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RESEARCH ARTICLE

Natural Cycloartane Triterpenoids from *Corypha utan* Lamk. with Anti-cancer Activity towards P388 Cell Lines and their Predicted Interaction with FLT3

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ARTICLE HISTORY

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Abstract: ***Background:*** Cancer is the second leading cause of death in the world. Leukemia is a type of cancer that accounts for 31.5% of all cancers in children under the age of 15 in industrialized countries and 15.7% in developing countries. The inhibition of FMS-like tyrosine kinase 3 (FLT3) is a suitable approach for acute myeloid leukemia (AML) therapy as it is overexpressed in AML.

Aim and Objective: This study intends to explore the natural constituents from the bark of *Cory- pha utan* Lamk., and assess their cytotoxicity on murine leukemia cell lines (P388) in addition to predicting their interaction with FLT3 as a studied target by computational methods.

Methods: Compounds **1** and **2** were isolated from *Corypha utan* Lamk using the stepwise radial chromatography method. These compounds were assessed for their cytotoxicity against *Artemia salina* using the BSLT and P388 cells and the MTT assay. The docking simulation was employed to predict the possible interaction between triterpenoid and FLT3.

Results: Isolation from the bark of *C. utan* Lamk. generated two triterpenoids, cycloartanol (**1**) and cycloartanone (**2**). Based on the *in vitro* and *in silico* studies, both compounds were found to have anticancer activity. The evaluation of cytotoxicity from this study reveals that cycloartanol (**1**) and cycloartanone (**2**) could inhibit P388 cell growth (IC_{50} value at 102.6 and 110.0 µg/mL, respectively). The binding energy of cycloartanone was -9.94 Kcal/mol with a Ki value of 0.051 µM, while the binding energy and Ki value of cycloartanol (**1**) were found to be

8.76 Kcal/mol and 0.38 µM, respectively. These compounds also demonstrate a stable interaction by forming hydrogen bonds with FLT3.

Conclusion: Cycloartanol (**1**) and cycloartanone (**2**) exhibit potency as anticancer agents by inhibiting P388 cells *in vitro* and the FLT3 gene *in silico*.

Keywords: *Corypha utan* Lamk., cycloartane triterpenoids, acute myeloid leukemia, FLT3.

1. INTRODUCTION

Cancer has the second-highest mortality rate, especially in developing countries [1]. Approximately 18.1 million newly diagnosed cases of cancer, followed by 9.9 million deaths, were reported in 2020 alone [2]. Acute myeloid leukemia (AML) is a cancer disease characterized by neoplastic transformation and the impaired differentiation of progenitor cells from myeloid cells. Overall, AML is the most common leukemia in adults, and is five times more common in people under 50 years of age [3,4]. The recent standard intervention for leukemia is limited to chemothera- py and the transplantation procedure of hematopoietic stem cells [5]. Although remission can be achieved with numerous approved chemo-drugs, various problems continually arise during treatment. Drug resistance, hepatotoxicity, and highly frequent relapse after transplantation may occur in the pa-

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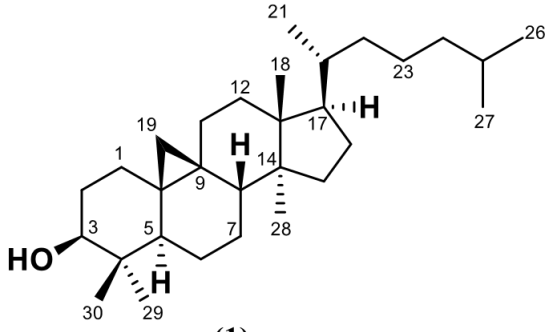
tient [6, 7]. In AML, there are heterogeneous disorders of hematopoietic progenitor cells, and these disorders have been identified to affect the balance between cell prolifera- tion, survival, and differentiation. This abnormality results in the expansion of the abnormal stem cell clone. Although many controls regulate hematopoiesis, mutations in regulato- ry genes are capable of promoting leukemogenesis. The FLT3 receptor tyrosine kinase plays an important role in controlling the survival, proliferation, and differentiation of hematopoietic cells [8]. Therefore, the search for compounds that can block these receptors can be an option for induction chemotherapy in AML patients. This method is estimated to be safer than classical chemotherapy, which can cause direct damage to the bone marrow and also reduce the production of platelets [9,10].

FMS-like tyrosine kinase 3 (FLT3), a class III receptor tyrosine kinase (RTK), generates between the normal and ferocious lymphohematopoietic cells [11]. This receptor plays a crucial part in the proliferation of stem cells and the activation of immune responses [12]. The deregulation of FLT3 activity owing to overexpression or mutation is associated with acute myeloid leukemia (AML) growth and poor prognosis [12,13]. Two groups of mutations occur in FLT3: an internal tandem duplication (ITD) located in the juxtamembrane domain and a point mutation in the kinase activation loop [14]. Typically, most mutated FLT3 receptor tyrosine kinases found in AML are induced by the ITD [7]. Cancer cells with FLT3 mutations are commonly dependent on FLT3 for development. This makes FLT3 a favorable inhibitor target for AML therapy.

Natural products are generated by organisms found in nature, such as plants. They are known to have abundant biological effects that have the potential for disease prevention or treatment. The utilization of herbal plants has long been recognized by our ancestors to relieve varied illnesses [15,16]. Therefore, herbal plants can be used as a promising source of medicine. This is in line with the purpose of drug research and development, which emphasizes the active and safe aspects of drug use [17]. Plant-derived compounds have obtained remarkable attention as alternative agents since they have demonstrated a broad range of pharmacological actions [18,19]. The applications of computational ap- proaches have been increasingly elevated to assess the bio- logical potency of multiple natural constituents and in the development of novel drug molecules [20]. Computational docking is a cogent strategy for designing new therapeutic molecules that are more efficient compared to conventional procedures and predicting their interaction with receptors [21]. Generally speaking, this method may help in selecting the important genes from a large volume of genomic data, providing possible target proteins for drug screening and design. It is feasible to explore the structural and thermody- namic properties of target proteins of various sizes using multiscale models in biomolecular simulations, which will aid in identifying drug binding sites and elucidating drug action mechanisms. Virtual screening helps in searching chemical libraries to find possible therapeutic possibilities using drug-binding sites on target proteins. With a substan- tially smaller pool of prospective drug candidates, *in vitro* cell assays can evaluate the effectiveness of these molecules further [22].

Triterpenoids, one of the natural products widespread in higher plants, have been known to show significant antioxidant [23,24], anti-inflammatory [25], antiproliferative [26-28], and anti-cancer properties [29-31]. Several studies have proved the cytotoxic activity of various types of triterpenoids. For example, (+)-barringtogenol B derived from *Cyrilla racemiflora* L. and (+)-cucurbitacin D from *Elaeocarpus chinensis* showed strong effects in inhibiting human colon cancer HT-29 cell lines with IC_{50} values of 1.7 and 0.12 µM, respectively [30-32]. The (+)- ursolic acid, a major constituent of *Syzygium corticosum,* displayed cytotoxicity toward MDA-MB-231 breast cancer cells with an IC_{50} value of 5.9 µM [33]. Ginsenoside derivatives from *Panax ginseng* were found to be active, showing IC_{50} values of 0.8-0.9 µM for HL-60 leukemia cells [34]. (+)-Acutissmatriterpene E of *Phyllanthus acutissima* Miq. could effectively inhibit murine leukemia P388 cell growth with an IC_{50} value of 0.005 µg/mL [35]. Thus, plant triterpenoids have a potential role as anticancer and anti-leukemic agents.

In our ongoing effort to find bioactive constituents from Indonesian plants, we focused on investigating the phytochemicals of *Corypha utan* Lamk. This species is a native tree and is found abundantly in Nusa Tenggara Province [36]. The prior study disclosed piceatannol from the seeds that demonstrated strong inhibition on P388 murine leuke- mia cells with an IC_{50} value of 1.56 µg/mL [37]. This paper presents two triterpenoids, cycloartanol (**1**) and cycloartanone (**2**), isolated from the bark of *C. utan* Lamk, and which belong to the family Arecaceae (Fig. **1**). The biologi- cal evaluation of these compounds was conducted using the MTT method. To understand their interaction with active sites of FLT3 as the target receptor, we employed molecular docking simulations.



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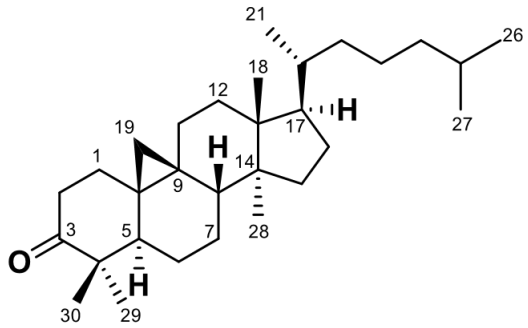

(2)

Fig. (1). Chemical structures of cycloartanol (**1**) and cycloartanone (**2**).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant

The bark of *C. utan* Lamk. was collected from Bu'at Soe, South Middle Timor, East Nusa Tenggara, Indonesia. The plant was authenticated at the Herbarium Bogoriense, Research Center for Biology in the Indonesian Institute of Sciences (LIPI), Bogor, Indonesia.

2.1.2. Cell lines

P388 murine leukemia cell lines were obtained from the Natural Product Chemistry Laboratory's collections at the Institut Teknologi Bandung (ITB), Indonesia, and their uses had been acceded by the Research Ethics Committee ITB.

2.1.3. Chemicals

Organic solvents of acetone, chloroform, dichloromethane, ethyl acetate, methanol, *n*-hexane, and water were used for extraction and purification procedures. Analytical grade chemicals (Merck) were used for spectroscopic analysis. Column chromatography separation employing silica G 60 (particle size 15-40 μm) and ODS RP-18 (particle size 0.040-0.63 mm), and then silica G 60 F_{254} and ODS RP-18 $F_{254}S$, was used for TLC monitoring. The plates were sprayed with 10% sulfuric acid solution in ethanol and heated for chemical detection.

Brine shrimp larvae of *A. salina* L. were used in this study as a preliminary test to determine the toxicity of extracts and compounds. The eggs were purchased from the research center at Universitas Padjadjaran, Indonesia.

2.1.4. Instruments

Several spectrometer measurements were used to identify the molecular structures of **1** and **2**, including a Varian Cary 100 UV-visible spectrophotometer and a Perkin-Elmer Spectrum One Fourier transform infrared (FT-IR) instrument.

NMR spectra were recorded at 25°C on a JEOL ECA-500 (Tokyo, Japan) spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Mass spectrometry (MS) was performed with a Waters LCT XE ESI-TOF instrument (Waters, Milford, MA, USA). UV detector lamps with wavelengths of 254 and 365 nm were used to visualize TLC. The bioassay procedure was carried out using 96-well microplates, micropipettes, microtubes, a centrifuge, an incubator, and a microplate reader.

2.2. Materials: *In silico* Assay

The crystal structure of the FLT3 gene (PDB ID: 4RT7 resolution 2.4 Å) with a co-crystallized ligand used for a molecular modeling study was freely accessible through the Protein Data Bank. The structures in the PDB format were downloaded from the RSCB Protein Data Bank page (https://www.rcsb.org/). The AutoDock Vina 1.5.6 in the PyRx 0.8 software was applied for the docking simulation. The ligands of compounds **1** (CID: 12760132) and **2** (CID: 21594789) with Artonin E (CID: 5481962) as a positive control ligand were downloaded in an SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and exchanged to a PDB format with Chem3D Pro 12.0 program.

3. EXPERIMENTAL

3.1. Isolation of Triterpenoids from the Bark of C. *utan* Lamk.

The air-dried *C. utan* Lamk. bark was ground to powder, obtaining two kilograms of bark powder of *C. utan* Lamk. The sample was macerated using methanol 95% for three days at room temperature (3 15 L) and evaporated under vacuum using a rotavapor (Büchi, Hamburg, Germany) to obtain the crude methanolic extract. The extract (20 g) was separated by vacuum liquid chromatography (VLC) using 10% gradient *n*-hexane-ethyl acetate, yielding seven fractions (A-G). Fraction A (7.48 g) was separated by VLC using a 10% gradient *n*-hexane-chloroform to give seven fractions (A1-A7). Fraction A4 was evaporated, giving a white crystal solid, identified as compound **1** (60 mg). Fraction A7 (60 mg) was further purified with the radial chromatography method using a stepwise *n*-hexane-chloroform system, obtaining compound **2** (13 mg).

3.2. Brine Shrimp Lethality Test (BSLT) for the Compounds

Brine shrimp larvae of *A. salina* L. were hatched in saline solution and distilled water (1:1) and then placed into a container. The larvae were incubated for 48 h, after which they could be used for the lethality bioassay. The examination of the compounds' lethality was conducted using BSLT against *A. salina* following the published procedure [38]. Each compound solution in DMSO was placed in 96-well plates at concentrations of 1, 10, 100, and 1000 ppm. Each concentration was made in three replications. Ten shrimp larvae were added to each well and observed after 24 h. The negative control used distilled water with 1% DMSO (v/v). The number of deaths was calculated after 24 hours of exposure, analyzed by the probit analysis method, and then converted to an LC_{50} value. The plant extracts were classified as lethal if they had an LC_{50} value lower than 1000 ppm.

3.3. Cytotoxic Evaluation Using the MTT Assay

For the MTT assay, P388 cells were placed in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were incubated in a 5% CO_2 incubator at 37°C for 24 h. The medium was replaced by fresh media and re-incubated in the same condition to achieve an adequate number of cells for testing. Afterward, the cells were rinsed with FBS and the RPMI medium was added. The cells were put into tubes and then centrifuged at 170 x *g* for 5 minutes. The pellets obtained were then replenished with RPMI medium containing 10% FBS. The cell density was determined by counting with a hemocytometer, and the dilution was made by adding the RPMI medium to obtain a cell suspension of 2×10^4 cells/mL.

The P388 murine leukemia cells in the RPMI medium (cell density of 2.0×10^4 cells/well) were sowed and then inoculated into the 96-well microplates. The cells were washed with FBS after 24 h of incubation, and samples were added to the wells.

DMSO was used to dissolve the samples, including a compounds test and artonin E as a positive control, at the

required concentration. Three replicates were prepared for each concentration. The well of the blank control was filled with DMSO in the media. The medium was removed after being incubated for 48h, and the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) solution was appended to each well. In a 37°C, 5% CO_2 incubator, the plate was incubated for four hours. The stop solution com- prising sodium dodecyl sulfate (SDS) was added and then incubated for 24 h. The measurement of the optical density of the cells was done by utilizing a microplate reader to de- termine the cell viability at 570 nm. The IC_{50} values were determined by plotting the percentage of viable cells versus the control against sample concentration (g/mL). The IC_{50} value is the concentration of a drug that half-inhibits a bio- logical process [39].

3.4. Evaluation of the Anticancer Activity by *In silico* Study

Virtual screening for predicting the interaction of compounds **1** and **2** was done by utilizing the AutoDock Vina (version 1.5.6) incorporated in the PyRx 0.8 software. The compounds and artonin E as ligands simulated to the FLT3 as the target receptor were evaluated, and the ligand was left unattached for docking simulations. Binding affinity was employed to determine the conformation. The root-mean-square deviation with a value of less than 2.5Å with the lowest binding energy was selected [40].

PYMOL was used to visualize the docking data, and the Discovery Studio 2020 Client software was used to assess them. The PYMOL program displays the interaction between the ligand and the residue and the docking position in the three-dimensional molecular figure. Those interactions were then visualized by applying the Discovery Studio 2020 Client software in three-dimensional molecular graphics for maximum display. Each complex of protein-ligand docking position was contrasted to the three-dimensional FLT3 that binds ligands. The most powerful compound was chosen as it has the lowest binding energy and stable hydrogen bond- ing [9].

4. RESULTS

4.1. Structural Determination of Compounds Isolated from the Bark of *C. utan* Lamk.

Spectral data of **1** (Supplementary Material) were as follows: IR: 3332, 2954, 1448, 1379 cm^{-1}; HR-TOFMS $[M+H]^+$ (*m/z*) 429.4062; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 1.26 (1H, dd, 5.5, 8.5 Hz, H-1a), 1.55 (1H, d, 6.5 Hz, H-1b), 1.56 (1H, s, 9.0 Hz, H-2a), 1.75 (1H, m, H-2b), 3.27 (1H, t, 5.5 Hz, H-3), 1.30 (1H, d, 6.5 Hz, H-5), 0.78 (1H, m, H-6a), 1.59 (1H, dd, 3.5, 10.0 Hz, H-6b), 1.07 (1H, dd, 2.5, 12.5 Hz, H-7a), 1.32 (1H, m, H- 7b), 1.52 (1H, d, 4.0 Hz, H-8), 1.11 (1H, m, H-11a), 1.99 (1H, m, H-11b), 1.62 (2H, dd, 7.0, 10.0 Hz, H-12), 1.29 (2H, m, H- 15), 1.28 (1H, m, H-16a), 1.88 (1H, m, H-16b), 1.57 (1H, t, 9.0 Hz, H-17), 0.96 (3H, s, H-18), 0.33 (1H, d, 4.0 Hz, H-19a), 0.55 (1H, d, 4.0 Hz, H-19b), 1.36 (1H, m, H-20), 0.86 (3H, d, 2.5 Hz, H-21), 1.00 (1H, m, H-22a), 1.37 (1H, dd, 1.5, 7.0 Hz, H-22b), 1.16 (1H, m, H-23a), 1.35 (1H, dd, 2.0, 12.0 Hz, H- 23b), 1.13 (2H, m, H-24), 1.51 (1H, d, 6.0 Hz, H-25), 0.88 (3H, d, 2.5 Hz, H-26), 0.87 (3H, d, 3.0 Hz, H-27), 0.81 (3H, s, H-28), 0.96 (3H, s, H-29), 0.89 ppm (3H, s, H-30); ^{13}C- NMR (125 MHz, $CDCl_3$): δ_C 31.9 (C-1), 30.3 (C-2), 78.8 (C- 3), 40.4 (C-4), 47.1 (C-5), 21.1 (C-6), 26.0 (C-7), 48.0 (C-8), 20.0 (C-9), 26.0 (C-10), 26.5 (C-11), 32.9 (C-12), 45.2 (C-13), 48.7 (C-14), 35.5 (C-15), 28.1 (C-16), 52.4 (C-17), 18.0 (C- 18), 29.9 (C-19), 36.1 (C-20), 18.3 (C-21), 36.4 (C-22), 24.1 (C-23), 39.5 (C-24), 28.0 (C-25), 22.8 (C-26), 22.5 (C-27), 14.0 (C-28), 25.4 (C-29), 19.3 ppm (C-30).

Spectral data of **2** (Supplementary Material) were as follows: IR: 2961, 1710, 1465, 1379 cm^{-1}; HR-TOFMS $[M+H]^+$(*m/z*) 427.3918; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 1.52 (1H, m, H- 1a), 1.85 (1H, td, 4.0, 13.5 Hz, H-1b), 2.28 (1H, dt, 3.5, 14.0 Hz, H-2a), 2.69 (1H, td, 6.5, 13.5 Hz, H-2b), 1.69 (1H, dd, 4.5, 12.5 Hz, H-5), 0.96 (1H, q, 12.0 Hz, H-6a), 1.54 (1H, m, H- 6b), 1.12 (1H, q, 12.0, H-7a), 1.3 (1H, m, H-7b), 1.58 (1H, dd, 5.0, 12.5 Hz, H-8), 1.18 (1H, m, H-11a), 2.02 (1H, m, H-11b), 1.64 (2H, m, H-12), 1.29 (2H, m, H-15), 1.28 (1H, m, H-16a), 1.89 (1H, m, H-16b), 1.57 (1H, d, 7.5 Hz, H-17), 0.98 (3H, s, H-18), 0.56 (1H, d, 4.5 Hz, H-19a), 0.77 (1H, d, 4.0, H-19b), 1.36 (1H, m, H-20), 0.85 (3H, d, 6.5 Hz, H-21), 1.00 (1H, t, 9.5 Hz, H-22a), 1.35 (1H, m, H-22b), 1.09 (1H, m, H-23a), 1.27 (1H, m, H-23b), 1.13 (2H, m, H-24), 1.52 (1H, q, 1.5 Hz, H-25), 0.86 (3H, s, H-26), 0.85 (3H, s, H-27), 1.03 (3H, s, H- 28), 1.08 (3H, s, H-29), 0.90 ppm (3H, s, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 33.4 (C-1), 37.4 (C-2), 216.4 (C-3), 50.2 (C-4), 48.4 (C-5), 21.5 (C-6), 25.8 (C-7), 47.9 (C-8), 21.0 (C-9), 25.9 (C-10), 26.7 (C-11), 32.8 (C-12), 45.2 (C-13), 48.7 (C-14), 35.5 (C-15), 28.1 (C-16), 52.4 (C-17), 18.0 (C-18), 29.5 (C-19), 36.0 (C-20), 18.3 (C-21), 36.4 (C-22), 24.1 (C- 23), 39.5 (C-24), 28.0 (C-25), 22.8 (C-26), 22.5 (C-27), 22.1 (C-28), 20.7 (C-29), 19.3 ppm (C-30).

Compound **1** was a white crystal solid and was established as $C_{30}H_{52}O$ from an HR-TOFMS peak at *m/z* 429.4062, $[M+H]^+$ (calcd. *m/z* 429.4096), representing five degrees of unsaturation. The IR spectrum exhibited hydroxyl groups at ν_{max} 3332 cm^{-1}, alkane C-H at ν_{max} 2954 $cm^{-1,}$ and *gem*-dimethyl groups at ν_{max} 1448 and 1379 cm^{-1}. A chemical shift in the ^{1}H-NMR revealed signals of typical protons for cyclopropane methylene at δ_H 0.33 and 0.55 ppm (1H, *d*, *J*=4.0 Hz), an oxymethine proton at δ_H 3.27 (1H, *t*, *J*=5.5 Hz), a secondary methyl proton at δ_H 0.86 (3H, *d*, *J*=2.5 Hz), and four tertiary methyl groups at δ_H 0.81 (3H, *s*), 0.89 (3H, *s*), and 0.96 ppm (3H, *s*, 2), respectively. These signal pro- tons were found to be identical to the triterpene structures for the cycloartane-type triterpenes [39]. The ^{13}C-NMR and DEPT spectra presented thirty carbon signals involving sev- en methyls, twelve methylenes, five methines, five quater- nary carbons, and an sp^3 oxygenated carbon, which also in- dicated a cycloartane-type triterpene with five cyclic pro- tons. The HMBC correlation (Fig. **2**) between H-1, H-2, H- 30, and C-3 revealed the position of a hydroxyl group at C-3. The presence of a cyclopropane ring attached to ring B sug- gested the characteristics of the cycloartane triterpenoids, revealed by the HMBC correlation between H- 19 and C-1, C-5, C-8, C-9, and C-10. Further identification was observed by the NOESY relationships between H-3 and H-5, H-28, and H_α-19 and H-8, H-18 shown in the hydroxyl groups at- tached to C-3 and the cyclopropane ring at C-19 in the β- configuration (Fig. **3**). The NMR data interpretation along with mass spectrometry data, in comparison to previously

published papers [41,42], suggested compound **1** to be cycloartanol (Fig. **1**). This compound was identified for the first time in the *Corypha* genus and Arecaceae family.

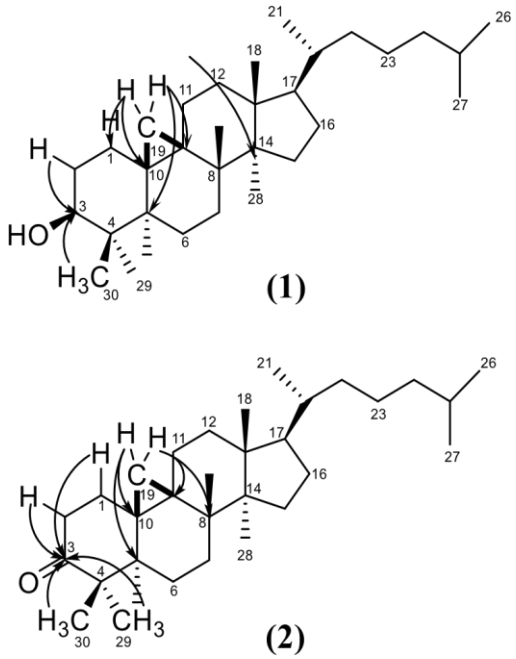

Fig. (2). Chosen hetero-nuclear multiple bond correlations (HMBC) of **1** and **2**.

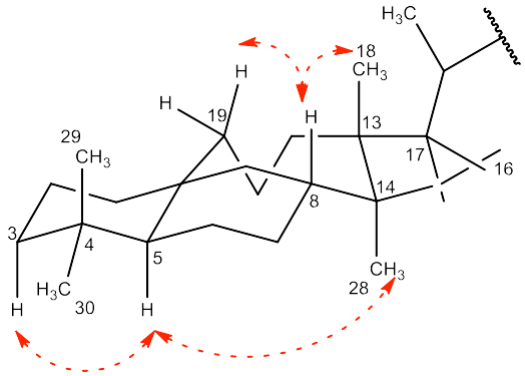

Fig. (3). Selected nuclear over-hauser effect spectroscopy (NOESY) correlations for **1**.

Compound **2** was achieved as a white crystal, and the HR-TOFMS showed (*m/z*) a value of 427.3918 (calcd. *m/z* 427.3940, established as $C_{30}H_{50}O$) based on the $[M+H]^+$ peak), displaying five degrees of unsaturation. The presence of alkane C-H was observed in the IR spectrum of **2** at ν_{max} 2961 cm^{-1}, ν_{max} 1710 cm^{-1} for the carbonyl group, and *gem*-dimethyl groups at ν_{max} 1465 and 1379 cm^{-1}, respectively. The ^1H and ^{13}C- NMR spectra indicated compound **2** to have a similar structure to compound **1**, except for the carbonyl group attached to C-3. This was also revealed by the HMBC correlation (Figure 2) between H-1, H-2, H-29, H-30, and C-3. Another HMBC correlation between H-19 and C-5, C-8, C-9, and C-10 indicated a typical cyclopropane ring in the B ring structure of cycloartane. Based on these data, compound **2** was identified as cycloartanone, as can be seen in Fig. (**1**). This compound was also successfully isolated first from the *Corypha* genus and the Arecaceae family.

4.2. Lethality Test of Cycloartane Triterpenoids Toward *Artemia salina*

BSLT was applied as a preliminary study to evaluate the toxicity of samples against *A. salina* larvae. The probit analysis results of each larva's lethality percentage are expressed as an LC_{50} value, as shown in Table **1**.

Table 1. Toxicity of methanol extract and compounds derived from the bark of *C. utan* Lamk.

Samples	LC_{50} (µg/mL)
Methanol bark extracts	81.40
Cycloartanol (1)	183.57
Cycloartanone (2)	197.23

Table **1** demonstrates methanol extract to have an LC_{50} value of 81.4 µg/mL, which indicates the bark extract of *C. utan* Lamk. to have a toxic effect on *A. salina* larvae. Hence, this extract was further isolated, resulting in compounds **1** and **2**. The LC_{50} value of each cycloartane was found to be higher than 100 µg/mL. These compounds exhibited toxic effects by eliminating 50% of the larvae population. A cyto- toxic assay is needed to ensure their potential as anticancer agents.

4.3. MTT Assay of the Cycloartanes from the Bark of C. *utan* Lamk.

The cytotoxicity profile of the compounds toward P388 murine leukemia cell lines was assessed following the reported studies [37]. The cytotoxicity profile of methanol extract and compounds derived from the bark of *C. utan* Lamk. is provided in Table **2** and Fig. (**4**) below. A previous study reported the cycloartane group to have some bioactivities, such as anti-proliferative and anti-cancer activity. Nevertheless, this group was developed to suppress inflammatory pathways linked to tumorigenesis [43]. Mangiferolic acid, a cycloartanol from propolis, inhibits the growth of the LU-1 cancer cell line with an IC_{50} value of 13.33 µg/mL [44].

Table 2. The IC_{50} values of methanol extract and compounds from *C. utan* Lamk. bark.

Samples	IC_{50} (µg/mL)
Methanol bark extracts	108
Cycloartanol (1)	102.6
Cycloartanone (2)	110.0
Artonin E	0.3

Based on Table **2**, compound **1** had a lower IC_{50} value than **2**. Although the IC_{50} value of each compound was much higher, it could be observed that compound **1** could restrain the growth of P388 cells. The anticancer effect of artonin E against P388 cell lines was also evaluated as a positive control. These assay results support the earlier lethality test data.

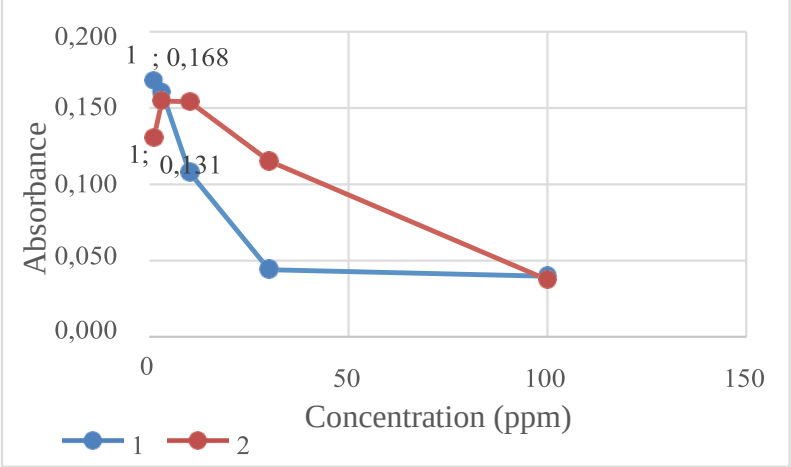

Fig. (4). Graph of the MTT assay for cycloartanol (**1**) and cycloartanone (**2**).

4.4. Molecular Docking Simulation of the Cycloartanes toward the FLT3 Gene

A docking simulation approach investigates the isolated compounds belonging to cycloartane triterpenoids for their inhibition activity against the FLT3 gene as molecular targets. The FLT3 gene is one of the receptor tyrosine kinase members, overexpressed largely on the AML [45]. The activated FLT3 had active site coordinates X, Y and Z of - 38.825, 11.685, and -15.423, respectively. It was used as a reference to investigate the potential of the FLT3 inhibitor in this docking investigation. Binding affinity and inhibition constants (Ki) were analyzed to identify the interactions between each compound and FLT3, as shown in Table **3**.

Table 3. Data of ligand-receptor interactions between the isolated compounds and the FLT3 gene.

Samples	Binding Affinity (Kcal/mol)	Ki Value (µM)
Cycloartanol (**1**)	-8.76	0.38
Cycloartanone (**2**)	-9.94	0.051
Artonin E	-11.25	0.0056

Compounds **1** and **2** interacted with the FLT3, as shown by the binding affinity between the triterpenoids and the receptor, respectively. Compound **2** exhibited stronger interaction with the FLT3 and a lower Ki value than **1**. Nevertheless, in this study, they did not form as strong as the interaction of artonin E, a positive control. The Ki value of both triterpenoids was also much higher than the positive control. Although artonin E exhibited maximum inhibition with the FLT3, it could be observed that compounds **1** and **2** belonging to triterpenoid also displayed a binding interaction with the FLT3 gene and could inhibit the target receptor with a low Ki value.

It is also supported by amino acid residues that interacted around the ligand-receptor complex, as can be seen in Figure 5 (3D interaction). Compound **2** exhibited a greater hydro- gen interaction with FLT3 than **1**. No amino acid residue formed hydrogen bonds with **1** as the ligand and FLT3 receptor. Amino acid residues that formed the bonds with the ligands are shown in Table **4**. A molecular docking program can identify the interaction type found in each compound-FLT3 receptor complex (Fig. **5**).

Table 4. Amino acid residue interactions in the ligand-receptor complex.

Ligand	Residue at Ligand–Receptor Complex
Cycloartanol (**1**)	Val624, Ala642, Val675, Leu818
Cycloartanone (**2**)	**Asp829**, Leu616
Artonin E	**Asp698,** Leu818, Leu616, **Cys694, Glu692,** Ala642, **Tyr693,** Val624, Val642, Val675

Bold text within the table reflects the hydrogen interaction formed between amino acid residues and each ligand (Fig. **5**).

4. DISCUSSION

This present study focused on exploring the natural constituents isolated from *C. utan* Lamk., and assessing their potential anticancer activity *in vitro* and *in silico*. A bioac- tive assessment was conducted as a guiding assay, which showed the compounds as anticancer agents, and their struc- tures were identified as cycloartanol (**1**) and cycloartanone (**2**) (Fig. **1**). These compounds were successfully isolated from the bark for the first time. Several approaches have been employed to investigate their cytotoxicity in inhibiting cancer cell growth. The toxicity assay was carried out first using *A. salina* and the BSLT method. This is an easy, rapid, and low-cost procedure to determine the toxicity level of extract or plant-derived compounds [46]. A positive linear relationship was detected between the BSLT and cytotoxic assay against various cell lines, although it did not represent certain cancer cells. Thus, this test may be utilized to evalu- ate the toxicity effect of natural products [47]. The methanol bark extract showed moderate toxicity with an LC_{50} value of 81.4 µg/mL (Table **1**). Cycloartanol (**1**) and cycloartanone (**2**) exhibited toxicity effects on *A. salina*, although they

were lower than the extract. The plant extract was consid- ered toxic if it had an LC_{50} range of 30-1000 µg/mL and no toxicity with an LC_{50} value >1000 µg/mL [48]. For further evaluation, the cytotoxic activity of **1** and **2** was examined against P388 cell lines.

Cycloartane triterpenoids derived from the bark of *C. utan* Lamk. were determined for their anticancer activity using the MTT assay. P388 murine leukemia cell lines were used in this study as cancer cell targets. The IC_{50} values of each sample used that was achieved from the MTT assay are provided in Table **2**. Both triterpenoids displayed cytotoxic effects, inhibiting the cancer cell viability with IC_{50} values of 102.6 and 110.0 µg/mL, respectively. The condition of a free hydroxyl group attached to C-3 made it more possible for compound **1** to contribute to inhibiting P388 cell lines than compound **2**. However, the anticancer activity of both compounds was substantially weaker than that of the positive control, artonin E (IC_{50} value of 0.3 µg/mL). It is a prenylated flavonoid that is known to have an antiproliferative activity on different cancer cells, including P388 cell lines [49, 50].

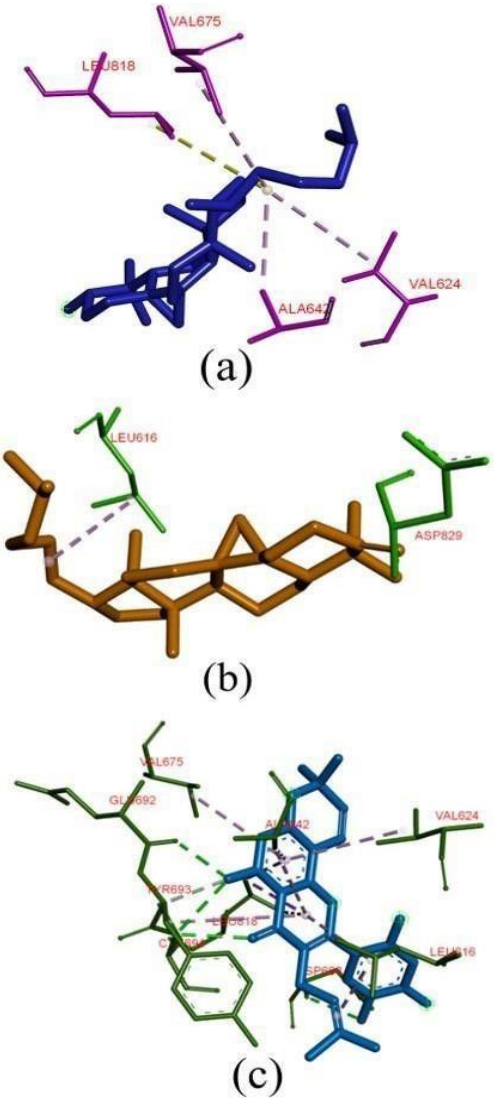

Fig. (5). Three-dimensional visualization of the binding position of each ligand: cycloartanol (**a**), cycloartanone (**b**), and artonin E (**c**) inside the FLT3 (PDB ID: 4RT7).

Essentially, the goal of molecular docking is to use computational techniques to anticipate the structure of the lig- and-receptor complex. To perform docking, two related processes must be taken: first, the ligand's conformations in the protein's active site must be sampled; next, these conformations must be ranked using a scoring function [51]. According to the "inducing fit" idea, the process of ligand and receptor recognition depends on spatial shape matching and energy matching. Roughly speaking, there are three types of molecular docking: rigid, semi-flexible, and flexible. The molecule structure is unaltered during rigid docking. Because of the calculating method's relative simplicity and focus on conformation matching, macromolecular systems, like protein-protein and protein-nucleic acid systems, are better suited for investigation. Semi-flexible docking is more suited to handle the interaction between proteins and small molecules since molecules' conformations can vary within a limited range. Small molecules generally have more flexible structures than macromolecules, which are fixed in place [52]. Molecular docking is a renewable method that is in line with technological developments. This method is widely developed at this time to save money and time and eviscerate the use of new compounds as drug candidates.

In this study, a molecular simulation of cycloartanol (**1**) and cycloartanone (**2**) (Fig. **6**) has been conducted to determine the functional group contributing to the FLT3 gene. This gene was chosen as the target of inhibitory protein be- cause FLT3, belonging to the receptor tyrosine kinases, is often found in AML patients [53]. The parameters observed were the binding affinity and the formation of several hydro- gen bonds. The binding affinity represents the free energy of a bond, symbolized with ΔG. The smaller or more negative ΔG value represents the more stable and stronger bond formed [54]. The free energy of these triterpenoids is -8.76 and -9.94 Kcal/mol, respectively. The bond of cycloartanone (**2**) formed with FLT3 was stronger than cycloartanol (**1**). Table 3 provides the calculated binding energy and Ki value of each compound. Inhibition constants (Ki) are defined as the ligand concentration needed to inhibit a substrate [55]. For the lower Ki value and the binding energy formed, the lower concentration required to inhibit the target indicates the highest ligand-receptor affinity [56,57]. Cycloartanone (**2**) exhibited a lower Ki value at 0.051 µM.

The lower binding free energy of **2** correlated with formed hydrogen bonds. Cycloartanone (**2**) exhibited a hy- drogen-bond interaction with the amino acid residue Asp829 and a hydrophobic interaction with Leu616, whereas cy- cloartanol (**1**) only demonstrated hydrophobic interactions on the FLT3 active site region (Table 4). Hydrogen bonding is the strongest interaction among several intermolecular interactions, including hydrophobic interactions, dispersion forces, and dipole-dipole interactions [58]. The hydrogen interaction of cycloartanone (**2**) through a ketone group with FLT3 makes the stability of **2** higher than **1**, as demonstrated by the lower binding free energy and the constant inhibition value [59]. Based on the computational results, as shown in Tables 3 and 4, we concluded cycloartanone (**2**) to be the strongest ligand that bound the FLT3 gene as an inhibition target. This information, which can be used as a foundation for additional testing, is the first to become accessible for cycloartanol (**1**) and cycloartanone (**2**).

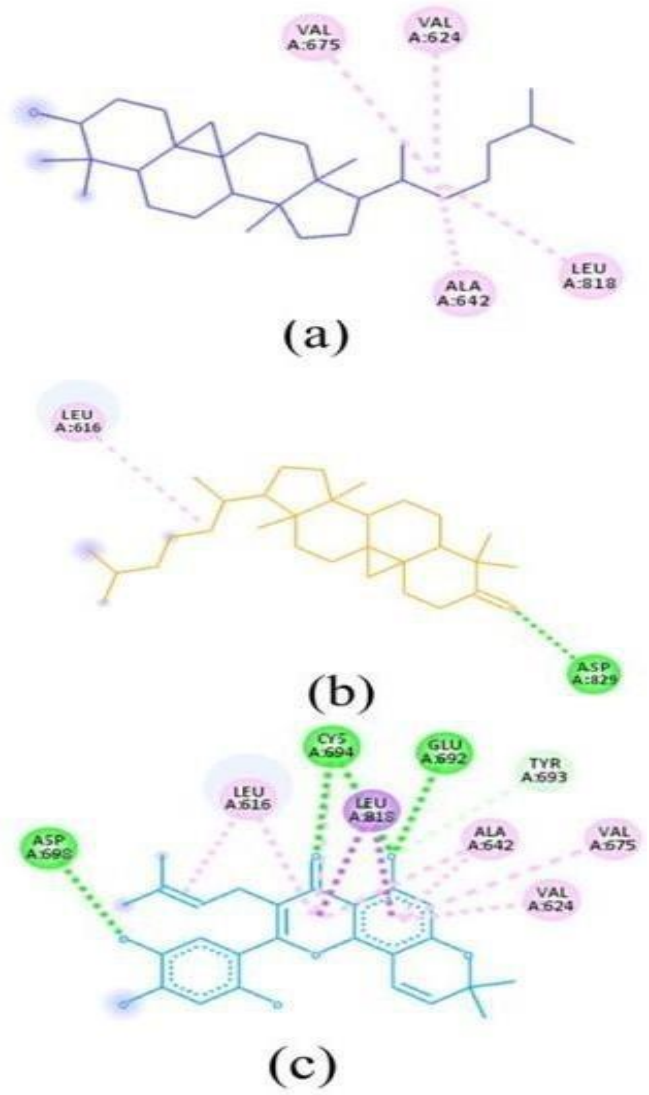

Fig. (6). Ligand-FLT3 interactions with the amino acids formed the hydrogen bonds for **1** (**a**), **2** (**b**), and artonin E (**c**).

Regarding structure-activity relationship, cycloartanol (**1**) and cycloartanone (**2**) exhibited different functional groups at the C-3 position. Cycloartanol (**1**) showed to have a hy- droxyl and cycloartanone (**2**) to have a carbonyl group. Ac- cording to bioactivity evaluation, both of them exhibited no significantly different value, although cycloartanol (**1**) has always been found to exert stronger activity than cycloar- tanone (**2**). In other words, the hydroxyl and carbonyl groups at C-3 do not have a different effect on the bioactivity of the triterpenoids.

Plant-derived cycloartane triterpenoids had shown inhibi- tion activity on various types of cancer cell lines. Cycloar- tane of *Euphorbia macrostegia* had demonstrated a cytotoxic effect on MDA-MB48 and MCF-7 breast cancer cells [60]. New cycloartanes, named neriifolius A-C, had been isolated from *Euphorbia neriifolia*, and they displayed cytotoxicity towards MDA-MB-231, MCF-7, and HCT-116 cell lines [61]. A combination of cycloartenol and 24-methylenecycloartanol from *Ficus krishnae* showed antidia- betic activity by lowering glucose activity in an oral glucose tolerance test (OGTT) [62]. Cycloartane triterpene was also detected in *Polyalthia longifolia* var. *pendula* leaves and exhibited anticancer activity on four human cancer cells, with the most active inhibition on cervical cancer cells [63].

The present study has described the anticancer effect of cycloartane triterpenoids isolated from the bark of *C. utan* Lamk. by *in vitro* and *in silico* evaluation. This is the first report regarding the cycloartane triterpenoids from this plant. The findings provide additional information regarding the cytotoxic effect of phytochemicals isolated from the Indone- sian Endemic plant *C. utan* Lamk.

CONCLUSION

Cycloartanol (**1**) and cycloartanone (**2**), parts of cycloar- tane triterpenoids, were first isolated from the bark of *C. utan* Lamk. (Arecaceae). These triterpenoids showed activity by inducing the inhibition of the P388 murine leukemia cell lines' growth, with IC_{50} values of 102.6 and 110.0 µg/mL, respectively. A computational docking study revealed cy- cloartanone (**2**) to effectively inhibit the FLT3 gene with a binding energy of -9.94 Kcal/mol and a Ki value of 0.051 µM. This compound was also observed to form hydrogen interactions with residual amino acid on the FLT3 site of action. Thus, cycloartanone demonstrated its cytotoxicity towards leukemia cancer cells based on the results of *in vitro* and *in silico* approaches. Our findings may support *C. utan* Lamk.'s functionality as a natural plant source that has the potential for bioactive compounds. Further studies on drug discovery from the *Corypha* genus and the validation of pre- clinical and clinical studies are very important to explore other anticancer compounds and evaluate the safety and tox- icity profile of these compounds to be used as cancer medi- cation.

LIST OF ABBREVIATIONS

AML - Acute Myeloid Leukemia

FLT3 = FMS-like Tyrosine Kinase 3

ITD = Internal Tandem Duplication

ITB = Institut Teknologi Bandung

FT-IR = Fourier transform infrared

MS = Mass spectrometry

VLC = Vacuum liquid chromatography

FBS = Fetal bovine serum

SDS = Sodium dodecyl sulfate

OGTT = Oral glucose tolerance test

ETHICS APPROVAL AND CONSENT TO PARTICI- PATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No humans/animals were used for studies that are the ba- sis of this research

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

A limited number of samples of the isolated compounds are available from the authors. Detailed data are included within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Elhady, S.S.; Eltamany, E.E.; Shaaban, A.E.; Bagalagel, A.A.; Muhammad, Y.A.; El-Sayed, N.M.; Ayyad, S.N.; Ahmed, A.A.M.; Elgawish, M.S.; Ahmed, S.A. Jaceidin flavonoid isolated from *Chiliadenus montanus* attenuates tumor progression in mice *via* vegf inhibition: *In vivo* and *in silico* studies. *Plants*, **2020**, *9*(8), 1031. http://dx.doi.org/10.3390/plants9081031 PMID: 32823927

[2] Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.*, **2021**, *71*(3), 209-249. http://dx.doi.org/10.3322/caac.21660 PMID: 33538338

[3] Vora, H.H.; Shukla, S.N.; Brahambhatt, B.V.; Mehta, S.H.; Patel, N.A.; Parikh, S.K.; Shah, K.N.; Shah, P.M. Clinical relevance of FLT3 receptor protein expression in Indian patients with acute leukemia. *Asia Pac. J. Clin. Oncol.*, **2010**, *6*(4), 306-319. http://dx.doi.org/10.1111/j.1743-7563.2010.01322.x PMID: 21114781

[4] Suzanna, E. Sirait, T.; Rahayu, P.S.; Shalmont, G.; Anwar, E.; Andalusia, R.; Harjati; Panigoro, S.S. Registrasi kanker berbasis rumah sakit di rumah sakit kanker dharmais. Pusat kanker nasional. *Ind. J. Cancer*, **2012**, *6*, 181-196. http://dx.doi.org/10.33371/ijoc.v6i4.299

[5] Hwang, D.; Kim, M.; Park, H.; Jeong, M.I.; Jung, W.; Kim, B. Natural products and acute myeloid leukimia: A review highlighting mechanisms of action. *Nutrients*, **2019**, *11*(5), 1010. http://dx.doi.org/10.3390/nu11051010 PMID: 31058874

[6] Yang, X.; Wang, J. Precision therapy for acute myeloid leukemia. *J. Hematol. Oncol.*, **2018**, *11*(1), 3. http://dx.doi.org/10.1186/s13045-017-0543-7 PMID: 29301553

[7] Liao, D.; Wang, M.; Liao, Y.; Li, J.; Niu, T. A review of efficacy and safety of checkpoint inhibitor for the treatment of acute myeloid leukemia. *Front. Pharmacol.*, **2019**, *10*, 609. http://dx.doi.org/10.3389/fphar.2019.00609 PMID: 31244654

[8] Garg, M.; Nagata, Y.; Kanojia, D.; Mayakonda, A.; Yoshida, K.; Haridas Keloth, S.; Zang, Z.J.; Okuno, Y.; Shiraishi, Y.; Chiba, K.; Tanaka, H.; Miyano, S.; Ding, L.W.; Alpermann, T.; Sun, Q.Y.; Lin, D.C.; Chien, W.; Madan, V.; Liu, L.Z.; Tan, K.T.; Sampath, A.; Venkatesan, S.; Inokuchi, K.; Wakita, S.; Yamaguchi, H.; Chng, W.J.; Kham, S.K.Y.; Yeoh, A.E.J.; Sanada, M.; Schiller, J.; Kreuzer, K.A.; Kornblau, S.M.; Kantarjian, H.M.; Haferlach, T.; Lill, M.; Kuo, M.C.; Shih, L.Y.; Blau, I.W.; Blau, O.; Yang, H.; Ogawa, S.; Koeffler, H.P. Profiling of somatic mutations in acute myeloid leukemia with FLT3-ITD at diagnosis and relapse. *Blood*, **2015**, *126*(22), 2491-2501. http://dx.doi.org/10.1182/blood-2015-05-646240 PMID: 26438511

[9] Lucas, D.; Scheiermann, C.; Chow, A.; Kunisaki, Y.; Bruns, I.; Barrick, C.; Tessarollo, L.; Frenette, P.S. Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. *Nat. Med.*, **2013**, *19*(6), 695-703. http://dx.doi.org/10.1038/nm.3155 PMID: 23644514

[10] May, J.E.; Donaldson, C.; Gynn, L.; Morse, H.R. Chemotherapy-induced genotoxic damage to bone marrow cells: long-term implications. *Mutagenesis*, **2018**, *33*(3), 241-251. http://dx.doi.org/10.1093/mutage/gey014 PMID: 30239865

[11] Bhujbal, S.P.; Keretsu, S.; Cho, S.J. Design of new therapeutic agents targeting FLT3 receptor tyrosine kinase using molecular docking and 3D-QSAR approach. *Lett. Drug Des. Discov.*, **2020**, *17*(5), 585-596. http://dx.doi.org/10.2174/1570180816666190618104632

[12] Zorn, J.A.; Wang, Q.; Fujimura, E.; Barros, T.; Kuriyan, J. Crystal structure of the FLT3 kinase domain bound to the inhibitor Quizartinib (AC220). *PLoS One*, **2015**, *10*(4), e0121177. http://dx.doi.org/10.1371/journal.pone.0121177 PMID: 25837374

[13] Sudhindra, A.; Smith, C.C. FLT3 inhibitors in AML: are we there yet? *Curr. Hematol. Malig. Rep.*, **2014**, *9*(2), 174-185. http://dx.doi.org/10.1007/s11899-014-0203-8 PMID: 24682858

[14] Jarusiewicz, J.A.; Jeon, J.Y.; Connelly, M.C.; Chen, Y.; Yang, L.; Baker, S.D.; Guy, R.K. Discovery of a diaminopyrimidine FLT3 inhibitor active against acute myeloid leukemia. *ACS Omega*, **2017**, *2*(5), 1985-2009. http://dx.doi.org/10.1021/acsomega.7b00144 PMID: 28580438

[15] Ji, H.F.; Li, X.J.; Zhang, H.Y. Natural products and drug discovery. *EMBO Rep.*, **2009**, *10*(3), 194-200. http://dx.doi.org/10.1038/embor.2009.12 PMID: 19229284

[16] Katz, L.; Baltz, R.H. Natural product discovery: past, present, and future. *J. Ind. Microbiol. Biotechnol.*, **2016**, *43*(2-3), 155-176. http://dx.doi.org/10.1007/s10295-015-1723-5 PMID: 26739136

[17] Karakaya, S.; Koca, M.; Yılmaz, S.; Yıldırım, K.; Pınar, N.; Demirci, B.; Brestic, M.; Sytar, O. Molecular docking studies of coumarins isolated from extracts and essential oils of *Zosima absinthifolia* link as potential inhibitors for Alzheimer's disease. *Molecules*, **2019**, *24*(4), 722. http://dx.doi.org/10.3390/molecules24040722 PMID: 30781573

[18] Bonini, S.A.; Premoli, M.; Tambaro, S.; Kumar, A.; Maccarinelli, G.; Memo, M.; Mastinu, A. *Cannabis sativa*: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.*, **2018**, *227*, 300-315. http://dx.doi.org/10.1016/j.jep.2018.09.004 PMID: 30205181

[19] Kurnia, D.; Hutabarat, G.S.; Windaryanti, D.; Herlina, T.; Herdiyati, Y.; Satari, M.H. Potential allylpyrocatechol derivatives as antibacterial agent against oral pathogen of *S. sanguinis* ATCC 10,556 and as inhibitor of MurA Enzymes: *In vitro* and *in silico* study. *Drug Des. Devel. Ther.*, **2020**, *14*, 2977-2985. http://dx.doi.org/10.2147/DDDT.S255269 PMID: 32801638

[20] Darwati, D.; Safitri, A.N.; Ambardhani, N.; Mayanti, T.; Nurlelasari, N.; Kurnia, D. Effectiveness and anticancer activity of a novel phenolic compound from *Garcinia porrecta* against the mcf-7 breast cancer cell line *in vitro* and *in silico*. *Drug Des. Devel. Ther.*, **2021**, *15*, 3523-3533. http://dx.doi.org/10.2147/DDDT.S321824 PMID: 34408404

[21] Abdelhameed, R.; Elgawish, M.S.; Mira, A.; Ibrahim, A.K.; Ahmed, S.A.; Shimizu, K.; Yamada, K. Anti-choline esterase activity of ceramides from the Red Sea marine sponge *Mycale euplectellioides. RSC Advances*, **2016**, *6*(24), 20422-20430. http://dx.doi.org/10.1039/C5RA26424C

[22] Lin, X.; Li, X.; Lin, X. A review on applications of computational methods in drug screening and design. *Molecules*, **2020**, *25*(6), 1375. http://dx.doi.org/10.3390/molecules25061375 PMID: 32197324

[23] Marica Bakovic, N.H. Biologically active triterpenoids and their cardioprotective and anti-inflammatory effects. *J. Bioanal. Biomed.*, **2015**, *1*(s12) http://dx.doi.org/10.4172/1948-593X.S12-005

[24] Ma, L.; Zhang, M.; Zhao, R.; Wang, D.; Ma, Y.; Ai, L. Plant natural products: Promising resources for cancer chemoprevention. *Molecules*, **2021**, *26*(4), 933.

http://dx.doi.org/10.3390/molecules26040933 PMID: 33578780

[25] Chen, F.; Liu, D.L.; Wang, W.; Lv, X.M.; Li, W.; Shao, L.D.; Wang, W.J. Bioactive triterpenoids from *Sambucus javanica* Blume. *Nat. Prod. Res.*, **2020**, *34*(19), 2816-2821. http://dx.doi.org/10.1080/14786419.2019.1596092 PMID: 30968700

[26] Patlolla, J.M.; Rao, C.V. Triterpenoids for cancer prevention and treatment: current status and future prospects. *Curr. Pharm. Biotechnol.*, **2012**, *13*(1), 147-155. http://dx.doi.org/10.2174/138920112798868719 PMID: 21466427

[27] Sultana, N.; Saeed Saify, Z. Naturally occurring and synthetic agents as potential anti-inflammatory and immunomodulants. *Anti-inflamm. Antiallergy Agents Med. Chem.*, **2012**, *11*(1), 3-19. http://dx.doi.org/10.2174/187152312803476264 PMID: 22934748

[28] Zhao, X.; Liu, M.; Li, D. Oleanolic acid suppresses the proliferation of lung carcinoma cells by miR-122/Cyclin G1/MEF2D axis. *Mol. Cell. Biochem.*, **2015**, *400*(1-2), 1-7. http://dx.doi.org/10.1007/s11010-014-2228-7 PMID: 25472877

[29] Bishayee, A.; Ahmed, S.; Brankov, N.; Perloff, M. Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. *Front. Biosci.*, **2011**, *16*(1), 980-996. http://dx.doi.org/10.2741/3730 PMID: 21196213

[30] Ren, Y.; Kinghorn, A.D. Natural product triterpenoids and their semi-synthetic derivatives with potential anticancer activity. *Planta Med.*, **2019**, *85*(11/12), 802-814. http://dx.doi.org/10.1055/a-0832-2383 PMID: 30658371

[31] Li, S.; Kuo, H.C.D.; Yin, R.; Wu, R.; Liu, X.; Wang, L.; Hudlikar, R.; Peter, R.M.; Kong, A.N. Epigenetics/epigenomics of triterpenoids in cancer prevention and in health. *Biochem. Pharmacol.*, **2020**, *175*, 113890. http://dx.doi.org/10.1016/j.bcp.2020.113890 PMID: 32119837

[32] Pan, L.; Yong, Y.; Deng, Y.; Lantvit, D.D.; Ninh, T.N.; Chai, H.; Carcache de Blanco, E.J.; Soejarto, D.D.; Swanson, S.M.; Kinghorn, A.D. Isolation, structure elucidation, and biological evaluation of 16,23-epoxycucurbitacin constituents from *Eleaocarpus chinensis*. *J. Nat. Prod.*, **2012**, *75*(3), 444-452. http://dx.doi.org/10.1021/np200879p PMID: 22239601

[33] Ren, Y.; Anaya-Eugenio, G.D.; Czarnecki, A.A.; Ninh, T.N.; Yuan, C.; Chai, H.B.; Soejarto, D.D.; Burdette, J.E.; de Blanco, E.J.C.; Kinghorn, A.D. Cytotoxic and NF-κB and mitochondrial transmembrane potential inhibitory pentacyclic triterpenoids from *Syzygium corticosum* and their semi-synthetic derivatives. *Bioorg. Med. Chem.*, **2018**, *26*(15), 4452-4460. http://dx.doi.org/10.1016/j.bmc.2018.07.025 PMID: 30057155

[34] Tung, N.H.; Song, G.Y.; Minh, C.V.; Kiem, P.V.; Jin, L.G.; Boo, H.J.; Kang, H.K.; Kim, Y.H. Steamed ginseng-leaf components enhance cytotoxic effects on human leukemia HL-60 cells. *Chem. Pharm. Bull. (Tokyo)*, **2010**, *58*(8), 1111-1115. http://dx.doi.org/10.1248/cpb.58.1111 PMID: 20686271

[35] Tuchinda, P.; Kornsakulkarn, J.; Pohmakotr, M.; Kongsaeree, P.; Prabpai, S.; Yoosook, C.; Kasisit, J.; Napaswad, C.; Sophasan, S.; Reutrakul, V. Dichapetalin-type triterpenoids and lignans from the aerial parts of *Phyllanthus acutissima*. *J. Nat. Prod.*, **2008**, *71*(4), 655-663. http://dx.doi.org/10.1021/np7007347 PMID: 18271551

[36] Nandika, D.; Darmawan, W.; Karlinasari, L.; Hadi, Y.S.; Abdillah, I.B.; Hiziroglu, S. Evaluation of color change and biodeterioration resistance of Gewang (*Corypha utan* Lamk.) wood. *Appl. Sci. (Basel)*, **2020**, *10*(21), 7501. http://dx.doi.org/10.3390/app10217501

[37] Heliawati, L.; Kardinan, A.; Mayanti, T.; Tjokronegoro, R. ati Piceatanol: Anti-cancer compound from Gewang seed extract. *J. Appl. Pharm. Sci.*, **2015**, *5*, 110-113. http://dx.doi.org/10.7324/JAPS.2015.50119

[38] Waghulde, S.; Kale, M.K.; Patil, V. Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. *Proceedings*, **2019**, *41*, 47. http://dx.doi.org/10.3390/ecsoc-23-06703

[39] Aykul, S.; Martinez-Hackert, E. Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. *Anal. Biochem.*, **2016**, *508*, 97-103. http://dx.doi.org/10.1016/j.ab.2016.06.025 PMID: 27365221

[40] Bell, E.W.; Zhang, Y. DockRMSD: an open-source tool for atom mapping and RMSD calculation of symmetric molecules through graph isomorphism. *J. Cheminform.*, **2019**, *11*(1), 40. http://dx.doi.org/10.1186/s13321-019-0362-7 PMID: 31175455

[41] Kim, N.; Park, S.; Nhiem, N.X.; Song, J.H.; Ko, H.J.; Kim, S.H. Cycloartane-type triterpenoid derivatives and a flavonoid glycoside from the burs of *Castanea crenata*. *Phytochemistry*, **2019**, *158*, 135-141. http://dx.doi.org/10.1016/j.phytochem.2018.11.001 PMID: 30529974

[42] Baniadam, S.; Rahiminejad, M.R.; Ghannadian, M.; Saeidi, H.; Ayatollahi, A.M.; Aghaei, M. Cycloartane triterpenoids from *Euphorbia macrostegia* with their cytotoxicity against MDA- MB48 and MCF-7 cancer cell lines. *Iran. J. Pharm. Res.*, **2014**, *13*(1), 135-141. http://dx.doi.org/10.22037/ijpr.2014.1419 PMID: 24734064

[43] Yadav, V.R.; Prasad, S.; Sung, B.; Kannappan, R.; Aggarwal, B.B. Targeting inflammatory pathways by triterpenoids for prevention and treatment of cancer. *Toxins (Basel)*, **2010**, *2*(10), 2428-2466. http://dx.doi.org/10.3390/toxins2102428 PMID: 22069560

[44] Thanh, L.N.; Thoa, H.T.; Oanh, N.T.T.; Giap, T.H.; Quyen, V.T.; Ha, N.T.T.; Phuong, D.T.L.; Lien, N.T.P.; Hang, N.T.M. Cycloartane triterpenoids and biological activities from the propolis of thestingless bee *Lisotrigona furva*. *Vietnam J. Chem.*, **2021**, *59*, 426-430. http://dx.doi.org/10.1002/vjch.201900143

[45] Kennedy, V.E.; Smith, C.C. FLT3 Mutations in acute myeloid leukemia: Key concepts and emerging controversies. *Front. Oncol.*, **2020**, *10*, 612880. http://dx.doi.org/10.3389/fonc.2020.612880 PMID: 33425766

[46] Wiji Prasetyaningrum, P.; Bahtiar, A.; Hayun, H. Synthesis and cytotoxicity evaluation of novel asymmetrical mono-carbonyl analogs of curcumin (AMACs) against vero, HeLa, and MCF7 cell lines. *Sci. Pharm.*, **2018**, *86*(2), 25. http://dx.doi.org/10.3390/scipharm86020025 PMID: 29880783

[47] Arcanjo, D.; Albuquerque, A.; Melo-Neto, B.; Santana, L.; Medeiros, M.G.F.; Citó, A. Bioactivity evaluation against *Artemia salina* Leach of medicinal plants used in Brazilian Northeastern folk medicine. *Braz. J. Biol.*, **2012**, *72*(3), 505-509. http://dx.doi.org/10.1590/S1519-69842012000300013 PMID: 22990821

[48] Meyer, B.; Ferrigni, N.; Putnam, J.; Jacobsen, L.; Nichols, D.; McLaughlin, J. Brine Shrimp: A convenient general bioassay for active plant constituents. *Planta Med.*, **1982**, *45*(5), 31-34. http://dx.doi.org/10.1055/s-2007-971236

[49] Rahman, M.A.; Ramli, F.; Karimian, H.; Dehghan, F.; Nordin, N.; Mohd Ali, H.; Mohan, S.; Mohd Hashim, N. Artonin E induces apoptosis *via* mitochondrial dysregulation in SKOV-3 ovarian cancer cells. *PLoS One*, **2016**, *11*(3), e0151466. http://dx.doi.org/10.1371/journal.pone.0151466 PMID: 27019365

[50] Etti, I.; Rasedee, A.; Mohd Hashim, N.; Bustamam Bin Abdul, A.; Abdul Kadir, A.; Yeap, S.K.; Waziri, P.; Malami, I.; Lim, K.L.; Etti, C. Artonin E induces p53-independent G1 cell cycle arrest and apoptosis through ROS-mediated mitochondrial pathway and livin suppression in MCF-7 cells. *Drug Des. Devel. Ther.*, **2017**, *11*, 865-879. http://dx.doi.org/10.2147/DDDT.S124324 PMID: 28356713

[51] Meng, X.Y.; Zhang, H.X.; Mezei, M.; Cui, M. Molecular docking: a powerful approach for structure-based drug discovery. *Curr. Comput. Aided Drug Des.*, **2011**, *7*(2), 146-157. http://dx.doi.org/10.2174/157340911795677602 PMID: 21534921

[52] Chen, G.; Seukep, A.J.; Guo, M. Recent advances in molecular docking for the research and discovery of potential marine drugs. *Mar. Drugs*, **2020**, *18*(11), 545. http://dx.doi.org/10.3390/md18110545 PMID: 33143025

[53] Musthapa, I.; Latip, J.; Takayama, H.; Juliawaty, L.D.; Hakim, E.H.; Syah, Y.M. Prenylated flavones from *Artocarpus lanceifolius* and their cytotoxic properties against P-388 cells. *Nat. Prod. Commun.*, **2009**, *4*(7), 1934578X0900400. http://dx.doi.org/10.1177/1934578X0900400710 PMID: 19731595

[54] Zhu, R.; Li, L.; Nguyen, B.; Seo, J.; Wu, M.; Seale, T.; Levis, M.; Duffield, A.; Hu, Y.; Small, D. FLT3 tyrosine kinase inhibitors synergize with BCL-2 inhibition to eliminate FLT3/ITD acute leu-

kemia cells through BIM activation. *Signal Transduct. Target. Ther.*, **2021**, *6*(1), 186. http://dx.doi.org/10.1038/s41392-021-00578-4 PMID: 34024909

[55] Gupta, A.; Chaudhary, N.; Kakularam, K.R.; Pallu, R.; Polamarasetty, A. The augmenting effects of desolvation and conformational energy terms on the predictions of docking programs against mPGES-1. *PLoS One*, **2015**, *10*(8), e0134472. http://dx.doi.org/10.1371/journal.pone.0134472 PMID: 26305898

[56] Cao, H.; Hu, H.; Colagiuri, B.; Liu, J. Medicinal cupping therapy in 30 patients with fibromyalgia: a case series observation. *Forsch. Komplement. Med.*, **2011**, *18*(3), 3. http://dx.doi.org/10.1159/000329329 PMID: 21701180

[57] Vargas, J.A.R.; Lopez, A.G.; Piñol, M.C.; Froeyen, M. Molecular docking study on the interaction between 2-substituted-4,5-difuryl Imidazoles with different Protein Target for antileishmanial activity. *J. Appl. Pharm. Sci.*, **2018**, *8*, 14-22. http://dx.doi.org/10.7324/JAPS.2018.8303

[58] Maulana Hidayatullah, I.; Setiadi, T.; Tri Ari Penia Kresnowati, M.; Boopathy, R. Xylanase inhibition by the derivatives of lignocellulosic material. *Bioresour. Technol.*, **2020**, *300*, 122740. http://dx.doi.org/10.1016/j.biortech.2020.122740 PMID: 31952895

[59] Vepuri, S.B.; Anbazhagan, S.; Divya, D.; Padmin, D. A review on supramolecular chemistry in drug design and formulation research. *Indones. J. Pharm.*, **2013**, *24*, 131-150. http://dx.doi.org/10.14499/INDONESIANJPHARM0ISS0PP131-150

[60] Katragadda, M.; Magotti, P.; Sfyroera, G.; Lambris, J.D. Hydrophobic effect and hydrogen bonds account for the improved activity of a complement inhibitor, compstatin. *J. Med. Chem.*, **2006**, *49*(15), 4616-4622. http://dx.doi.org/10.1021/jm0603419 PMID: 16854067

[61] Sashidhara, K.V.; Singh, S.P.; Kant, R.; Maulik, P.R.; Sarkar, J.; Kanojiya, S.; Ravi Kumar, K. Cytotoxic cycloartane triterpene and rare isomeric bisclerodane diterpenes from the leaves of *Polyalthia longifolia* var. *pendula*. *Bioorg. Med. Chem. Lett.*, **2010**, *20*(19), 5767-5771. http://dx.doi.org/10.1016/j.bmcl.2010.07.141 PMID: 20732814

[62] Choodej, S.; Pudhom, K. Cycloartane triterpenoids from the leaves of *Euphorbia neriifolia*. *Phytochem. Lett.*, **2020**, *35*, 1-5. http://dx.doi.org/10.1016/j.phytol.2019.10.005

[63] Nair, A.N.S.; Nair, R.V.R.; Nair, A.P.R.; Nair, A.S.; Thyagarajan, S.; Johnson, A.J.; Baby, S. Antidiabetes constituents, cycloartenol and 24-methylenecycloartanol, from *Ficus krishnae*. *PLoS One*, **2020**, *15*(6), e0235221. http://dx.doi.org/10.1371/journal.pone.0235221 PMID: 32584888