International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 2, 2025

Original Article

MOLECULAR DOCKING AND PHARMACOKINETIC EVALUATION OF NANOHERBAL SENDUDUK BULU (*MICONIA CRENATA* (VAHL.) MICHELANG.) COMPOUNDS AS AKT1 INHIBITORS

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Received: 14 Oct 2024, Revised and Accepted: 10 Dec 2024

ABSTRACT

Objective: This study seeks to investigate the potential of 36 nanoherbal compounds extracted from senduduk bulu (*Miconia crenata* (Vahl) Michelang.) as inhibitors of v-akt murine thymoma viral oncogene homolog 1 (AKT1) using molecular docking techniques, pharmacokinetic analysis, safety evaluation, and bioactivity assessment.

Methods: Senduduk bulu leaves were nanoparticle-processed and analyzed via Gas Chromatography-Mass Spectrometry (GC-MS). Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profiles and biological activities were predicted, and molecular docking assessed compound interactions with AKT1 using borussertib as a reference.

Results: Findings indicate that 20 out of 36 compounds meet the criteria as drug candidates, demonstrating favorable interactions with the AKT1 protein, although their affinity did not surpass that of the positive control, borussertib. Several compounds exhibited high oral bioavailability, showed no interaction with the liver enzyme Cytochrome P450 2D6 (CYP2D6), and did not inhibit the Organic cation transporter 2 (OCT2) protein in the kidneys. In terms of toxicity, these compounds displayed a range of effects, from non-hazardous to hazardous, with some potentially posing risks of hepatotoxicity, carcinogenicity, and mutagenicity.

Conclusion: This research highlights the potential of nanoherbal senduduk bulu in cancer therapy development; however, further validation through *in vitro* and *in vivo* studies is necessary to comprehensively ensure their efficacy and safety.

Keywords: AKT1 inhibition, Breast cancer, Insilico analysis, Miconia crenata, Molecular docking, Nanoherbal

DOI: https://dx.doi.org/10.22159/ijap.2025v17i2.52932 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Breast cancer is one of the most prevalent types of cancer worldwide and serves as a leading cause of death among women, with an estimated 2.3 million new cases reported in 2020, resulting in approximately 685,000 fatalities [1]. This disease is characterised by the abnormal growth of cells within breast tissue, which can metastasise to other parts of the body if not addressed promptly. Risk factors for breast cancer include genetic predispositions, environmental influences, and lifestyle choices, such as diet and physical activity [2, 3]. Current treatment options for breast cancer comprise surgery, chemotherapy, and targeted therapies, including Human Epidermal Growth Factor Receptor 2 (HER2) monoclonal antibodies, antibody-drug conjugates (ADCs), and inhibitors of poly(adenosine diphosphate-ribose) polymerase (PARPi) that target mutations in the Breast Cancer Susceptibility Gene (BRCA) [4]. Consequently, with limited treatment alternatives available, there is an urgent need to identify more effective and safer therapies to tackle the challenges of resistance to existing treatments.

One of the key targets in cancer therapy development is the AKT1 protein (serine/threonine kinase 1), which is part of the Phosphatidylinositol 3-Kinase (PI3K)/AKT/mTOR signalling pathway. This pathway plays a crucial role in regulating various cellular processes, including growth, proliferation, metabolism, and cell survival [5, 6]. Abnormal activation of this pathway is often associated with cancer progression. In the context of breast cancer, AKT1 is frequently overexpressed or mutated, leading to resistance against conventional therapies such as chemotherapy and hormone treatments [7]. These mutations are commonly observed in cancers with alterations in the estrogen receptor (ER), contributing to the development and progression of more aggressive disease [8]. Consequently, AKT1 presents an attractive target for therapeutic intervention, where inhibiting its activity is expected to slow down or halt tumour growth. Several synthetic inhibitors have been developed, showing promising results; however, the long-term impacts and side effects of these drugs remain a significant concern [9]. In the realm of drug research, molecular docking techniques have emerged as essential computational methods for predicting interactions between molecules, such as drug compounds and target proteins. This technique is particularly valuable for identifying new drug candidates capable of inhibiting specific protein activities, like that of AKT1 [10, 11]. The use of docking software allows for the evaluation of binding affinities between tested compounds and the target protein, providing vital information for developing more effective cancer therapies [12]. Additionally, molecular docking enables the simulation of molecular interactions, thereby predicting the potential of compounds as inhibitors and assessing their mechanisms of action [13].

An innovative approach in cancer treatment involves the utilisation of natural compounds derived from biological resources, such as medicinal plants. Senduduk bulu (*Miconia crenata*) is known to possess various biological activities, including anti-inflammatory, antioxidant, and potential anti-cancer effects [14, 15]. Preliminary studies indicate that the leaf extracts of senduduk bulu contain bioactive compounds that may serve as anti-cancer agents. Through the technique of nanoformulation, these compounds can be obtained in the form of nanoherbal preparations, which are expected to enhance bioavailability, stability, and efficacy, thereby improving the penetration of active ingredients into cancer cells and minimising unwanted side effects [16].

This study aims to explore the potential of nano herbal compounds derived from the leaves of senduduk bulu as AKT1 inhibitors through in silico analysis. By employing a molecular docking approach, it is anticipated that compounds with a high affinity for AKT1 will be identified. Additionally, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis will be conducted to evaluate the safety profile and bioavailability of these compounds. The findings from this research are expected to make a significant contribution to the development of more effective cancer therapies while also highlighting the importance of exploring natural resources in the discovery of new drugs.

MATERIALS AND METHODS

Plant collection and preparation of nanoherbal senduduk bulu (*Miconia crenata* (Vahl) Michelang.)

The leaves of senduduk bulu were collected from Padangsidimpuan, North Sumatra, Indonesia. Subsequently, the plant was identified at the Herbarium of Andalas University, under identification number 28/K-ID/ANDA/I/2024. The collected leaves were then dried and ground using a blender. Following this, the leaves underwent nanoparticle processing using a Planetary Ball Mill (PBM) (Tokyo, Japan) at Nanotech Indonesia.

Gas chromatography-mass spectrometry (GC-MS) analisys

Bioactive compounds from the nanoherbal senduduk bulu in 96% ethanol were identified using GC-MS (Shimadzu QP 2010S), employing Wiley/NIST library software for data analysis. The analysis was conducted with an Rtx-5 ms column measuring 30 metres (Restek Corp). The injector and detector temperatures were maintained at 250 °C, while the operating temperature ranged from 50 to 300 °C. The column temperature was programmed to increase from 50 °C to 120 °C at a rate of 4 °C per minute, held for one minute, and then raised from 120 °C to 300 °C at a rate of 6 °C per minute, held for five minutes, resulting in a total retention time of 80 min. Helium was used as the carrier gas, with a mass-to-charge ratio range of 50-500 AMU. Electron ionization was performed at 70 eV [17].

Bioactive compounds from nano herbal sen duduk bulu

This study identified 36 bioactive compounds from the nanoherbal senduduk bulu, which include: 2-Cyclopenten-1-one, 2-hydroxy- (PubChem CID: 82674), Glycerin (PubChem CID: 753), 2-Hydroxy-gamma-butyrolactone (PubChem CID: 545831), Thymine (PubChem CID: 1135), 2-Bromo-octane (PubChem CID: 79046), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(PubChem CID: 119838), Octanal dimethyl acetal (PubChem CID: 61431), Catechol (PubChem CID: 289), 5-Hydroxymethylfurfural (PubChem CID: 237332), 1,3-Bis(1,1-dimethylethyl)benzene (PubChem CID: 136810), Isobornyl acetate (PubChem CID: 6950273), 3-Ethyl-2-hydroxy-2-cyclopenten-1-one (PubChem CID: 62752), α-Terpinyl acetate (PubChem CID: 111037), Sulfurous acid, cyclohexylmethyl hexadecyl ester (PubChem CID: 6421705), 1,2,3-Benzenetriol (PubChem CID: 1057), Tetradecane (PubChem CID: 12389), 2-(Hydroxymethyl)-2-nitro-1,3-propanediol (PubChem CID: 31337), D-Allose (PubChem CID: 439507), 3-Deoxy-D-mannoic lactone (PubChem CID: 541561), Diethyltoluamide (PubChem CID: 4284), Hexadecane (PubChem CID: 11006), α-Methyl-L-sorboside (PubChem CID: 219886), Octadecyl ester of undec-10-ynoic acid (PubChem CID: 91692469), Tricyclohexyl methane (PubChem CID: 137109), 2-(Phenylmethylene)-octanal (PubChem CID: 1715135), Benzyl benzoate (PubChem CID: 2345), Heptadecane (PubChem CID: 12398), Methyl hexadecanoate (PubChem CID: 8181), n-Hexadecanoic acid (PubChem CID: 985), n-Nonadecanol-1 (PubChem CID: 80281), Methyl 9,12-octadecadienoate (Z,Z)- (PubChem CID: 5284421), Methyl 9-octadecenoate (PubChem CID: 5280590), (Z,Z,Z)-7,10,13-hexadecatrienoic acid (PubChem CID: 5312428), Phytol (PubChem CID: 5280435), γ-Sitosterol (PubChem CID: 457801), and 2-Butoxyethanol (PubChem CID: 8133). The 2D structures of the bioactive chemical molecules from nanoherbal senduduk bulu are depicted in fig. 1.

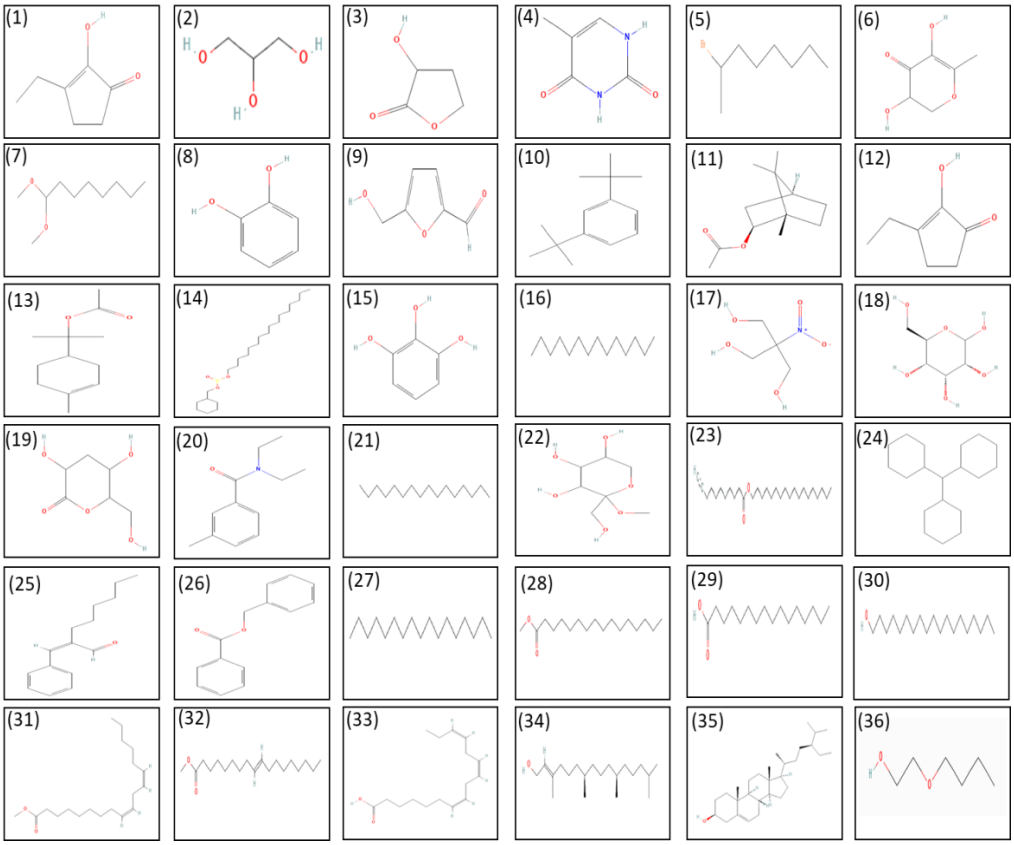

Fig. 1: 2D structures of the compounds from nanoherbal senduduk bulu (1) 2-Cyclopenten-1-one, 2-hydroxy-; (2) Glycerin; (3) 2-Hydroxy-gamma-butyrolactone; (4) Thymine; (5) Octane, 2-bromo-; (6) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; (7) Octanal dimethyl acetal; (8) Catechol; (9) 5-Hydroxymethylfurfural; (10) Benzene, 1,3-bis(1,1-dimethylethyl)-; (11) Isobornyl acetate; (12) 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-; (13) α-Terpinyl acetate; (14) Sulfurous acid, cyclohexylmethyl hexadecyl ester; (15) 1,2,3-Benzenetriol; (16) Tetradecane; (17) 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; (18) D-Allose; (19) 3-Deoxy-d-mannoic lactone; (20) Diethyltoluamide; (21) Hexadecane; (21) α-Methyl-l-sorboside; (22) Undec-10-ynoic acid, octadecyl ester; (23) Methane, tricyclohexyl-; (24) Octanal, 2-(phenylmethylene)-; (25) Benzyl Benzoate; (26) Heptadecane; (27) Hexadecanoic acid, methyl ester; (29) n-Hexadecanoic acid; (30) n-Nonadecanol-1; (31) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; (32) 9-Octadecenoic αSitosterol; (36) Ethanol, 2-butoxy

Prediction of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles for different compounds

To assess the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profile of the compounds, their 2D structures were downloaded in. sdf format, and the SMILES structures were retrieved from the PubChem database (http://pubchem.ncbi.nlm.nih.gov). ADME analysis was performed using the SwissADME platform (http://www.swissadme.ch/index.php), which identifies key pharmacokinetic parameters. The compounds' ability to cross the blood-brain barrier (BBB) was then predicted using the pkcSM tool (https://biosig.lab.uq.edu.au/pkcsm/prediction). Descriptors such as the number of hydrogen bond donors and acceptors, molecular weight, bioavailability, and compliance with Lipinski's Rule of Five were considered to evaluate the compounds' potential for oral absorption and pharmacological activity. Finally, toxicity predictions were carried out using the ProTox-II server (https://tox.charite.de/protox3/), based on toxicity classifications in line with the Globally Harmonised System (GHS) for Classification and Labelling of Chemicals [18].

Assessment of the compounds biological activities using the PASS online tool

The biological activity screening of compounds derived from nanoherbal senduduk bulu was performed using the Prediction of Activity Spectra of Substances (PASS) test through the Way2drug online platform (http://way2drug.com/PassOnline/). Initially, the compound names were entered into the PubChem database (http://pubchem.ncbi.nlm.nih.gov) to retrieve their SMILES structures. These structures were then submitted to the Way2drug server for the prediction of biological activities. The PASS online test results provided Pa values, classified into three categories: (i) Pa>0.7 indicates a high probability of the compound being biologically active, (ii) 0.5<Pa<0.7 suggests moderate biological activity, and (iii) Pa<0.5 signifies low biological activity [19].

Molecular docking evaluation

The preparation of ligands was carried out using the BIOVIA Discovery Studio software (https://discover.3ds.com/discovery-studio-visualizer-download). Ligand structures were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov) and saved in Standard Data Format (SDF) for further analysis. These ligands were also subjected to energy minimization via Open Babel, integrated with Pyrx v.0.8. Molecular docking simulations were carried out to evaluate the potential of bioactive compounds from senduduk bulu as AKT1 inhibitors. The AKT1 protein (PDB ID: 6HHF) was retrieved from the RCSB Protein Data Bank (PDB ID: 6HHF; https://www.rcsb.org/structure/6HHF) and docked with each nanoherbal senduduk bulu compound [20]. Borussertib (PubChem CID: 122668091), a standard commercial inhibitor, served as a reference control to compare the inhibitory effects of the bioactive compounds, as summarized in table 1. Docking was performed using AutoDock Vina within Pyrx, and the results were visualized with PyMOL.

Table 1: Active site and grid position of specific docking

Protein	PDB ID	Active site	Ref.	Grid coordinate (Å)	
				Center	Dimension
Akt1	6hhf	Trp80, Ile84, Glu85, Leu210, Leu264, Lys268, Val270, Tyr272, Arg273, Asp292		X: 7.4778 Y: 6.1953 Z: 12.6163	X: 27.3485 Y: 27.4698 Z: 28.6818

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS is a vital analytical technique in the field of chemistry, combining two powerful methods to effectively identify and quantify volatile and semi-volatile compounds within complex mixtures [21]. In the context of this study, GC-MS is employed to identify and quantify bioactive compounds in herbal extracts. The results from GC-MS provide valuable insights into the chemical composition of the samples, which can then be further analysed to understand the potential bioactivity of these compounds [22, 23]. Raw data from the GC-MS analysis and individual chromatograms were evaluated using Origin software. The GC-MS analysis identified 36 bioactive compounds, one of which is a solvent in the nanoherbal senduduk bulu, including Ethanol, 2-butoxy-; 2-Cyclopenten-1-one, 2-hydroxy-; Glycerin; 2-Hydroxy-gamma-butyrolactone; Thymine; Octane, 2-bromo-; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; Octanal dimethyl acetal; Catechol; 5-Hydroxymethylfurfural; Benzene, 1,3-bis(1,1-dimethylethyl)-; Isobornyl acetate; 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-; α-Terpinyl acetate; sulfurous acid, cyclohexylmethyl hexadecyl ester; 1,2,3-Benzenetriol; Tetradecane; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; D-Allose; 3-Deoxy-d-mannoic lactone; Diethyltoluamide; Hexadecane; α-Methyl-l-sorboside; Undec-10-ynoic acid, octadecyl ester; Methane, tricyclohexyl-; Octanal, 2-(phenylmethylene)-; Benzyl benzoate; Heptadecane; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; n-Nonadecanol-1; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; 9-Octadecenoic acid, methyl ester; 7,10,13-Hexadecatrienoic acid; Phytol; and γ-Sitosterol. The chromatogram shown in fig. 2 and the retention times of these compounds are presented in table 2. According to screening results from PubChem, 31 of these compounds possess 3D structures. Consequently, these 31 compounds will be further selected for docking analysis to evaluate their potential as AKT1 inhibitors.

The GC-MS analysis revealed the presence of various compounds with different retention times. Ethanol, specifically 2-butoxy, was identified as the primary solvent component, exhibiting a retention time of 4.58 min and a concentration of 4.59%. Commonly referred to as butoxyethanol or 2-butoxyethanol, this solvent is widely employed in various industrial and commercial applications. It belongs to the glycol ether group and possesses the ability to dissolve a range of substances, including oils, fats, and resins [24]. The detection of compounds such as catechol, with a retention time of 9.180 min, and hexadecanoic acid, with a retention time of 18.307 min, at significant concentrations suggests the presence of biologically active compounds, as these substances are frequently associated with antioxidant and antimicrobial properties [25, 26]. Additionally, compounds such as 2-cyclopenten-1-one, 2-hydroxy- and α-terpinyl acetate, detected at relatively short retention times of 4.893 min and 11.423 min, demonstrate good stability under the analytical conditions, making them attractive candidates for further development in pharmaceutical or cosmetic formulations. The identification of longer retention time compounds like 1,2,3-benzenetriol and γ-sitosterol, with retention times of 11.691 min and 35.441 min respectively, indicates the presence of high molecular weight substances that may contribute to the structure or function of more complex materials within the sample. Overall, the chemical composition of nanoherbal senduduk bulu highlights its broad potential applications in the chemical industry, pharmaceuticals, and as a raw material for the development of new products.

Prediction of drug-likeness

Predicting the drug-likeness of compounds is a crucial initial step in drug development, as it provides essential insights into the potential of a compound to serve as an effective therapeutic agent [27]. Evaluating the physicochemical properties of these compounds against established criteria, such as Lipinski's Rule of Five, allows for the assessment of favourable pharmacokinetic profiles. Based on the physicochemical analysis of compounds from the nanoherbal senduduk bulu and their adherence to Lipinski's criteria, as detailed in table 3, it was found that 20 out of a total of 36 selected

compounds meet the criteria for drug-like properties. Among these, one compound, 2-butoxyethanol (ethanol), along with 15 other compounds, did not fully satisfy the drug-likeness criteria. Compounds such as sulfurous acid, cyclohexylmethyl hexadecyl ester, tetradecane, hexadecane, under-10-yonic acid, octadecyl ester, tricyclohexylmethane, heptadecane, hexadecanoic acid methyl ester, n-hexadecanoic acid, n-nonadecanol-1, 9,12-octadecadienoic acid (Z,Z)-methyl ester, 9-octadecenoic acid methyl ester, and phytol failed to meet the criteria due to their high MlogP values exceeding ≤ 4.15. Additionally, compounds such as D-allose and α-methyl-l-sorboside did not comply because they surpassed the minimum threshold of 6. These deviations highlight the need for further optimisation of these compounds to enhance their pharmacokinetic properties. Elevated MlogP values indicate excessive lipophilicity, which can lead to poor solubility in aqueous environments, resulting in reduced absorption in the gastrointestinal tract when administered orally [28]. Modifications that can be implemented to address these discrepancies involve optimising the balance between hydrophobic and hydrophilic properties, which may enhance solubility, bioavailability, and the drug-likeness of these compounds, thereby making them more suitable candidates for therapeutic use [29].

Twenty compounds exhibited high potential as drug candidates with clinical viability. These compounds not only demonstrated MlogP values within acceptable limits but also maintained an optimal number of hydrogen bonds and molecular weights conducive to good bioavailability. Adherence to Lipinski's Rule indicates that these compounds possess favourable pharmacokinetic profiles, encompassing absorption, distribution, metabolism, and excretion, thereby increasing the likelihood of their success in further development as therapeutic agents, particularly in clinical applications [30, 31].

Table 2: Identified compounds of nanoherbal senduduk bulu by GC-MS

No	Compounds name	Mol. formula	Mass (g/mol)	R. Time (min)	Concentration (%)	Smiles	PubChem CID
1	Ethanol, 2-butoxy-	C6H14O2	118	4.58	4.59	CCCCOCCO	8133
2	2-Cyclopenten-1-one, 2-hydroxy-	C5H6O2	98	4.893	1.47	C1CC(=O)C(=C1)O	82674
3	Glycerin	C3H8O3	92	5.555	2.8	C(C(CO)O)O	753
4	2-Hydroxy-gamma-butyrolactone	C4H6O3	102	5.961	1.19	C1COC(=O)C1O	545831
5	Thymine	C5H6N2O2	126	7.284	0.85	CC1=CNC(=O)NC1=O	1135
6	Octane, 2-bromo-	C8H17Br	192	7.694	0.52	CCCCCCC(C)Br	79046
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	8.434	1.12	CC1=C(C(=O)C(CO1)O)O	119838
8	Octanal dimethyl acetal	C10H22O2	174	8.864	0.76	CCCCCCCC(OC)OC	61431
9	Catechol	C6H6O2	110	9,180	1.96	C 1=CC=C(C(=C1)O)O	289
10	5-Hydroxymethylfurfural	C6H6O3	126	9,648	13.13	C1=C(OC(=C1)C=O)CO	237332
11	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190	10,071	0.87	CC(C)(C)C1=CC(=CC=C1)C(C)(C)C	136810
12	Isobornyl acetate	C12H20O2	196	10,610	0.5	CC(=O) OC1CC2CCC1(C2(C)C)C	6950273
13	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	C7H10O2	126	10,727	0.63	CCC1=C(C(=O) CC1) O	62752
14	α-Terpinyl acetate	C12H20O2	196	11,423	0.59	CC1=CCC(CC1)C(C)(C)OC(=O)C	111037
15	Sulfurous acid, cyclohexylmethyl hexadecyl ester	C23H46O3S	402	11,490	1.3	CCCCCCCCCCCCCCCCCOS(=O)OCC1CCCCC1	6421705
16	1,2,3-Benzenetriol	C6H6O3	126	11,691	4.04	C1=CC(=C(C(=C1)O)O)O	1057
17	Tetradecane	C14H30	198	11,983	0.88	CCCCCCCCCCCCCC	12389
18	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	C4H9NO5	151	12,387	4.99	C(C(CO)(CO)[N+](=O)[O-])O	31337
19	D-Allose	C6H12O6	180	13,085	1.35	C(C1C(C(C(C(O1)O)O)O)O)O	439507
20	3-Deoxy-d-mannoic lactone	C6H10O5	162	14,192	1.02	C1C(C(OC(=O)C1O)CO)O	541561
21	Diethyltoluamide	C12H17NO	191	14,392	0.82	CCN(CC)C(=O)C1=CC=CC(=C1)C	4284
22	Hexadecane	C16H34	226	14,454	0.84	CCCCCCCCCCCCCCCC	11006
23	α-Methyl-l-sorboside	C7H14O6	194	14,640	13.29	COC1(C(C(C(CO1)O)O)O)CO	219886
24	Undec-10-ynoic acid, octadecyl ester	C29H54O2	434	15,235	0.54	CCCCCCCCCCCCCCCCCCCOC(=O)CCCCCCCCC#C	91692469
25	Methane, tricyclohexyl-	C19H34	262	15,786	0.55	C1CCC(CC1)C(C2CCCCC2)C3CCCCC3	137109
26	Octanal, 2-(phenylmethylene)-	C15H20O	216	16,292	0.98	CCCCCCC(=CC1=CC=CC=C1)C=O	1715135
27	Benzyl Benzoate	C14H12O2	212	16,535	1.75	C1=CC=C(C=C1)COC(=O)C2=CC=CC=C2	2345
28	Heptadecane	C17H36	240	16.671	0.64	CCCCCCCCCCCCCCCCC	12398
29	Hexadecanoic acid, methyl ester	C17H34O2	270	17.985	2.37	CCCCCCCCCCCCCCCC(=O)OC	8181
30	n-Hexadecanoic acid	C16H32O2	256	18.307	1.96	CCCCCCCCCCCCCCCC(=O)O	985
31	n-Nonadecanol-1	C19H40O	284	19.507	0.73	CCCCCCCCCCCCCCCCCCCO	80281
32	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	19.636	1.12	CCCCCC=CCC=CCCCCCCCC(=O)OC	5284421
33	9-Octadecenoic acid, methyl ester	C19H36O2	296	19.680	1.02	CCCCCCCCC=CCCCCCCCC(=O)OC	5280590
34	7,10,13-Hexadecatrienoic acid	C16H26O2	250	19.706	1.11	CCC=CCC=CCC=CCCCCCC(=O)O	5312428
35	Phytol	C20H40O	296	19.802	16.7	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	5280435
36	γ-Sitosterol	C29H50O	414	35.441	3.53	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C	457801

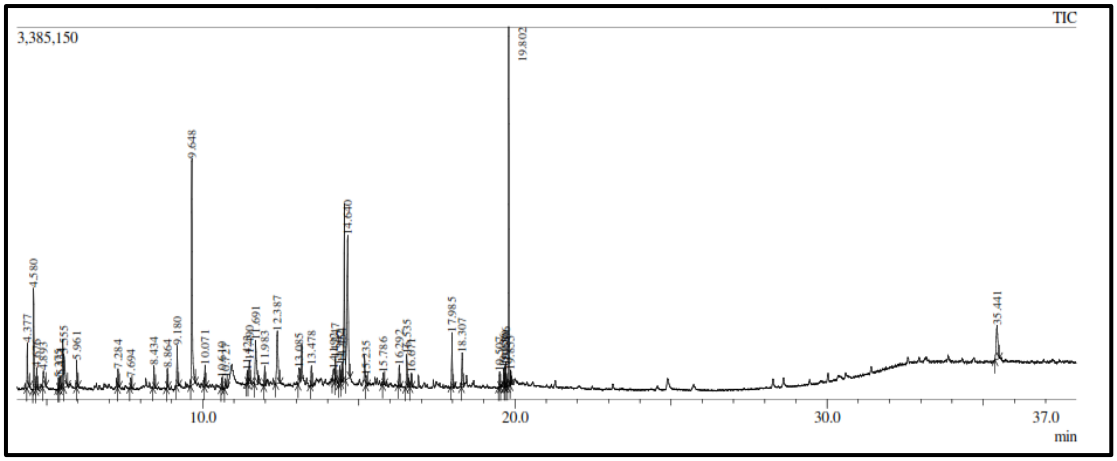

Fig. 2: GC-MS chromatogram of nanoherbal senduduk bulu

Table 3: The physicochemical characteristic of compounds nanoherbal senduduk bulu were assessed based on lipinski's rule of five

No	Compound name	Lipinski				Violasi
		MW	MlogP ≤ 4.15	NorO ≤ 10	NHorOH ≤ 5	
Positive control						
1.	Borussertib	596	3.66	3	5	Yes (1)
Bioactive components of the nanoherbal *Clidemia crenata*						
2.	Ethanol, 2-butoxy-	118	0.61	1	2	Yes (0)
3.	2-Cyclopenten-1-one, 2-hydroxy-	98	-0.42	1	2	Yes (0)
4.	Glycerin	92	-1.51	3	3	Yes (0)
5.	2-Hydroxy-gamma-butyrolactone	102	-0.79	1	3	Yes (0)
6.	Thymine	126	-0.39	2	2	Yes (0)
7.	Octane, 2-bromo-	192	3.76	0	0	Yes (0)
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	-1.77	2	4	Yes (0)
9.	Octanal dimethyl acetal	174	2.32	0	2	Yes (0)
10.	Catechol	110	0.79	2	2	Yes (0)
11.	5-Hydroxymethylfurfural	126	-1.06	1	3	Yes (0)
12.	Benzene, 1,3-bis(1,1-dimethylethyl)-	190	-1.06	1	3	Yes (0)
13.	Isobornyl acetate	196	2.76	0	2	Yes (0)
14.	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	126	0.31	1	2	Yes (0)
15.	α-Terpinyl acetate	196	2.65	0	2	Yes (0)
16.	Sulfurous acid, cyclohexylmethyl hexadecyl ester	402	4.92	0	3	Yes (1)
17.	1,2,3-Benzenetriol	126	0.18	3	3	Yes (0)
18.	Tetradecane	198	5.93	0	0	Yes (1)
19.	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	151	-2.41	3	5	Yes (0)
20.	D-Allose	180	-2.75	5	6	Yes (0)
21.	3-Deoxy-d-mannoic lactone	162	-1.68	3	5	Yes (0)
22.	Diethyltoluamide	191	2.72	0	1	Yes (0)
23.	Hexadecane	226	6.44	0	0	Yes (1)
24.	α-Methyl-l-sorboside	194	-2.4	4	6	Yes (0)
25.	Undec-10-ynoic acid, octadecyl ester	434	6.9	0	2	Yes (1)
26.	Methane, tricyclohexyl-	262	6.76	0	0	Yes (1)
27.	Octanal, 2-(phenyl methylene)-	216	3.68	0	1	Yes (0)
28.	Benzyl Benzoate	212	3.41	0	2	Yes (0)
29.	Heptadecane	240	6.68	0	0	Yes (1)
30.	Hexadecanoic acid, methyl ester	270	4.44	0	2	Yes (1)
31.	n-Hexadecanoic acid	256	4.19	1	2	Yes (1)
32.	n-Nonadecanol-1	284	5.17	1	1	Yes (1)
33.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	4.7	0	2	Yes (1)
34.	9-Octadecenoic acid, methyl ester	296	4.8	0	2	Yes (1)
35.	7,10,13-Hexadecatrienoic acid	250	3.9	1	2	Yes (0)
36.	Phytol	296	5.25	1	1	Yes (1)
37.	γ-Sitosterol	414	6.73	1	1	Yes (1)

Predicted pharmacokinetic properties of the nano herbal senduduk bulu compounds

Absorption of the compounds

The analysis of the absorption of compounds from nanoherbal senduduk bulu, as presented in table 4 indicates that the majority of these compounds can be efficiently absorbed by the human intestine, with 17 out of a total of 20 compounds exhibiting absorption rates exceeding 80%. For instance, the compound 2-cyclopenten-1-one, 2-hydroxy-demonstrated the highest absorption rate at 95.862%. Conversely, some compounds, such as 3-deoxy-D-mannoic lactone and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, showed lower absorption rates of 52.503% and 65.925%, respectively. Compounds with intestinal absorption rates above

80% indicate high oral bioavailability, suggesting a favourable pharmacokinetic profile that includes elevated plasma concentrations and prolonged action duration following oral administration [32, 33]. Additionally, the Caco-2 permeability assay, used to estimate the rate of oral drug absorption, corroborates these findings, as the majority of compounds exhibited high permeability, with Papp values exceeding 1.0 x 10^{-6} cm/s in 16 out of the 20 compounds assessed. Compounds with high permeability values are considered capable of efficiently crossing the intestinal membrane, which correlates with good oral bioavailability. Those with Papp values above 1.0x10^{-6} cm/s frequently demonstrate sufficiently high absorption rates in *in vivo* models, supporting their use in predicting drug absorption in humans [34, 35].

Distribution of the compounds

To understand the distribution of compounds from nanoherbal senduduk bulu, an analysis was conducted on their oral bioavailability in humans, specifically, the ability of compounds to enter the bloodstream after oral consumption and their capacity to cross the Blood-Brain Barrier (BBB). The results presented in table 4 indicate that the majority of compounds exhibit negative BBB permeability values, leading to the conclusion that these compounds are unlikely to directly affect the central nervous system. Compounds with low or negative BBB permeability may require additional strategies, such as chemical modifications, to enhance their potential to penetrate the BBB. However, if the therapeutic target is not located in the brain, low BBB permeability is considered advantageous, as it may reduce the risk of undesirable side effects on the central nervous system [35, 36].

Metabolism of the compounds

Cytochrome P450 2D6 (CYP2D6) enzymes play a crucial role in the metabolism of various medications, predominantly located in the liver. This enzyme is responsible for the metabolism of approximately 20-25% of all clinically used drugs [37]. Interactions with this enzyme can lead to significant alterations in the pharmacokinetics of medications, potentially resulting in undesirable drug interactions. The results presented in table 4 indicate that these compounds are unlikely to interfere with the metabolism of other drugs via this enzymatic pathway. This suggests that compounds that do not act as substrates or inhibitors of CYP2D6 generally exhibit simpler drug interaction profiles, which may help mitigate the risk of side effects associated with pharmacokinetic interactions [38].

Excretion of the compounds

Organic cation transporter 2 (OCT2) is a membrane protein that plays a critical role in the transport of organic cations in the kidneys, particularly in the absorption and excretion of compounds in the proximal tubules. It is capable of transporting a wide range of organic cations, including drugs, metabolites, and endogenous compounds [39, 40]. Based on the results shown in table 4, no compounds were identified as substrates for the OCT2 transporter involved in the renal transport of organic cations. This suggests that these compounds are less likely to interact with other drugs via competitive transport mechanisms in the kidneys, thereby reducing the potential for harmful pharmacokinetic interactions [41].

Water solubility of the compounds

Water solubility is a critical physicochemical property in pharmacokinetics, as it affects the absorption, distribution, metabolism, and excretion of drugs in the body. As indicated in table 4 the compounds exhibit considerable variation in their solubility. Nine compounds fall into the highly soluble category, including glycerin, 2-hydroxy-gamma-butyrolactone, thymine, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, catechol, 5-hydroxymethylfurfural, 2-cyclopenten-1-one, 3-ethyl-2-hydroxy-, 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, and 3-deoxy-D-mannoic lactone. High solubility typically indicates that these compounds are easily absorbed in the gastrointestinal tract following oral administration [42]. Two compounds, 2-cyclopenten-1-one, 2-hydroxy-and 1,2,3-benzenetriol, are classified as moderately soluble. Another two compounds, α-terpinyl acetate and diethyltoluamide, are categorised as sparingly soluble, and seven compounds are classified as poorly soluble. These include octane, 2-bromo-, octanal dimethyl acetal, benzene, 1,3-bis(1,1-dimethylethyl)-, isobornyl acetate, octanal, 2-(phenylmethylene)-, benzyl benzoate, and 7,10,13-Hexadecatrienoic acid. Low solubility can present a significant barrier to absorption within the body, as these compounds may not dissolve quickly enough in the gastrointestinal tract, potentially leading to low bioavailability. Compounds with poor water solubility often require specialised formulations, such as complex formation or the use of solubilising excipients, to improve their solubility [43].

Table 4: Pharmakokinetics properties of compounds from nano herbal senduduk bulu

No	Compounds name	Human intestinal absorption	Caco-2 permeability	BBB permanent	CYP2D6 substrate	CYP2D6 inhibitor	OCT2 substrate	Log S (ESOL)
1	2-Cyclopenten-1-one, 2-hydroxy-	95.862	1.577	-0.27	No	No	No	Moderately soluble
2	Glycerin	74.246	1.073	-0.362	No	No	No	Highly soluble
3	2-Hydroxy-gamma-butyrolactone	95.746	1.109	-0.28	No	No	No	Highly soluble
4	Thymine	86.806	0.617	-0.441	No	No	No	Highly soluble
5	Octane, 2-bromo-	92.954	1.391	0.776	No	No	No	Very poorly soluble
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	83.475	0.476	-0.3	No	No	No	Highly soluble
7	Octanal dimethyl acetal	94.608	1.614	0.704	No	No	No	Poorly soluble
8	Catechol	86.856	1.682	-0.318	No	No	No	Highly soluble
9	5-Hydroxymethylfurfural	95.848	1.172	-0.361	No	No	No	Highly soluble
10	Benzene, 1,3-bis(1,1-dimethylethyl)-	94.226	1.503	0.561	No	No	No	Very poorly soluble
11	Isobornyl acetate	95.366	1.855	0.553	No	No	No	Poorly soluble
12	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	93.736	1.588	0.362	No	No	No	Highly soluble
13	α-Terpinyl acetate	96.295	1.626	0.429	No	No	No	Slightly soluble
14	1,2,3-Benzenetriol	83.549	1.122	-0.441	No	No	No	Moderately soluble
15	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	52.503	-0.325	-0.908	No	No	No	Highly soluble
16	3-Deoxy-d-mannoic lactone	65.925	0.389	-0.43	No	No	No	Highly soluble
17	Diethyltoluamide	93.683	1.192	0.358	No	No	No	Slightly soluble
18	Octanal, 2-(phenylmethylene)-	94.782	1.652	0.686	No	No	No	Very poorly soluble
19	Benzyl Benzoate	95.835	1.43	0.282	No	No	No	Poorly soluble
20	7,10,13-Hexadecatrienoic acid	93.523	1.579	1.579	No	No	No	Very poorly soluble

Predicted toxicity profiles of the compounds

The toxicity prediction results shown in table 5 indicate that the 20 selected compounds from nanoherbal senduduk bulu, which passed clinical suitability based on Lipinski's rule, range from the category of harmful if swallowed (Class 3) to non-toxic (Class 6). Two compounds classified as harmful, if swallowed, are catechol and 1,2,3-benzenetriol, with LD50 values of 100 and 300 mg/kg body weight, respectively. Catechol, a phenolic compound, is known to cause liver and kidney damage at high doses, as well as irritation to the skin and respiratory tract [44]. Meanwhile, one compound is classified as non-toxic, namely 7,10,13-Hexadecatrienoic acid, with an LD50 value of 10,000 mg/kg body weight. This compound is a fatty acid commonly found in plants and vegetable oils. The remaining 17 compounds are predicted to have potential toxicity, falling within class 4 and 5 categories.

Based on the toxicity predictions shown in table 6, 11 selected compounds were found to have no toxic potential, while 9 others were predicted to pose toxicity risks. α-Terpinyl acetate is the only compound exhibiting hepatotoxic activity, indicating that it may cause liver damage either through oxidative stress pathways or disruption of liver enzymes [45]. 6 compounds, including thymine, octane, 2-bromo-, catechol, 5-hydroxymethylfurfural, benzene, 1,3-bis(1,1-dimethylethyl)-, and 1,2,3-benzenetriol, were identified as having potential carcinogenicity. This carcinogenicity suggests that these compounds may promote uncontrolled cell growth and increase cancer risk [46]. Meanwhile, compounds such as 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-hydroxymethylfurfural, 1,2,3-benzenetriol, and benzyl benzoate were found to exhibit mutagenic activity, meaning they have the potential to induce mutations in genetic material. These mutations are linked to an increased risk of genetic disorders due to changes in DNA that can affect key proteins regulating the cell cycle [47]. On the other hand, the predictions showed no compounds causing immunotoxicity or cytotoxicity, which is a favourable outcome, as these toxicities often pose significant risks in long-term therapies.

The toxicity assessment results of the 20 compounds derived from nanoherbal senduduk bulu demonstrate a significant variation in toxicity risk. This highlights the considerable differences in the pharmacological and toxicological potential of these compounds, necessitating thorough evaluation before therapeutic use [6]. Overall, while some selected compounds from nanoherbal senduduk bulu exhibit favourable safety profiles, others that show toxicity potential require further investigation. *In vivo* studies are crucial to validate these predictions and to gain a deeper understanding of the toxicity mechanisms and safe dosage levels of these compounds before their application in clinical therapy.

Predicted bioactivity of the nanoherbal senduduk bulu compounds based on the PASS online test

The predicted biological activities using the PASS online assay, based on probability of activity (Pa) and probability of inactivity (Pi) shown in table 7, reveal various levels of biological activities that can be harnessed for therapeutic development, particularly for cancer and inflammation treatments [48]. Probabilities were calculated for each compound and its biological targets, such as JAK2 expression inhibitors, apoptosis inducers, and others. The Pa value indicates the likelihood of a compound exhibiting a specific biological activity, while the Pi value reflects the probability of inactivity. Compounds like 2-Cyclopenten-1-one, 2-hydroxy-and Benzene, 1,3-bis(1,1-dimethylethyl)-exhibit potential as JAK2 inhibitors, a known target in cancer therapy, with respective Pa values of 0.806 and 0.749. The JAK2/STAT pathway plays a key role in cancer cell proliferation, and inhibiting JAK2 can suppress tumour growth and promote apoptosis, particularly in cases of leukaemia and solid tumours [49]. Additionally, compounds such as 7,10,13-Hexadecatrienoic acid and Octanal, 2-(phenylmethylene)-show potential as BRAF inhibitors, commonly found in melanoma, with Pa values of 0.892 and 0.825, respectively. Furthermore, several compounds, including Glycerin, Thymine, 1,2,3-Benzenetriol, and 3-Deoxy-d-mannoic lactone, are predicted to enhance the expression of TP53, a tumour suppressor gene. TP53 regulates cell cycles and apoptosis, and increasing TP53 expression can induce the death of mutated cancer cells [50]. Apoptosis induction is also a primary strategy in cancer therapy, with 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-showing strong activity as an apoptosis inducer (Pa = 0.858) and antineoplastic agent (Pa = 0.737). Additionally, the compound with the highest anti-inflammatory and TNF inhibition properties (Pa = 0.804) is 7,10,13-Hexadecatrienoic acid, highlighting its potential in mitigating chronic inflammation, often linked with cancer progression, by suppressing pro-inflammatory cytokines [51].

The screening results highlight several compounds with potential as anticancer agents through diverse mechanisms. 7,10,13-Hexadecatrienoic acid and 1,2,3-Benzenetriol emerge as strong candidates, exhibiting various antitumor activities with high values. Although the screening suggests these compounds hold promise, further exploration is needed through *in vitro* and *in vivo* studies in the laboratory to assess their efficacy and safety.

Table 5: Predicted toxicology levels of the compounds nanoherbal senduduk bulu

No	Compounds name	Predicted LD_{50}	Predicted toxicity class	Average similarity	Prediction accuracy
1	2-Cyclopenten-1-one, 2-hydroxy-	400 mg/kg	Four (harmful)	66.52%	68.07%
2	Glycerin	4090 mg/kg	Five (possibly hazardous)	100%	100%
3	2-Hydroxy-gamma-butyrolactone	1510 mg/kg	Four (harmful)	71.67%	69.26%
4	Thymine	1923 mg/kg	Four (harmful)	57.72%	67.38%
5	Octane, 2-bromo-	1190 mg/kg	Four (harmful)	100%	100%
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	595 mg/kg	Four (harmful)	60.38%	68.07%
7	Octanal dimethyl acetal	5000 mg/kg	Five (possibly hazardous)	100%	100%
8	Catechol	100 mg/kg	Three (harmful if ingested)	100%	100%
9	5-Hydroxymethylfurfural	2500 mg/kg	Five (possibly hazardous)	100%	100%
10	Benzene, 1,3-bis(1,1-dimethylethyl)-	3100 mg/kg	Five (possibly hazardous)	100%	100%
11	Isobornyl acetate	3100 mg/kg	Five (possibly hazardous)	100%	100%
12	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	2000 mg/kg	Four (harmful)	78%	69.26%
13	α-Terpinyl acetate	4800 mg/kg	Five (possibly hazardous)	100%	100%
14	1,2,3-Benzenetriol	300 mg/kg	Three (harmful if ingested)	100%	100%
15	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	845 mg/kg	Four (harmful)	63.56%	68.07%
16	3-Deoxy-d-mannoic lactone	1590 mg/kg	Four (harmful)	75.34%	69.26%
17	Diethyltoluamide	1170 mg/kg	Four (harmful)	100%	100%
18	Octanal, 2-(phenylmethylene)-	2300 mg/kg	Five (possibly hazardous)	100%	100%
19	Benzyl Benzoate	1000 mg/kg	Four (harmful)	100%	100%
20	7,10,13-Hexadecatrienoic acid	10000 mg/kg	Six (nontoxic)	100%	100%

Table 6: Predicted organ toxicity of the compounds from nanoherbal senduduk bulu

No	Compounds	Organ toxicity				
		Hepatotoxicity	Carcinogenecity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	2-Cyclopenten-1-one, 2-hydroxy-	Inactive	Inactive	Inactive	Inactive	Inactive
2	Glycerin	Inactive	Inactive	Inactive	Inactive	Inactive
3	2-Hydroxy-gamma-butyrolactone	Inactive	Inactive	Inactive	Inactive	Inactive
4	Thymine	Inactive	Active	Inactive	Inactive	Inactive
5	Octane, 2-bromo-	Inactive	Active	Inactive	Inactive	Inactive
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Inactive	Inactive	Inactive	Active	Inactive
7	Octanal dimethyl acetal	Inactive	Inactive	Inactive	Inactive	Inactive
8	Catechol	Inactive	Active	Inactive	Inactive	Inactive
9	5-Hydroxymethylfurfural	Inactive	Active	Inactive	Active	Inactive
10	Benzene, 1,3-bis(1,1-dimethylethyl)-	Inactive	Active	Inactive	Inactive	Inactive
11	Isobornyl acetate	Inactive	Inactive	Inactive	Inactive	Inactive
12	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	Inactive	Inactive	Inactive	Inactive	Inactive
13	α-Terpinyl acetate	Active	Inactive	Inactive	Inactive	Inactive
14	1,2,3-Benzenetriol	Inactive	Active	Inactive	Active	Inactive
15	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	Inactive	Inactive	Inactive	Inactive	Inactive
16	3-Deoxy-d-mannoic lactone	Inactive	Inactive	Inactive	Inactive	Inactive
17	Diethyltoluamide	Inactive	Inactive	Inactive	Inactive	Inactive
18	Octanal, 2-(phenylmethylene)-	Inactive	Inactive	Inactive	Inactive	Inactive
19	Benzyl Benzoate	Inactive	Inactive	Inactive	Active	Inactive
20	7,10,13-Hexadecatrienoic acid	Inactive	Inactive	Inactive	Inactive	Inactive

Table 7: Prediction biological activity compounds of nanoherbal senduduk bulu by PASS online test

No	Compound name	Biological activity	Pa	Pi	Criteria
1	2-Cyclopenten-1-one, 2-hydroxy-	JAK2 expression inhibitor	0.806	0.008	High
		MMP9 expression inhibitor	0.696	0.007	Low
		TP53 expression enhancer	0.690	0.028	Low
		Macrophage colony stimulating factor agonist	0.677	0.017	Low
		Apoptosis agonist	0.665	0.019	Low
		Antineoplastic (multiple myeloma)	0.509	0.009	Low
		Cytochrome P450 stimulant	0.486	0.030	Low
		BRAF expression inhibitor	0.437	0.011	Low
		Caspase 3 stimulant	0.441	0.038	Low
		Antineoplastic (breast cancer)	0.430	0.027	Low
		Antimetastatic	0.387	0.050	Low
2	Glycerin	Macrophage colony stimulating factor agonist	0,878	0,003	High
		TP53 expression enhancer	0,736	0,019	High
		BRAF expression inhibitor	0.712	0,003	High
		JAK2 expression inhibitor	0,624	0,028	Low
		Antiinflammatory	0,558	0,041	Low
		MMP9 expression inhibitor	0,531	0,024	Low
		TNF expression inhibitor	0,514	0,027	Low
		Caspase 8 stimulant	0,454	0,029	Low
		Caspase 3 stimulant	0,421	0,044	Low
		Cytochrome P450 stimulant	0,418	0.048	Low
3	2-Hydroxy-gamma-butyrolactone	Macrophage colony stimulating factor agonist	0.699	0.014	Low
		TP53 expression enhancer	0.636	0.040	Low
		Caspase 8 stimulant	0.573	0.009	Low
		MMP9 expression inhibitor	0.519	0.026	Low
4	Thymine	TP53 expression enhancer	0.728	0.021	High
		Cytochrome P450 stimulant	0.705	0.006	High
		TNF expression inhibitor	0.441	0.044	Low
5	Octane, 2-bromo-	JAK2 expression inhibitor	0.481	0.058	Low
		MMP9 expression inhibitor	0.381	0.058	Low
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Apoptosis agonist	0.868	0.005	High
		Antineoplastic	0.737	0.020	High
		JAK2 expression inhibitor	0.730	0.014	High
		Antioxidant	0.587	0.005	Low
		TP53 expression enhancer	0.603	0.049	Low
		MMP9 expression inhibitor	0.571	0.019	Low
		Macrophage colony stimulating factor agonist	0.581	0.037	Low
		Caspase 8 stimulant	0.544	0.011	Low
7	Octanal dimethyl acetal	BRAF expression inhibitor	0.608	0.005	Low
		JAK2 expression inhibitor	0.559	0.040	Low
		Antineoplastic	0.502	0.071	Low
		TP53 expression enhancer	0.504	0.088	Low
		Caspase 3 stimulant	0.365	0.066	Low
8	Catechol	JAK2 expression inhibitor	0.910	0.003	High
		TP53 expression enhancer	0.731	0.020	High

No	Compound name	Biological activity	Pa	Pi	Criteria
		BRAF expression inhibitor	0.553	0.006	Low
		Caspase 8 stimulant	0.552	0.010	Low
		Antioxidant	0.501	0.007	Low
		Antiinflammatory	0.521	0.051	Low
9	5-Hydroxymethylfurfural	Macrophage colony stimulating factor agonist	0.655	0.021	Low
		Antineoplastic	0.644	0.036	Low
		TP53 expression enhancer	0.636	0.039	Low
		Apoptosis agonist	0.409	0.069	Low
10	Benzene, 1,3-bis(1,1-dimethylethyl)-	JAK2 expression inhibitor	0.749	0.013	High
		BRAF expression inhibitor	0.591	0.005	Low
		Antiinflammatory	0.614	0.029	Low
		HERG 1 channel blocker	0.558	0.014	Low
		MMP9 expression inhibitor	0.539	0.023	Low
		TNF expression inhibitor	0.539	0.022	Low
11	Isobornyl acetate	Transcription factor NF kappa B stimulant	0.521	0.019	Low
		TNF expression inhibitor	0.517	0.026	Low
		MMP9 expression inhibitor	0.513	0.026	Low
		Antineoplastic	0.474	0.079	Low
		Apoptosis agonist	0.441	0.055	Low
12	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	Apoptosis agonist	0.708	0.014	High
		Macrophage colony stimulating factor agonist	0.661	0.019	Low
		TP53 expression enhancer	0.664	0.033	Low
		Antineoplastic	0.648	0.035	Low
		MMP9 expression inhibitor	0.615	0.014	Low
		JAK2 expression inhibitor	0.633	0.026	Low
		TNF expression inhibitor	0.514	0.027	Low
13	α-Terpinyl acetate	Antiinflammatory	0.693	0.017	Low
		Antineoplastic	0.576	0.050	Low
		Macrophage colony stimulating factor agonist	0.506	0.062	Low
		Cytochrome P450 stimulant	0.335	0.089	Low
14	1,2,3-Benzenetriol	JAK2 expression inhibitor	0.898	0.003	High
		Apoptosis agonist	0.755	0.010	High
		Macrophage colony stimulating factor agonist	0.746	0.008	High
		TP53 expression enhancer	0.744	0.018	High
		MMP9 expression inhibitor	0.705	0.006	High
		Antioxidant	0.655	0.004	Low
		TNF expression inhibitor	0.581	0.015	Low
		Antineoplastic	0.572	0.051	Low
		BRAF expression inhibitor	0.511	0.008	Low
		Caspase 3 stimulant	0.507	0.026	Low
15	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	Macrophage colony stimulating factor agonist	0.776	0.006	High
		Chemosensitizer	0.520	0.017	Low
		JAK2 expression inhibitor	0.541	0.044	Low
16	3-Deoxy-d-mannoic lactone	Antineoplastic	0.822	0.009	High
		TP53 expression enhancer	0.775	0.014	High
		Immunostimulant	0.654	0.018	Low
		Chemosensitizer	0.504	0.019	Low
		MMP9 expression inhibitor	0.415	0.046	Low
		TNF expression inhibitor	0.419	0.051	Low
		Apoptosis antagonist	0.371	0.010	Low
		Antioxidant	0.348	0.017	Low
17	Diethyltoluamide	Macrophage colony stimulating factor agonist	0.600	0.032	Low
		MMP9 expression inhibitor	0.416	0.046	Low
		JAK2 expression inhibitor	0.416	0,080	Low
		Caspase 3 stimulant	0.324	0.098	Low
		Caspase 8 stimulant	0.336	0.101	Low
18	Octanal, 2-(phenylmethylene)-	BRAF expression inhibitor	0.825	0.002	High
		Macrophage colony stimulating factor agonist	0.746	0.009	High
		MMP9 expression inhibitor	0.651	0.010	Low
		JAK2 expression inhibitor	0.621	0.028	Low
		TP53 expression enhancer	0.623	0.041	Low
		Apoptosis agonist	0.536	0.034	Low
19	Benzyl Benzoate	JAK2 expression inhibitor	0.615	0,029	Low
		Caspase 8 stimulant	0.446	0.032	Low
		Antiinflammatory	0.459	0.070	Low
		Caspase 3 stimulant	0.401	0.050	Low
20	7,10,13-Hexadecatrienoic acid	Macrophage colony stimulating factor agonist	0.892	0.002	High
		BRAF expression inhibitor	0.828	0.002	High
		Antiinflammatory	0.804	0.006	High
		TP53 expression enhancer	0.731	0.020	High
		TNF expression inhibitor	0.713	0.006	High
		Apoptosis agonist	0.628	0.023	Low
		MMP9 expression inhibitor	0.600	0.016	Low
		Caspase 8 stimulant	0.579	0.008	Low
		Chemoprotective	0.349	0.030	Low

Table 8: Residues and binding 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	Borussertib (control)	TYR18 TYR272 THR291 PHE293	ASN279	GLY294 PHE293 LYS297 LYS20	LYS276	-11.5
Bioactive components of the nanoherbal senduduk bulu						
2	2-Cyclopenten-1-one, 2-hydroxy-	LEU275	-	LEU316 VAL330	-	-5.1
3	Glycerin	GLY294 TYR272 ASN279 THR291	-	-	-	-4.2
4	2-Hydroxy-gamma-butyrolactone	ASN279 THR291 PHF293 ASP292 GLY294	-	-	-	-4.8
5	Thymine	ALA317 LEU275	GLY334	LYS276 TYR315 LEU316	-	-5.5
6	Octane, 2-bromo-	-	-	TRP333 TYR18 VAL330 LEU316 VAL320 ALA317	-	-4.0
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-	-	ALA317 VAL330 VAL320 LEU316 TYR18	-	-4.1
8	Octanal dimethyl acetal	LEU275	GLY334 VAL330	TYR18	-	-4.4
9	Catechol	-	-	LEU316	-	-5.7
10	5-Hydroxymethylfurfural	LEU275	-	LEU316	-	-5.1
11	Benzene, 1,3-bis(1,1-dimethylethyl)-	-	-	-	ASP274	-5.7
12	Isobornyl acetate	ARG15	GLU17	ARG15	-	-5.5
13	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	-	-	LEU316 LYS276	-	-5.4
14	α-Terpinyl acetate	-	-	TYR272	-	-5.7
15	1,2,3-Benzenetriol	ASP274 LEU275	-	LEU316	-	-5.9
16	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	ARG15 GLY16 GLU85	-	-	-	-3.9
17	3-Deoxy-d-mannoic lactone	PHE293 THR291 ASP274	ASP292	-	-	-5.3
18	Diethyltoluamide	-	-	PHE293 MET281 LEU156 VAL164	GLU234 MET281	-5.0
19	Octanal, 2-(phenylmethylene)-	LYS276	-	TYR272 PHE293	-	-5.4
20	Benzyl Benzoate	ASN279 GLY294 PHE293	-	PHE293	ASP274	-7.0
21	7,10,13-Hexadecatrienoic acid	THR291 TYR272 PHE293 GLY294	-	VAL164 LEU156	-	-5.7

Molecular interactions of bioactive compounds from nanoherbal senduduk bulu with AKT1

Based on the results of molecular docking analysis comparing the control compound borussertib with various bioactive compounds from nanoherbal senduduk bulu as shown in table 8 (fig. 3 and 4), there is a significant variation in the types of interactions and binding energies with the target protein AKT1. Borussertib, with the highest binding energy (-11.5 kcal/mol), forms strong interactions through hydrogen bonds, electrostatic, and hydrophobic interactions with key residues such as TYR18, TYR272, THR291, and PHE293. This indicates that borussertib has a very strong affinity for

the AKT1 protein. The effectiveness of borussertib as a potent AKT1 inhibitor is supported by its interactions with important residues that play a role in inhibiting phosphorylation activity, which in turn affects the cell cycle and apoptosis. In previous studies, borussertib has been shown to suppress AKT1 phosphorylation activity, particularly in cancer cells dependent on AKT1, and significantly induce apoptosis [52–54]. The high binding energy of borussertib highlights its ability to stabilize the AKT1 structure in its inactive form, thereby inhibiting the signalling pathway regulated by AKT1. In the context of cancer therapy, AKT1 inhibition has significant clinical implications, as AKT1 is a key protein in signalling pathways that regulate cell growth and survival, which are often overactivated in cancer cells [55].

When compared to the bioactive compounds from senduduk bulu, compounds such as 1,2,3-Benzenetriol (-5.9 kcal/mol) and Benzyl benzoate (-7.0 kcal/mol) exhibit relatively moderate binding energies, although not as high as borussertib. These compounds still demonstrate significant interactions with key residues, including ASP274, LEU275, ASN279, GLY294, and ASP274. This suggests that, despite their lower binding affinities compared to borussertib, these compounds may still have potential to modulate AKT1 activity, particularly in the context of combination therapies where they could be used alongside stronger inhibitors to achieve synergistic effects.

Other compounds, such as glycerin (-4.2 kcal/mol) and octane, 2-bromo (-4.0 kcal/mol), show much lower binding energies and limited interactions with the target protein, mostly through hydrophobic interactions. This indicates a low affinity for AKT1, which is likely to result in less significant biological activity. Meanwhile, compounds like thymine (-5.5 kcal/mol) and 5-Hydroxymethylfurfural (-5.1 kcal/mol) demonstrate moderate binding energies, with a broader range of hydrogen and hydrophobic interactions, suggesting these compounds may have moderate activity towards AKT1.

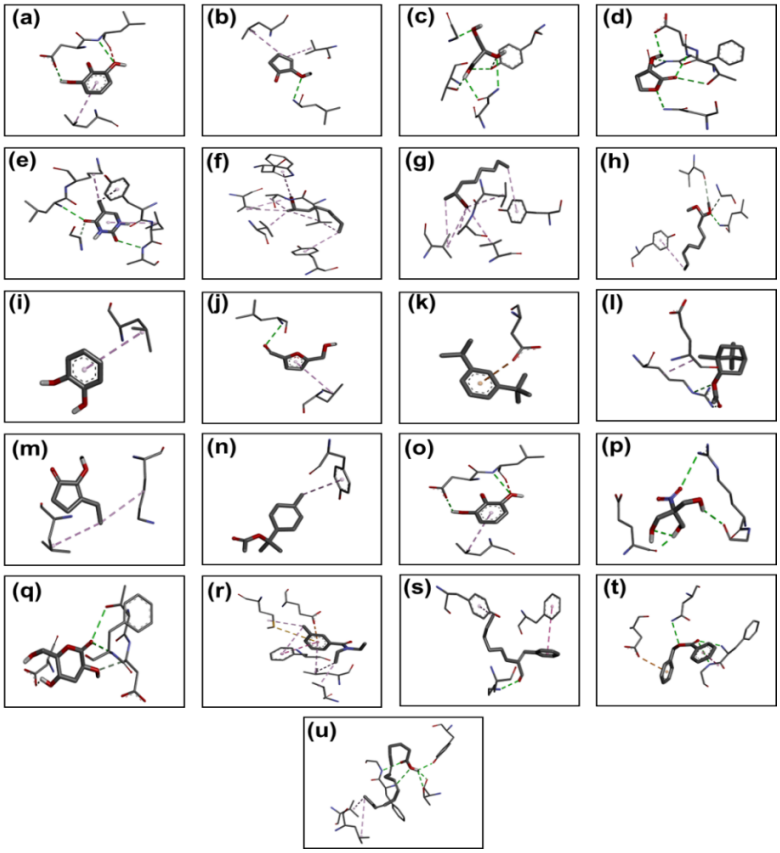

Fig. 3: Visualization 3D of interactions borussertib and bioactive compounds from nanoherbal senduduk bulu against the AKT1 protein. (a) borussertib; (b) 2-Cyclopenten-1-one, 2-hydroxy-; (c) Glycerin; (d) 2-Hydroxy-gamma-butyrolactone; (e) Thymine; (f) Octane, 2-bromo-; (g) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; (h) Octanal dimethyl acetal; (i) Catechol; (j) 5-Hydroxymethylfurfural; (k) Benzene, 1,3-bis(1,1-dimethylethyl)-; (l) Isobornyl acetate; (m) 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-; (n) α-Terpinyl acetate; (o) 1,2,3-Benzenetriol; (p) 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; (q) 3-Deoxy-d-mannoic lactone; (r) Diethyltoluamide; (s) Octanal, 2-(phenylmethylene)-; (t) Benzyl benzoate; (u) 7,10,13-Hexadecatrienoic acid

From these results, it can be concluded that compounds with higher binding energies, such as borussertib, generally exhibit stronger affinity and interactions with the target protein, making them more effective as AKT1 inhibitors. In contrast, compounds with lower binding energies, such as glycerin and octane, 2-bromo, demonstrate weaker affinity and are predicted to have limited biological activity. However, compounds with moderate affinity, such as 1,2,3-Benzenetriol and Benzyl benzoate, although not as potent as borussertib, still hold potential for contributing to AKT1 modulation, particularly when used in combination with other compounds. Overall, while the bioactive compounds from senduduk bulu may not possess the same potential as borussertib as standalone AKT1 inhibitors, they still show promising potential for modulating the AKT1 signalling pathway, which could be utilised in multi-target or combination therapeutic approaches. This study provides valuable preliminary insights into the potential interactions of bioactive compounds from senduduk bulu with the AKT1 protein through computational approaches, such as molecular docking and virtual screening. Although these findings are intriguing, the study also presents several important limitations, primarily the reliance on in silico methods, which may yield predictions that do not accurately reflect the actual biological activity within the body. Therefore, further experimental validation, including *in vitro* and *in vivo* studies, is essential to ascertain the therapeutic potential and safety of these compounds. Additionally, while Lipinski's rule is employed to predict the viability of compounds as drug candidates, successful drug development requires a more nuanced consideration of various factors.

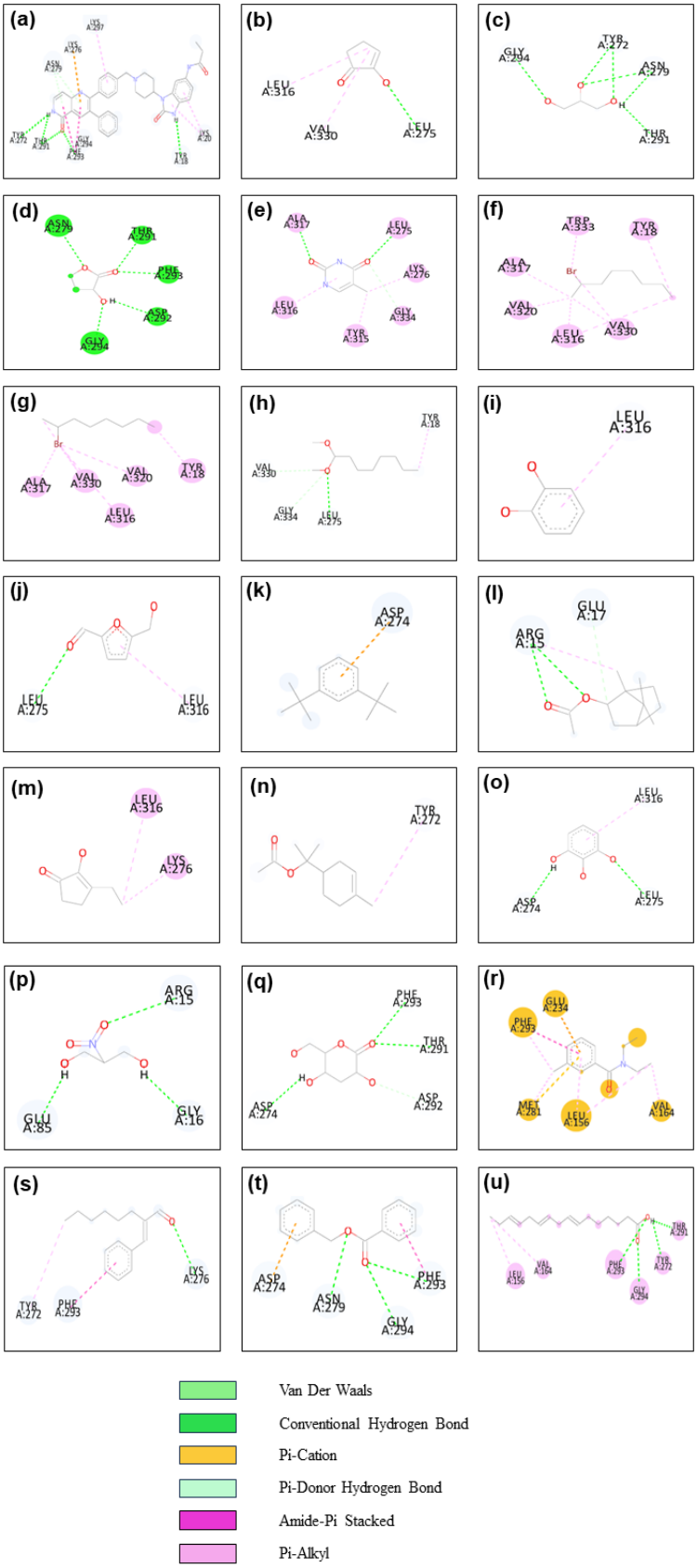

Fig. 4: Visualization 2D interactions borussertib and bioactive compounds from nanoherbal senduduk bulu against the AKT1 protein. (a) borussertib; (b) 2-Cyclopenten-1-one, 2-hydroxy-; (c) Glycerin; (d) 2-Hydroxy-gamma-butyrolactone; (e) Thymine; (f) Octane, 2-bromo-; (g) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; (h) Octanal dimethyl acetal; (i) Catechol; (j) 5-Hydroxymethylfurfural; (k) Benzene, 1,3-bis(1,1-dimethylethyl)-; (l) Isobornyl acetate; (m) 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-; (n) α-Terpinyl acetate; (o) 1,2,3-Benzenetriol; (p) 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; (q) 3-Deoxy-d-mannoic lactone; (r) Diethyltoluamide; (s) Octanal, 2-(phenylmethylene)-; (t) Benzyl Benzoate; (u) 7,10,13-Hexadecatrienoic acid

CONCLUSION

This research successfully identified 20 out of 36 compounds from senduduk bulu that meet the criteria to be considered as potential drugs and demonstrated their potential as AKT1 inhibitors in the context of cancer treatment through in silico analysis. Molecular docking tests revealed promising interactions between these compounds and the target protein AKT1, with affinity values that, while not surpassing that of the control drug borussertib, remain encouraging. ADMET analysis further highlighted that several compounds possess characteristics indicative of high oral bioavailability. Metabolically, these compounds are not substrates for the CYP2D6 enzyme in the liver, and none function as inhibitors of the OCT2 protein in the kidneys. The majority of the compounds from senduduk bulu exhibit good water solubility and are likely to be well absorbed by the human intestine. Regarding toxicity, there are indications of varying toxicity risks ranging from non-hazardous to hazardous, with some presenting potential risks for hepatotoxicity, mutagenicity, and carcinogenicity.

These findings underscore the potential of compounds from senduduk bulu in the development of innovative cancer therapies, while also highlighting the need for further research to experimentally explore the biological effects of these compounds. Therefore, this study provides a solid foundation for future development, which could include *in vitro* and *in vivo* studies to validate the efficacy and safety of these compounds. Through these efforts, it is hoped that these findings can be translated into clinical applications, potentially enhancing cancer treatment strategies and improving outcomes for patients battling this disease.

ACKNOWLEDGMENT

We extend our deepest gratitude to the Ministry of Education, Culture, Research, and Technology for their generous support through the PMDSU (Master to Doctoral Education) Batch VII Scholarship Program. This invaluable assistance has greatly facilitated our research and academic progress at the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. We are sincerely thankful for this opportunity and for the guidance provided, which has been instrumental in our academic journey.

FUNDING

This work received financial support from The Directorate of Research, Technology, and Community Service (DRTPM) Kemendikbudristek grant number: 87/UN5.4.10. S/PPM/KP-DRTPM/2024.

AUTHORS CONTRIBUTIONS

As the lead author, Dina Khairani spearheaded the project, designed the research framework, drafted the initial manuscript, and ensured its coherence. Syafruddin Ilyas, as the corresponding author, polished the content, ensured the manuscript's accuracy and proper English gmar, and managed the submission process and journal correspondence. Dini Prastyo Waty as co-author contributed writing and editing. All authors reviewed and approved the final manuscript, contributed to critical discussions, and provided valuable input to enhance the paper's quality.

CONFLICT OF INTERESTS

The authors hereby declare that there are no conflicts of interest, either financial or non-financial, that could have influenced the research outcomes or the interpretation of data in this study.

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