibandingkan dengan inhibitor NOS2 kontrol, yang menunjukkan potensi yang kuat sebagai inhibitor NOS2.

Kata kunci: bioaktivitas, *docking*, farmaseutika, ligan, teripang

INTRODUCTION

Sea cucumbers are an aquatic resource that has not been fully utilized. Because sea cucumbers possess promising bioactive components, the pharmaceutical and fisheries product processing industries may employ them as an alternative source of raw materials for medications. Sea cucumbers contain compounds that have been linked to biological activities such as antioxidant (Wargasetia *et al*., 2021; Hanifaturahmah *et al*., 2024; Sukmiwati *et al.*, 2024), anti-inflammatory (Carletti *et al*., 2022), anti-diabetic (Wang *et al*., 2018), antimicrobial (Sukmiwati *et al*., 2018), pro-apoptotic (Liu *et al.*, 2023), anti-obesity (Seo *et al*., 2024), anticancer (Sukmiwati *et al*., 2024), and others.

Sea cucumbers are marine invertebrates with great potential for use as an anti-breast cancer agent. Previous studies have suggested that sea cucumber isolates (*P. australis*) may inhibit the growth of T47D breast cancer cells. Previous studies have suggested that isolates of *P. australis* contain several bioactive compounds, such as steroids, phenols, triterpenoids, and saponins, and may inhibit the growth of T47D breast cancer cells (Sukmiwati *et al*., 2024). These bioactive compounds exhibit promising pharmacological activities and therefore hold significant potential for development as standalone natural drug candidates or as key components in the formulation of marine-derived pharmaceutical products. Their unique chemical structures and mechanisms of action, which are often distinct from those of terrestrial sources, further highlight their value in the ongoing search for novel therapeutic agents.

In silico testing refers to experiments conducted using computer simulations, typically to explore the interaction between a substance and a target molecule, such as a receptor. This approach allows researchers to use computational techniques to analyse and predict the phytochemical content of substances (Setiawan & Istyastono, 2015). This study employed a multi-method in silico computational approach, integrating bioactivity prediction, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling, molecular docking, and molecular dynamics simulation, to comprehensively evaluate the therapeutic potential and interaction stability of selected compounds with target proteins.

AKT1 has emerged as a promising anticancer target for breast cancer treatment because of its significant role in regulating cellular behaviors associated with malignancy. Although AKT1 activation may contribute to enhanced cell proliferation, studies have shown that its inhibition can effectively suppress key processes involved in cancer progression, particularly cell invasion and motility. This suggests that targeting AKT1 could be beneficial in limiting metastatic potential, even though it may have complex effects on tumor growth. Therefore, therapeutic strategies aimed at selectively modulating AKT1 activity hold considerable potential for breast cancer management, especially in combating tumor spread and invasion (Chandran *et al*., 2024).

Akt/Protein Kinase B acts as an emerging breast cancer inhibitor. Among the three isoforms of the Akt family, Akt1 is an excellent anticancer target against breast cancer cells by regulating apoptosis and proliferation. The activation of the Akt1 may lead to cell proliferation, but cell invasion and motility are suppressed by Akt1 inhibition (Li *et al*., 2018). AKT is a serine-threonine kinase involved in various downstream signaling pathways that control mechanisms such as cell metabolism, division, differentiation, proliferation, and apoptosis (Chandran *et al*., 2024). Each of these is associated with many cancers, including ovarian, breast, colon, stomach, hormone-insensitive breast, and prostate cancers.

One protein that contributes to the development of breast cancer cells is AKT1 (Wargasetia *et al.,* 2021).AKT1 protein may be inhibited by compounds found in sea cucumber isolates. AKT1 is overexpressed and overactivated in many types of breast cancer cells and is involved in cell growth, metabolism and proliferation. To prevent the action of this protein, various inhibitory compounds have been developed (Yang *et al.,* 2015). According to Song *et al.* (2019), Inhibiting AKT1 can increase the activity of antiproliferative proteins, such as FOXO1 and GSK3β, while decreasing the activity of proteins that are crucial for cell proliferation, such as mTOR, IKK, and Cyclin D1.

The study of the extensive physiological effects of nitric oxide (NO) has given rise to a substantial biomedical field over the last few decades. Its influence extends across numerous physiological and pathological processes, including chronic inflammatory diseases, cancer, cardiovascular diseases, and neurodegeneration (Coutinho *et al*., 2024). There are three types of NOSs in mammalian systems. Ca2+ binding to calmodulin regulates the generation of relatively small amounts of NO (nM levels) by two of them, which are constitutively produced in neurons (nNOS, encoded by the NOS1 gene) and epithelial cells (eNOS, encoded by the NOS3 gene) (Qin *et al.,* 2021). Third, pro-inflammatory cytokines trigger the expression of inducible NOS (iNOS), which is encoded by the NOS2 gene and is expressed in inflammatory tissues (Wang *et al.,* 2018). In ER-breast cancer, tumor NOS2 co-expression has recently demonstrated remarkable predictive power, with higher expression associated with a lower survival rate. NO plays a crucial role in two stages of carcinogenesis: tumor suppression and metastatic growth. Numerous cancer types express NOS2, which produces more NO (Hays *et al.,* 2019); (Baghban *et al.,* 2020); (Watkins *et al.,* 2019).

Numerous studies have explored the chemical constituents of sea cucumbers and their potential medicinal properties. However, research specifically focusing on the bioactive compounds from *Paracaudina australis* as anti-inflammatory, anticancer, anti-proliferative, pro-apoptotic, anti-diabetic (type 2), and anti-obesity agents remains limited. This study employed a bioinformatics approach to demonstrate the potential of *P. australis* isolates in inhibiting the activity of key target proteins—AKT1 and NOS2—which are involved in various pathological processes, including cancer progression, cell proliferation, inflammation, type 2 diabetes mellitus, and obesity. The primary objective of this study was to analyze the interaction between the target proteins RAC-alpha serine/threonine-protein kinase (AKT1) and Nitric Oxide Synthase 2 (NOS2) with the compounds identified in *P. australis*.

MATERIALS AND METHODS

Ligand and Ligand Control Preparation

The CADD Group Chemoinformatics Tools and User Services (CACTUS) website (https://cactus.nci.nih.gov/translate) was also used to obtain Canonical SMILES from the ligand chemicals in ChemSpider, which were downloaded in. mol file format (Wicaksono *et al.,* 2021). The ligand structure is flexible in its ideal conformation (Onyango *et al.,* 2022). The compounds were then saved. thepdbpdb format, following repair.

Compounds that can activate or inhibit proteins are known as control compounds. The control compound was prepared in the same manner as the active molecule and was derived from the initial ligand of the target

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protein. The natural ligands of AKT1 and NOS2 are sources of inhibitory chemicals.

Bioactivity Prediction

The PASS Online webserver (www.way2drug.com/passonline) was used to predict the bioactivity. Bioactivity is classified into several areas, including anti-inflammatory, anti-cancer, anti-proliferation, pro-apoptosis, anti-diabetes mellitus type 2 (anti-DM type 2), and anti-obesity. Pa is the value used in the table. If a compound's Pa value is greater than Pi and Pa > 0.3, it is considered to have a particular bioactivity potential (Aini *et al.,* 2023).

ADMET Test

The pharmacokinetic characteristics and toxicity of a ligand compound as a potential medication were assessed using the ADMET test (Rudrapal *et al.,* 2022). ADMET factors, which typically comprise absorption, distribution, metabolism, excretion, and toxicity, are crucial for evaluating the safety and toxicity of drugs (Pradeepkiran *et al.,* 2021; Christina *et al.,* 2021).

Pathway Prediction

Cytoscape 3.7.2 software was used to investigate the proteins that interact with the three target proteins using the STRING database (https://string-db.org/). The proteins identified in the protein-protein interaction analysis were entered into the DAVID (Database for Annotation, Visualization and Integrated Discovery) web server (https://david.ncifcrf.gov/) to perform pathway predictions.

Protein Preparation, Target Protein Modeling Analysis, Active or Functional Site Prediction

The RCSB PDB database (https://www.rcsb.org/) provided the 3D structures of the AKT1 and NOS2 proteins along with their corresponding IDs. Using Biovia Discovery Studio 2019 software, the protein was produced by eliminating contaminating compounds (Widyananda *et al.,* 2021).

Molecular Docking and Chemical Interaction

The PyRx 0.8 software's AutoDock Vina was used for molecular docking. The docking findings with the lowest binding affinity values for each mode were selected. Only ligand interactions in the active site region of the protein were predicted by the docking that was performed. The stability of the association between the ligand and protein is indicated by the binding affinity value. The protein-ligand association is more stable when the binding affinity value is lower (Tripathy *et al.,* 2019).

Structural Vizualitation and Molecular Dynamics

Molecular dynamics simulations were performed using the WebGro web server (https://simlab.uams.edu/index.php). The Swiss PDB Viewer (SPDBV) program was used to clean and add conformers to the protein-ligand complex from molecular docking. Pre-processing or input parameters for MD simulations of proteins in water using 0.15M neutral NaCl, SPC water model, triclinic box type, and GROMACS96 43a1 forcefield. Furthermore, the Steepest Descent integrator was used 5,000 times to minimize the parameters. A common type of equilibrium parameter, NVT/NPT, was simulated at 310 K, 1 bar of pressure, and within 20 ns. The Leap integrator was used for the simulation parameters, and an average of 1,000 simulation frames were used (Grahadi *et al.,* 2023).

RESULT AND DISCUSSION

Compounds Structure from *P. australis* Isolates

Table 1 lists the compounds present in the extract, including the compound name, CID, and the source website from which the compound was sourced. The three-dimensional structures of the compounds are shown using atom coloring and in stick form. The colors of the C, O, and H atoms are green, red, and white, respectively. Certain compounds were not included in the database because the database could not identify them. In-source collision-induced dissociation (CID) is commonly used with single-stage

Table 1 Identified compounds from *P. australis*, their PubChem CIDs, and data sources

Compounds	CID	Sources
Benzoic acid, 3,4-dimethyl-	12073	PubChem
3-Hydroxy-1-phenylpropan-1-one	569605	PubChem
Mexacarbate	9414	PubChem
Hexanedioic acid, dioctyl ester	31271	PubChem
Hexadecanoic acid, 3-hydroxy-, methyl ester	103553	PubChem
Octadecanoic acid, 3-hydroxy-, met hyl ester	538801	PubChem
1,2-benzenedicarboxylic acid	1017	PubChem
Diisooctyl phthalate	33934	PubChem
Methyl 3-hydroxydodecanoate	155752	PubChem
Valeric acid, octadecyl ester	568510	PubChem
Spongi-13-en-16-one	FsGTBMzqok0	SpectraBase
2,4-Cyclohexadien-1-one, 2,3,4,5-t etramethyl-6,6-diphenyl-	627423	PubChem
Cholestanol	6665	PubChem
5.alpha.-Cholestan-3.beta.-ol	3240	PubChem
1,3-dimethyl-4-azaphenanthrene	610182	PubChem
5.alpha.-Cholest-7-en-3.beta.-ol	6432730	PubChem
Lathosterol	65728	PubChem
Cholest-7-en-3-ol, (3.beta.)-	9930097	PubChem
Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	91691444	PubChem
Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	5283669	PubChem
ERGOSTAN-3-OL	3080559	PubChem
Ergostanol	5283641	PubChem
2H-Cyclotrideca[b]furan-2-one, 6-a cetyl-3,3a,4,7,8,11,12,14a-octahydro-9,13-dimethyl-3-methylene-	Fp9Uf8t2xEc	SpectraBase
Ergostan-3-one, 12-hydroxy-, (5.be ta.,12.alpha.)-	91740131	PubChem
Ergost-7-en-3-ol, (3.beta.,5.alpha.)-	5283646	PubChem
Stigmasta-7,16-dien-3-ol, (3.beta. ,5.alpha.)-	22295562	PubChem
STIGMAST-7-EN-3-OL	3080632	PubChem
5.alpha.-Stigmast-7-en-3.beta.-ol, (24S)-	5283639	PubChem
Stigmast-8(14)-en-3.beta.-ol	22296811	PubChem

high-resolution mass spectrometers to obtain both molecular formula and structural information through the collisional activation of analytes with residual background gas in the source region of the mass spectrometer (Davidson *et al.*, 2020).

Bioactivity Prediction

The lack of a highlighter in the Pa score indicated that 38 compounds had metabolic activity associated with the target protein (Pa> Pi and Pa> 0.3), according to bioactivity predictions made using the PASS Online

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Table 2 Predicted bioactivities of ligand compounds using PASS Online

Compounds	Bioactivity (Pa)
Benzoic acid, 3,4-dimethyl-	Cancer associated disorders treatment 0.356 NOS2 expression inhibitor 0.301
3-Hydroxy-1-phenylpropan-1-one	Cancer associated disorders treatment 0.380 NOS2 expression inhibitor 0.336
Mexacarbate	Cancer associated disorders treatment 0,315
Hexanedioic acid, dioctyl ester	Cancer associated disorders treatment 0.339 Proliferative diseases treatment 0.328
Hexadecanoic acid, 3-hydroxy-, methyl ester	Cancer associated disorders treatment 0.338 Proliferative diseases treatment 0.307
Octadecanoic acid, 3-hydroxy-, methyl ester	Apoptosis agonist 0.434 NOS2 expression inhibitor 0.365
1,2-benzenedicarboxylic acid	Cancer associated disorders treatment 0,338 Cancer associated disorders treatment 0.362 NOS2 expression inhibitor 0.321
Diisooctyl phthalate	Cancer associated disorders treatment 0.316
Methyl 3-hydroxydodecanoate	Apoptosis agonist 0.434 Cancer associated disorders treatment 0,338 NOS2 expression inhibitor 0.365 Proliferative diseases treatment 0,307
Valeric acid, octadecyl ester	Cancer associated disorders treatment 0.339 Apoptosis agonist 0.334
Spongi-13-en-16-one	Apoptosis agonist 0.839 Proliferative diseases treatment 0.437
2,4-Cyclohexadien-1-one, 2,3,4,5-tetramethyl-6,6-diphenyl-	Apoptosis agonist 0.566 NOS2 expression inhibitor
Cholestanol	Apoptosis agonist 0.606 Proliferative diseases treatment 0.762
5.alpha.-Cholestan-3.beta.-ol	Apoptosis agonist 0.606 Cancer associated disorders treatment 0.326
5.alpha.-Cholest-7-en-3.beta.-ol	Apoptosis agonist 0.687 Proliferative diseases treatment 0.748
Lathosterol	Apoptosis agonist 0.687 Proliferative diseases treatment 0.748
Cholest-7-en-3-ol, (3.beta.)-	Apoptosis agonist 0.687
Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	Apoptosis agonist 0.892 Proliferative diseases treatment 0.653

Table 2 Predicted bioactivities of ligand compounds using PASS Online (cont.)

Compounds	Bioactivity (Pa)
Ergosta-7,22-dien-3-ol, (3.beta.,5. alpha.,22E)-	Apoptosis agonist 0.892
	Proliferative diseases treatment 0.653
ERGOSTAN-3-OL	Apoptosis agonist 0.668
	Proliferative diseases treatment 0.693
Ergostanol	Apoptosis agonist 0.668
	Proliferative diseases treatment 0.693
2H-Cyclotrideca[b]furan-2-one, 6-a cetyl-3,3a,4,7,8,11,12,14a-octahydro-9,13-dimethyl-3-methylene-	Apoptosis agonist 0.865
	NOS2 expression inhibitor
Ergostan-3-one, 12-hydroxy-, (5.be ta.,12. alpha.)-	Apoptosis agonist 0.617
	Proliferative diseases treatment 0.684
Ergost-7-en-3-ol, (3.beta.,5.alpha .)-	Apoptosis agonist 0.738
	Proliferative diseases treatment 0.679
Stigmasta-7,16-dien-3-ol, (3.beta. ,5.alpha.)-	Apoptosis agonist 0.736
	Proliferative diseases treatment 0.693
STIGMAST-7-EN-3-OL	Apoptosis agonist 0.747
	Proliferative diseases treatment 0.699
5.alpha.-Stigmast-7-en-3.beta.-ol, (24S)-	Apoptosis agonist 0.747
	Proliferative diseases treatment 0.699
Stigmast-8(14)-en-3.beta.-ol	Apoptosis agonist 0.697
	Proliferative diseases treatment 0.581

webserver (Aini *et al.,* 2023). One molecule, 2-iso-propyl-3-amino-1-thia-3-aza-cyclopentane ($C_7H_{16}N_2S$), did not, however, have any promise for anticancer, anti-proliferation, and pro-apoptosis. ADMET is then predicted for new compounds that exhibit the anticipated metabolic activity (Srivastava *et al.,* 2022), (Sternbeck *et al.,* 2022).

ADMET Test

Drug distribution in the body is one of the characteristics that are employed (Pradeepkiran *et al.,* 2021), (Abdullahi *et al.,* 2022). Additionally, the ability to suppress phase 1 of CYP450 was used to evaluate the metabolic characteristics. This is due to the fact that 90% of enzymatic reaction metabolism involves CYP (Esteves *et al.,* 2021). As a CYP450 family inhibitor, metabolism is classified as either "yes" or "no"

Meanwhile, all distribution parameter markers were met by 35 substances. Drug concentrations in plasma at steady state affect the volume distribution (D1) in humans (Lombardo *et al*., 2021). Furthermore, the ability of a medication to bind to blood proteins is demonstrated by the unbound fraction (D2), which results in varying levels of drug efficacy when diffusing through cell membranes (Atkinson *et al*., 2022). Meanwhile, surface exchange between the blood and the central nervous system is influenced by the enhanced permeability of the blood-brain barrier (D3) (Zhao *et al*., 2022). The ability of drugs to enter the central nervous system can be estimated using the BBB value (log PS, D4) (Kang *et al.,* 2023). Furthermore, the highest overall clearance values were observed for the octadecyl ester of valeric acid, dioctyl ester of hexanedioic acid, and 3-hydroxy-

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methyl ester of hexadecanoic acid. According to Pantaleão *et al.* (2022), total clearance means that the medication can be eliminated from the body's central compartment without the need for major mechanisms. However, organic cation transporter 2 (OCT2) plays an important role in renal clearance and drug disposal. A drug has a negative impact if it inhibits OCT2.

Because they are not CYP450 enzyme inhibitors (M1–7), which have no detrimental effects on the body when ingested, a total of 26 substances that have been examined have been found to meet metabolic parameters (Klomp *et al.*, 2020) shown CYP450 activity inhibition, which results in poor metabolite clearance and buildup in the body. Meanwhile, at least one CYP450 enzyme was inhibited by the remaining 12 substances. The 33 compounds were projected to pass the toxicity test, according to the data, while five additional compounds failed the Ames test and liver toxicity. The highest dose tolerance in sequence for humans was demonstrated by phthalic acid, di(2-propylpentyl) ester; 2,4-cyclohexadien-1-one, 2,3,4,5-t etramethyl-6,6-diphenyl-; and hexadecanoic acid, 3-hydroxy-, methyl ester.

Protein-protein Interaction, Pathway Prediction

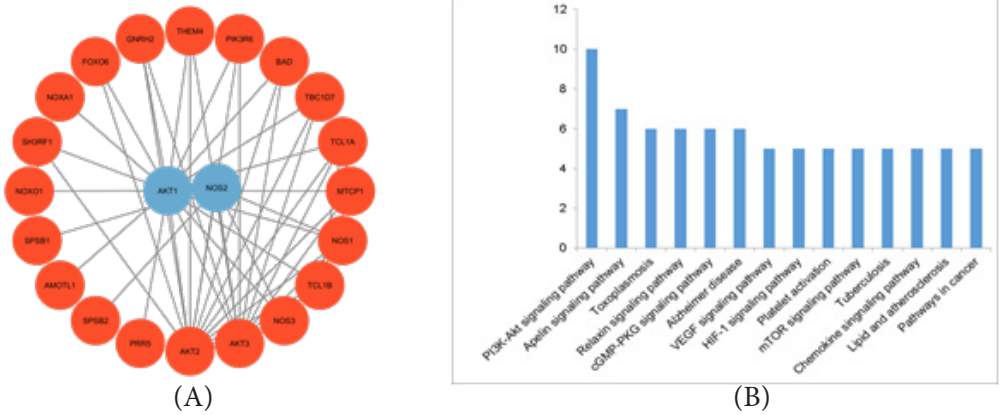
Protein interaction analysis revealed that AKT1 and NOS2 interacted with MTCP1, TCL1A, and NOX1 (Figure 1A). These target proteins are involved in cancer, apelin, the PIK3K-Akt signalling pathway, and other processes (Figure 1B).

Molecular Docking, Chemical Interactions, and Visualization

Binding affinity refers to the energy required to create a bond between a protein and ligand. A lower binding affinity value indicates that the ligand can more readily attach to the protein, thus having a greater potential to affect the protein (Cao *et al.*, 2022). The results of docking between proteins and various compounds yielded binding affinity values, as shown in Table 3.

Ergosta-7,22-dien-3-ol compounds, specifically (3. beta.,5. alpha.,22E)-; ERGOSTAN-3-OL; Ergost-7-en-3-ol, (3.beta.,5.alpha.)-; and Stigmasta-7,16-dien-3-ol, (3. beta.,5.alpha.) exhibited the most consistent binding to AKT1, despite the control inhibitor showing a lower binding affinity or improved interaction. In contrast, Ergosta-7,22-dien-3-ol (3. beta.,5.alpha.,22E)- and Ergosta-7,22-dien-3-ol, (3.beta.,22E)- compounds displayed binding affinity values lower than those of the NOS2 control inhibitor, as simulated through molecular docking. The interactions between selected ligands and target proteins will be illustrated using tables and images (Table 3, Figure 2).

The docking results between AKT1 and the test compound indicated that chemical



(A) (B)

Figure 1 Protein-protein interactions and predicted pathways; (A) Protein-protein interactions, light blue indicates target proteins, orange indicates proteins that interact with target proteins; (B) Fourteen pathways affected by target proteins and proteins that interact with target proteins.

Table 3 Binding affinity results of molecular docking

Compound	AKT1	NOS2
Benzoic acid, 3,4-dimethyl-	-7.0	-8.0
3-Hydroxy-1-phenylpropan-1-one	-6.6	-7
Mexacarbate	-6.5	-6.7
Hexanedioic acid, dioctyl ester	-6.5	-6.5
Hexadecanoic acid, 3-hydroxy-, methyl ester	-6.4	-6.3
Octadecanoic acid, 3-hydroxy-, met hyl ester	-6.4	-6.8
1,2-benzenedicarboxylic acid	-7.0	-7.6
Diisooctyl phthalate	-7.8	-9.2
Methyl 3-hydroxydodecanoate	-6.1	-6.3
Valeric acid, octadecyl ester	-6.7	-6.4
Spongi-13-en-16-one	-10.3	-9.2
2,4-Cyclohexadien-1-one, 2,3,4,5-t etramethyl-6,6-diphenyl-	-6.7	-9.9
Cholestanol	-10.6	-10.5
1,3-dimethyl-4-azaphenanthrene	-9.8	-9.9
Tetrasiloxane, decamethyl-	-6.6	-6.3
5.alpha.-Cholest-7-en-3.beta.-ol	-8.7	-9.9
Lathosterol	-10.8	-10.2
Cholest-7-en-3-ol, (3.beta.)-	-10.1	-10.3
Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	-10.2	**-11.2**
Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	**-11.5**	**-11.4**
ERGOSTAN-3-OL	**-11.1**	-10.7
Ergostanol	-10.9	-10.1
2H-Cyclotrideca[b]furan-2-one, 6-a cetyl-3,3a,4,7,8,11,12,14a-octahydro-9,13-dimethyl-3-methylene-	-8.9	-9.0
Ergostan-3-one, 12-hydroxy-, (5.be ta.,12.alpha.)-	-7.9	-9.0
Ergost-7-en-3-ol, (3.beta.,5.alpha.)-	**-11.1**	-10.8
Stigmasta-7,16-dien-3-ol, (3.beta. ,5.alpha.)-	**-11.1**	-9.6
STIGMAST-7-EN-3-OL	-10.6	-9.4
5.alpha.-Stigmast-7-en-3.beta.-ol, (24S)-	-11.0	-9.4
Stigmast-8(14)-en-3.beta.-ol	-10.7	-9.5

interactions occurred at the active or functional site and were characterized by inhibitors obtained from the database (Figure 4). The test compound occupied the same binding position as the inhibitor, suggesting that it exhibited similar activity in inhibiting AKT1 protein (Table 4). The interaction between the inhibitor and target protein occurs at multiple sites on the active site of the protein. Generally, the control ligand demonstrates a stronger electrostatic bond than the hydrophobic bond, resulting in a more negative binding affinity value and a more stable ligand-protein complex (Widyananda *et al.,* 2022).

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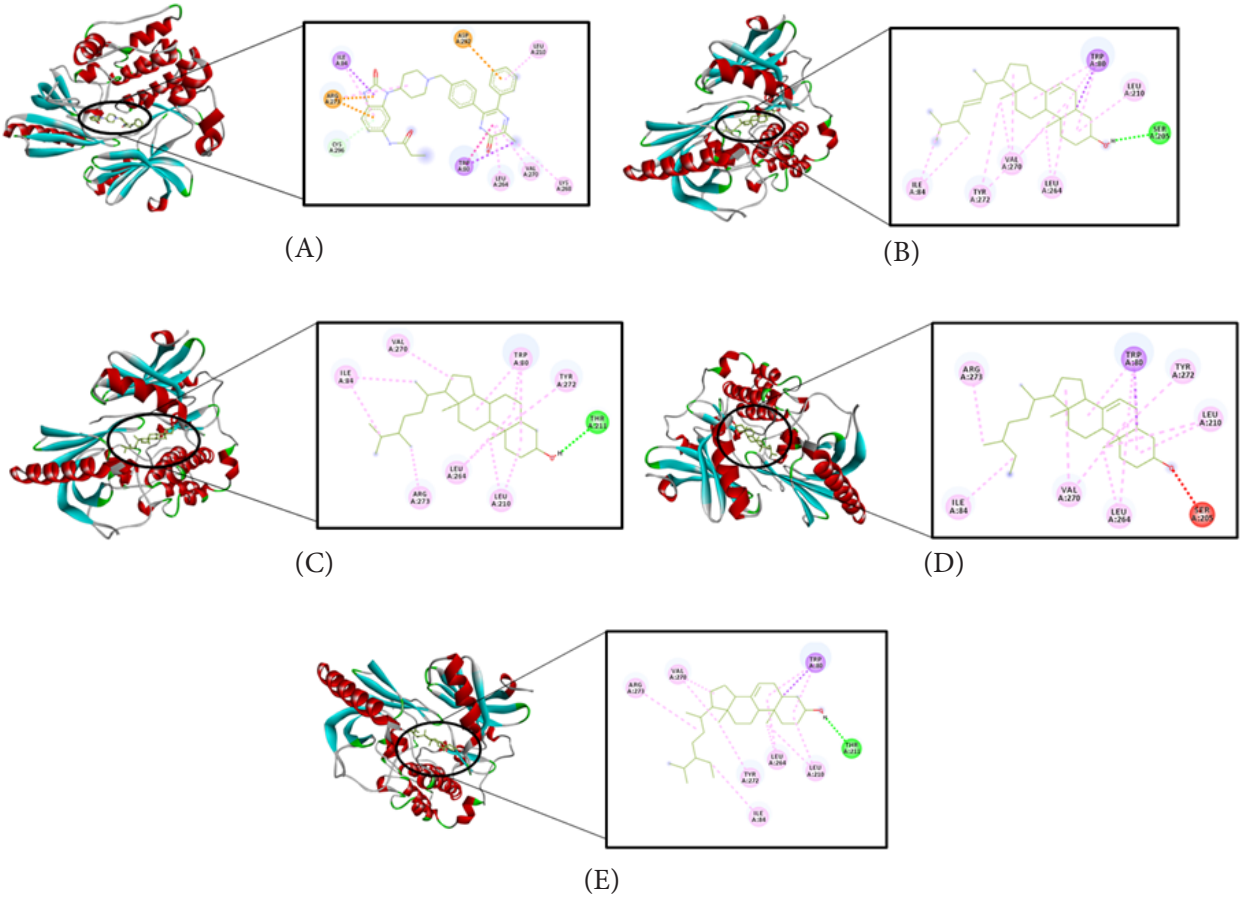


Figure 2 Interaction between the AKT1 complex and selected ligands; (A) ~{N}-[3-[1-[[4-methyl-6-oxidanylidene-3-phenyl-1~{H}-pyrazin-2-yl)phenyl]methyl]piperidin-4-yl]-2-oxidanylidene-1~{H}-benzimidazol-5-yl]propanamide (control ligand); (B) Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-; (C) ERGOSTAN-3-OL; (D) Ergost-7-en-3-ol, (3.beta.,5.alpha.)-; and (E) Stigmasta-7,16-dien-3-ol, (3.beta. ,5.alpha.)-.

Table 4 Details of protein-ligand interactions with the best results

Protein	Ligand	Bond category	Bond type	Residue
AKT1	~{N}-[3-[1-[[4-(5-methyl-6-oxidanylidene-3-phenyl-1~{H}-pyrazin-2-yl) phenyl]methyl]piperidin-4-yl]-2-oxidanylidene-1~{H}-benzimidazol-5-yl] propanamide (ligand control)	Hydrogen	Pi-donor hydrogen bond	Cys296
		Electrostatic	Pi-kation	Arg273, Arg273
			Pi-anion	Asp292
		Hydrophobic	Pi-sigma	Trp80, Ile84
			Pi-pi stacked	Trp80, Trp80
			Alkyl	Lys268, Arg273
			Pi-alkyl	Trp80, Ile84, Leu210, Leu264, Val270

Table 4 Details of protein-ligand interactions with the best results (cont)

Protein	Ligand	Bond category	Bond type	Residue
AKT1	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	Hydrogen	Conventional	Ser205
		Hydrophobic	Pi-sigma	Trp80
			Alkyl	Ile84, Ile84, Leu210, Leu264, Leu264, Val270, Val270, Val270
			Pi-alkyl	Trp80, Trp80, Trp80, Trp80, Tyr272, Tyr272
	ERGOSTAN-3-OL	Hydrogen	Conventional	Thr211
		Hydrophobic	Alkyl	Ile84, Ile84, Leu210, Leu210, Leu264, Leu264, Val270, Arg273
			Pi-alkyl	Trp80, Trp80, Trp80, Trp80, Tyr272
	Ergost-7-en-3-ol, (3.beta.,5.alpha.)-	Hydrophobic	Pi-sigma	Trp80
			Alkyl	Ile84, Leu210, Leu210, Leu264, Leu264, Val270, Val270, Arg273
			Pi-alkyl	Trp80, Trp80, Trp80, Tyr272
		Others	Unwanted acceptors	Ser205
	Stigmasta-7,16-dien-3-ol, (3. beta. ,5.alpha.)-	Conventional	Hydrogen	Thr211
		Hydrophobic	Pi-sigma	Trp80
			Alkyl	Ile84, Leu210, Leu210, Leu264, Leu264, Val270, Val270, Arg273
			Pi-alkyl	Trp80, Trp80, Tyr272
NOS2	Imidazole	Hydrogen	Carbon-hydrogen	Glu377
		Hydrophobic	Pi-alkyl	Pro350

http://dx.doi.org/10.17844/m8s22h81

Table 4 Details of protein-ligand interactions with the best results (cont)

Protein	Ligand	Bond category	Bond type	Residue
NOS2	Imidazole	Hydrogen	Carbon-hydrogen	Glu377
		Hydrophobic	Pi-alkyl	Pro350
	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	Hydrophobic	Pi-sigma	Trp194, Trp194, Trp194, Phe369
			Alkyl	Ala197, Arg199, Cys200, Cys200, Leu209, Val352, Val352, Met355
			Pi-alkyl	Trp194, Trp194, Trp194, Phe369, Phe369
	Ergosta-7,22-dien-3-ol, (3.beta.,22E)-	Hydrogen	Conventional	Trp372
		Hydrophobic	Pi-sigma	Tyr491
			Alkyl	Leu125, Ala197, Ala197, Arg199, Arg199, Cys200, Cys200, Val352, Met355
			Pi-alkyl	Phe369, Phe369, Phe369, Tyr489, Tyr491, Tyr491

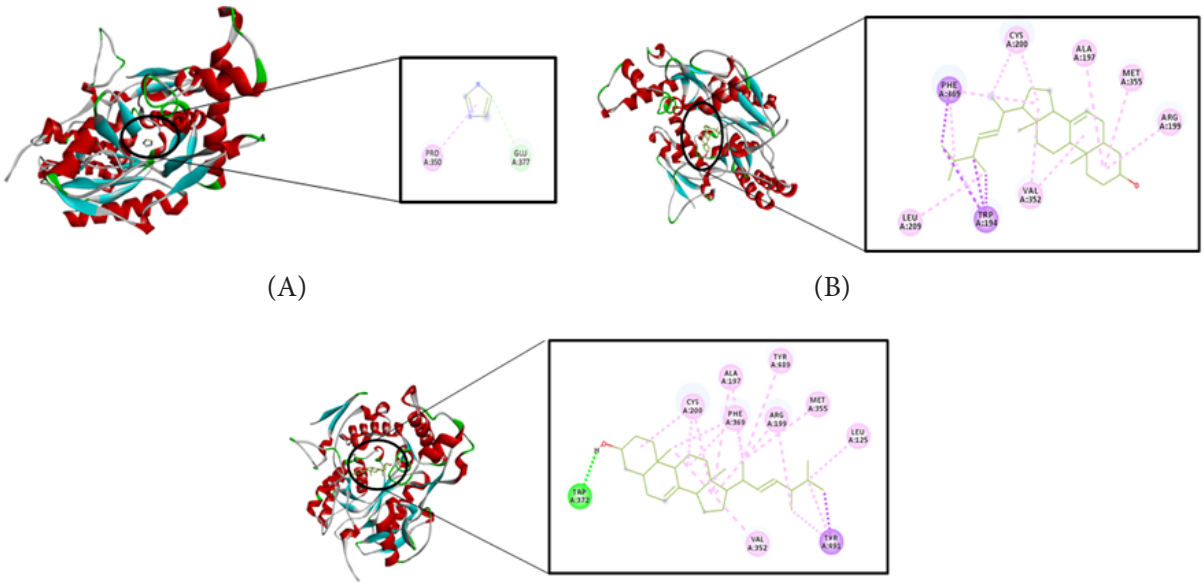
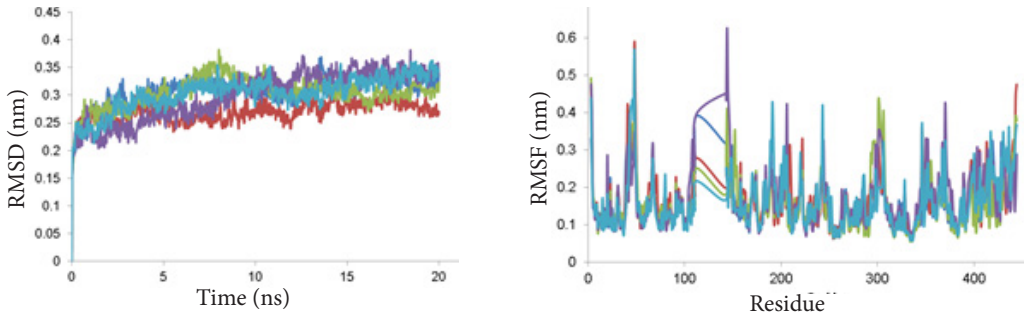


Figure 3 Interactions between NOS2 complexes with selected ligands; (A) imidazole (control ligand), B) Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-; and C) Ergosta-7,22-dien-3-ol, (3.beta.,22E)-.

Additionally, hydrogen bonds are crucial for the stability and turnover of cellular protein activity. The interactions between NOS2 and the test compound, as well as with AKT1, indicate that the chemical interaction with the test compound is more stable than that of the inhibitor. Imidazole exhibited only two interactions that significantly influenced the high binding affinity. This observation is further substantiated by the presence of a greater number of hydrophobic interactions in the test compounds than in the inhibitors (Verma *et al.,* 2022), (Ebenso *et al.,* 2021), (Verma *et al.,* 2022), (Chen *et al.,* 2022).

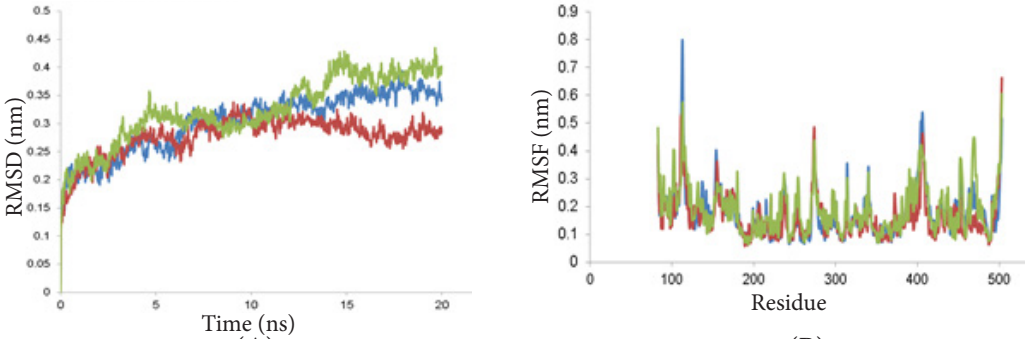
Molecular Dynamics

Figure 4 and 5 displays the molecular dynamics findings of the optimal docking outcomes. The RMSD values of the remaining protein-ligand complexes, both from the backbone category, are less than 3 Å (0.3 nm). According to Groeneveld *et al.* (2024), these findings indicate that the protein is unstable when interacting with the relevant ligand (Figure 4A-B). The NOS2-ergosta-7,22-dien-3-ol, (3. beta.,22E)- and AKT1-ergost-7-en-3-ol, (3beta.,5-alpha.) In contrast, the-complexes exhibited an average RMSD value of less than 0.3 nm, indicating that these complexes are stable during molecular dynamics predictions of interactions in human cells (Zrieq *et al.*, 2021). A previous study (Hosen *et al.,* 2023) showed that The compounds Ergosta-7,22-dien-3-ol, (3. beta.,5. alpha.,22E)- exhibited better binding affinity against AChE as potential cholinesterase inhibitors.



(A) (B)

Figure 4 Results of AKT1 molecular dynamic analysis in the form of RMSD (A) and RSMF (B) of proteins with selected ligands; (—) control ligand, (—) ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E), (—) ergostan-3-ol, (—) ergost-7-en-3-ol, (3.beta.,5 alpha.)-, (—) stigmasta-7,16-dien-3-ol, (3. beta. ,5.alpha.)-



(A) (B)

Figure 5 Results of NOS2 molecular dynamic analysis in the form of RMSD (A) and RMSF (B) of proteins with selected ligands; (—) control ligand, (—) ergosta-7,22-dien-3-ol, (3.beta.,22E)-, (—) ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)

In the meantime, the RMSF values of AKT1 and NOS2, which are less than 3 Å, show stability. However, during the 20 ns simulation, the complex also exhibited a peak RMSF value at several residues that was greater than 0.5 nm. This suggests that the interaction between the target protein complex and the chosen ligand is generally stable, with some residues showing RMSF peaks (Figure 5A-B) fluctuations (Tayye *et al*., 2024).

CONCLUSION

This study presents strong in silico evidence that bioactive compounds from *Paracaudina australis* have the potential to inhibit proteins linked to cancer, diabetes, obesity, inflammation, and cell proliferation. Using bioinformatics approaches, including bioactivity screening, ADMET profiling, molecular docking, and molecular dynamics, several compounds showed favorable interactions with AKT1 and NOS2. Specifically, ergosta-7,22-dien-3-ol (3β,5α,22E), ergostan-3-ol, ergost-7-en-3-ol (3β,5α), and stigmasta-7,16-dien-3-ol (3β,5α) consistently bound to AKT1, indicating anticancer potential. Meanwhile, ergosta-7,22-dien-3-ol (3β,22E) and ergosta-7,22-dien-3-ol (3β,5α,22E) showed higher stability than control ligands in targeting NOS2, suggesting anti-inflammatory effects. These findings position *P. australis* as a promising marine source of novel drug leads, although further in vitro and in vivo validation is required for clinical development.

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