# Study In-vitro and in-silico of ethylacetate extract and fractions of soft coral *Lobophytum* sp. towards *Artemia salina* Brine Shrim (BSLT)

*Sahidin* Sahidin[1*], *Adryan* Fristiohady[1], Wahyuni[1], Arfan[1], *Baru* Sadarun[2], *Nur Syifa* Rahmatika[3], *Agung Wibawa Mahatva* Yodha[4], *Nur Upik En* Masrika[5], *Andini* Sundowo[6], *Sofa* Fajriah[7]

[1]Faculty of Pharmacy, Universitas Halu Oleo, Kendari, INDONESIA
[2]Faculty of Marine Sciences and Fisheries, Universitas Halu oleo, Kendari, INDONESIA
[3]Faculty of Medicine, Universitas Halu Oleo, Kendari, INDONESIA
[4]Health Polytechnics of Bina Husada, Kendari, INDONESIA
[5]Faculty of Medicine, Universitas Khairun, Ternate, INDONESIA
[6]Research Centre for Chemistry, [7]Research Centre for Pharmaceutical Ingredient and Traditional medicine, BRIN, Kawasan Puspitek, Tangerang Selatan 15314, Banten, INDONESIA

**Abstract**. The article aims to describe the findings of chemical and pharmaceutical aspects of *Lobophytum* sp. from Southeast Sulawesi, Indonesia. Ethylacetate extract was fractionated by Vacuum liquid chromatography (VLC). Toxicity was evaluated by BSLT test, and the phytochemical screening and LCMSMS method were used to determine the chemical composition and molecular docking for in-silico study. The results showed that the ethylacetate extract was produced seven fractions namely Fraction A-G. The weight of each fraction was A (12.8% w/w), B (9.7%), C (10.1%), D (2.0%), E (7.0%), F (25, 3%) and G (11.5%). The toxicity potency of Fraction B is the most toxic with $LC_{50}$ (mg/L) 26.70 ± 0.58. LCMSMS data indicated that the fraction B contains 19β-glukocyl-14-deoxy-11,12-didehydrographoside, 3-isoazmalicine, abietraticine, arachidonic acid, neociwujiaphenol, oxyphyliacinol, saurufuran B and some unidentified compounds with molecular formulas $C_{37}H_{46}O_7$, $C_{35}H_{44}O_5$, and $C_{20}H_{26}O_2$. Based on computational simulations, Ar-Abietatriene and 3-Iso-ajmalicine have the potential to inhibit CDK-6. These compounds hinder the progression of the cell cycle and the proliferation of cancer cells by forming molecular interactions with residues Ile19, Val27, Ala41, Val77, Phe98, Val101, Leu152, and Ala162. This suggests their potential as anticancer agents. Thus, Fraction B can be continued for the anticancer evaluation.

* Corresponding author: sahidin02@uho.ac.id

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

# 1 Introduction

In continuing our study on the chemical and pharmaceutical aspects of marine natural resources, we selected soft coral, particularly *Lobophytum* sp., as a sample. Before studying soft corals, we have isolated and identified the structure of a novel molecule from a sponge *Chlatria* sp., called Clathruohate [1], and chemically screened a few sponges [2]. Anti-hyperlipidemia [3], anti-inflammatory [4], antioxidant capabilities and acute toxicity [5], as well as chemical and medicinal characteristics of soft coral *Nepthea* sp. [6] are just a few of the biological activities of sponges that have been noted.

Southeast Sulawesi's waters are home to soft corals, one of them is the *Lobophytum* sp. [7]. A few studies on the chemical and pharmaceutical aspects of this genus have been reported, including three cembrene diterpenoids named Lobophylins F-H from *L. crassum* growing in Taiwan [8] and lobocrasols A-D were identified from Vietnamese's *L. crassum* which have potential as anti-inflammatory [9], and Chinese's *L. crassum* produced polyoxygenated cembranoids namely lobophycrasins A-D and hum

ulisin A [10]. Some Vietnamese's soft corals also produce interesting compounds including laevigatol A-D which was active as anti-inflammatory and anticancer agents from *L. laevigatum* [11], lobocompactol A-B and sterols from *L. compactum* is active as antioxidant and cytotoxic against HL-60 (leukaemia) and A549 (lung) cell lines [12]. The South China Sea-dwelling *Lobophytum* sp. has also been investigated for its chemical and pharmacological properties, including the production of Lobophytrols A-C [13], the anti-inflammatory and antibacterial activity of durumolides A to E from *L. durum* [14], and the activity of lobophysterols A-D against the cell lines HT-29, SNU-398, and Capan-1 [15]. The cembranolide chemicals generated by an Okinawan-based *Lobophytum* sp. were cytotoxic to the HeLa, A459, B16-F10, and RAW246.7 cell lines [16].

The study of soft corals from Eastern Indonesia is still in the mapping stage [17, 18]. However, some research reports on marine organisms from the area are interesting, for example, *Lobophytum* sp. from Selayar (South Sulawesi) has potential as an anti-bacterial and antioxidant [19]. Lobozoathamine, an alkaloid from *Lobophytum* sp, growing in Manado, North Sulawesi, has been successfully isolated and identified [20]. This paper will present the Antioxidant, toxicity and secondary metabolites of ethyl acetate fraction from soft coral *Lobophytum* sp. growing in South East Sulawesi.

# 2 Materials and Methods

## *2.1 Soft Coral Collection, Preparation and Extraction*

Southeast Sulawesi's Hoga Island, located at 5º27' 54' 36" LS and 123º45' 20" LE, was the site of a soft coral collection of *Lobophytum* sp. 3.0 kg of dried soft coral powder was macerated in 10 L of ethyl acetate (EtOAc) for 3 x 24 hours and concentrated in a vacuum rotary evaporator. Furthermore, the ethyl acetate extract was fractionated using liquid vacuum chromatography (VLC) with Si-gel adsorbent and a mixture of n-hexane: ethyl acetate and methanol as the eluent. [6].

## *2.2 Phytochemical Screening*

To identify the secondary metabolite of the ethyl acetate fractions of the soft coral *Lobophytum* sp., phytochemical screening was done. The Harborne Method was used to

assess the secondary metabolites, which included flavonoids, saponins, tannins/polyphenols, alkaloids, and terpenoids [6]. Additionally, utilizing this instrument's regular operating method, LC-MS/MS analysis was used to determine the precise chemical composition of *Lobophytum* sp. [2].

### *2.3 Toxicity Assays*

*Brine Shrimp Lethality Test* (BSLT) assay was used for toxicity evaluation of the sample [21].

### *2.4 Protein and Ligand Preparation*

We chose the three-dimensional structures of CDK-6 (PDB ID: 5L2I) [22] from the Protein Data Bank (https://www.rcsb.org/). The compounds identified from the LC-MS/MS results were gathered from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). AutoDockTool v1.5.6 was utilized to prepare the protein and ligands. Water molecules were removed for the protein, and protonations and the Kollman charges were added [23]. The ligand was configured to rotate freely, and the Gasteiger charges were incorporated [24].

### *2.5 Molecular Docking Simulation*

The docking process was run with the assistance of AutoDock Vina v.1.1.2 [25]. The binding site is set following the position of Palbociclib in CDK-6 with a grid area of 20 x 20 x 20 Å and 0,375 Å point spacing. The validated procedures were identified with a root mean square deviation (RMSD) of after redocking process of Palbociclib was below 2 Å [26]. Other docking procedures are set by default. Lastly, the protein and compounds' interactions were analyzed and visualized with help of Discovery Studio Visualizer software v17.2.0.16349.

## 3. Result and Discussion

Ethyl acetate was used to extract the *Lobophytum* sp. powder (3 kg), producing a 75 g concentrated extract (2.50%). In fractionation, the ethyl acetate extract was produced in seven fractions namely Fraction A-G. The weight of each extract was A (12.8% w/w), B (9.7%), C (10.1%), D (2.0%), E (7.0%), F (25.3%) and G (11.5%). Fraction F is the largest fraction in terms of weight so it was chosen for further study. The phytochemical screening of the fraction F gave information which showed in **Table 1**.

**Table 1**. Weight and chemical composition of fraction F ethyl acetate extract

| Weight (g) | | Chemical Contents | | | | |
|---|---|---|---|---|---|---|
| Ethyl acetate extract | Fraction B | Terpenoids | saponins | Tannins/ Phenolics | Flavonoids | alkaloids |
| 75 | 12.93 | + | - | + | - | + |

**Table 1** reveals that the content of secondary metabolites in soft corals of *Lobophytum* sp, especially those which are the polar part of the ethyl acetate extract, called fraction B as

much as 12.93 g (9.7% w/w). Its composition includes saponins, tannins/phenolic and alkaloids, while terpenoids and flavonoids are not found in the fraction F of *Lobophytum* sp. The composition of secondary metabolites in more detail was carried out by translating the LC-MS/MS data as shown in **Table 2.**

**Table 2**. Compounds of Fraction B of *Lobophytum* sp based on LC-MS/MS data

| Sample | No | Rt (min) | Observed [M+H]/ [M+Na] (m/z) | Experimental Neutral Mass (Da) | Theoretical Neutral Mass (Da) | $MS^n$ Fragmentation | Component Name |
|---|---|---|---|---|---|---|---|
| Fraction B | 1 | 6.87 | 333.2066 | 332.19876 | 332.19876 | 315.20; 297.18; 268.19; 165.07 | 19β-Glucosyl-14-deoxy-11,12-didehydroand-rographoside [27] |
| | 2 | 7.16 | 403.1761 | 402.16785 | 402.16785 | 315.19; 299.19; 203.17 | Neociwujiaphenol [28] |
| | 3 | 7.24 | 315.1962 | 314.18819 | 314.18819 | 297.19; 165.18 | Oxyphyllacinol [29] |
| | 4 | 7.72 | 317.2119 | 316.20384 | 316.20384 | 299.20; 203.18 | Saurufuran B [30] |
| | 5 | 8.17 | 353.1886 | 352.17869 | 352.17869 | 397.20; 271.24 | 3-Iso-ajmalicine [31] |
| | 6 | 9.33 | 271.2425 | 270.23475 | 270.23475 | 173.13 | ar-Abietatriene [32] |
| | 7 | 9.80 | 305.2477 | 304.24023 | 304.24023 | 287.24 | Arachidonic acid [33] |

The LC-MS/MS data informs that there are 10 main compounds contained in the Fraction B of ethyl acetate extract of *Lobophytum* sp., of which 7 compounds have been identified and the rest have not been identified. The seven compounds that have been identified are 19β-Glucosyl-14-deoxy-11,12-didehydroand-rographoside, Neociwujiaphenol, Oxyphyllacinol, Saurufuran B, 3-Iso-ajmalicine, ar-Abietatriene, Arachidonic acid and some unidentified compounds with molecular formulas $C_{37}H_{46}O_7$, $C_{35}H_{44}O_5$, and $C_{20}H_{26}O_2$. The structure of the seven compounds that have been identified is shown in **Fig. 1**.

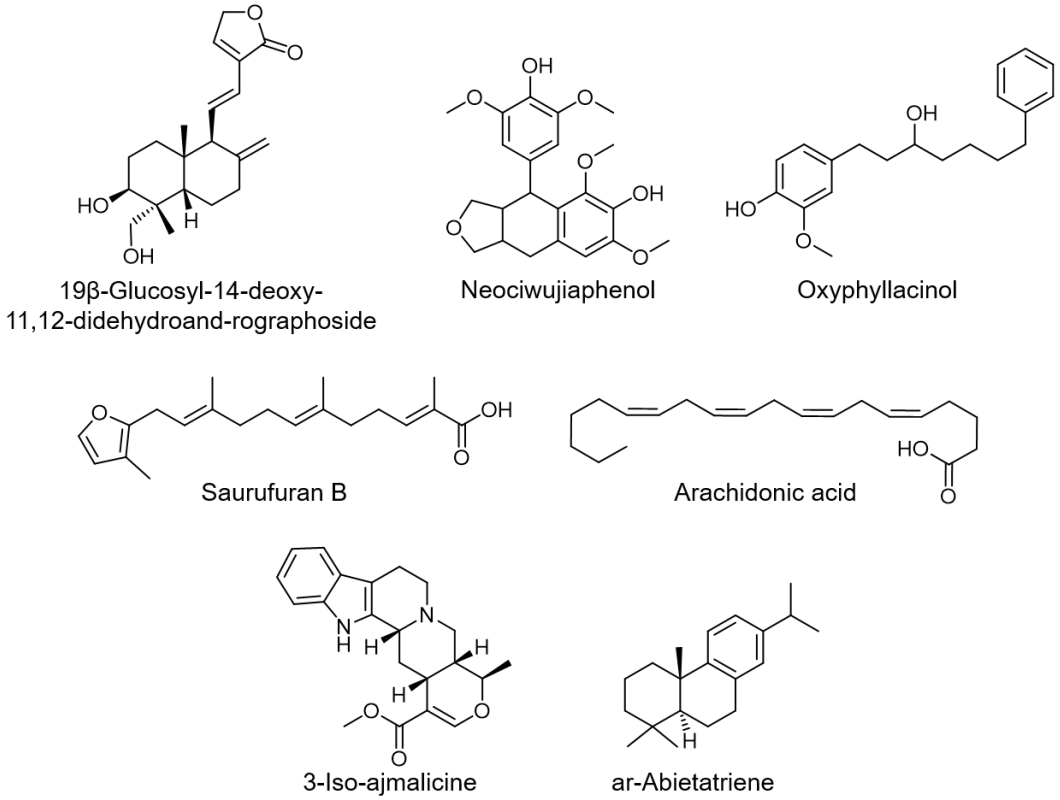



**Fig. 1**. Identified compounds of Fraction B of *Lobophytum* sp. ethylacetate extract

The structure and molecular formula data from LC-MS/MS strengthen the chemical data of phytochemical screening results. The presence of terpenoids are supported by 19β-Glucosyl-14-deoxy-11,12-didehydroand-rographoside, Saurufuran B, ar-Abietatriene, and Arachidonic acid. Phenolic compounds are Neociwujiaphenol and Oxyphyllacinol. The last, 3-Iso-ajmalicine represents the presence of alkaloids.The unidentified compounds with molecular formulas $C_{37}H_{46}O_7$, $C_{35}H_{44}O_5$, and $C_{20}H_{26}O_2$ are predicted phenolic compounds. The diversity of compounds possessed by the Fraction B of ethyl acetate extract of *Lobophytum* sp. causes this fraction to have various biological activities, especially in toxicity as shown in **Table 3**.

**Table 3**. Toxicity of Ethylacetate extract and Fraction B ethyl acetate extract of *Lobophytum* sp.

| | Sample(s) | | |
|---|---|---|---|
| | Ethylacetate extract | Fraction B of ethylacetate extract | Potassium dichromate (positive control) |
| $LC_{50}$ in mg/L | 50.07 ± 1.24 | 26.70 ± 0.58 | 5.96 ± 0.68 |

**Table 3** shows that the toxicity of an extract and fraction of *Lobophytum* sp. using BSLT was evaluated $LC_{50}$ (mg/mL) value. If the $LC_{50}$ value is less than 1000 mg/mL, it is categorized as active [22]. Thus, all samples have high toxic properties and Fraction B of the ethyl acetate extract is more active than ethylacetate extract of *Lobophytum* sp. In general, extracts or compounds that have high toxicity have the opportunity to be developed as anticancer drugs. Anticancer potential testing was carried out in-silico using molecular docking analysis.

## 4 Mole cular Docking Analysis

Cyclin-Dependent Kinase 6 (CDK-6) plays a significant role in cancer by regulating the cell cycle and promoting cell proliferation. Hyperactivation of CDK-6 is commonly observed in various types of cancers [35]. This hyperactivation can result from mutations, gene amplification, or dysregulation of pathways that control CDK-6 expression [36]. High CDK-6 activity can lead to uncontrolled cell division and tumor formation. Given its critical role in cell cycle progression and proliferation, CDK-6 has become a target for cancer therapy [35]. CDK-6 inhibitors, such as palbociclib, have been developed to block the activity of CDK-6 selectively. These inhibitors aim to slow down or halt the proliferation of cancer cells, particularly in hormone receptor-positive breast cancers [37].

This research aims to present insights into the potential of compounds derived from the soft coral *Lobophytum* sp. as molecular inhibitors of CDK-6. To validate the accuracy of our simulations, we conducted a redocking procedure using the palbociclib crystal structure back into CDK-6 and evaluated the Root Mean Square Deviation (RMSD). A lower RMSD value signifies that the docking parameters successfully replicated the conformation of the co-crystal ligand, as observed through x-ray crystallography. The most optimal palbociclib conformation within CDK-6 is depicted in Figure 1. The re-docking analysis exhibited a pose similar to the reported x-ray crystallography, showing an RMSD of 1.821 Å.

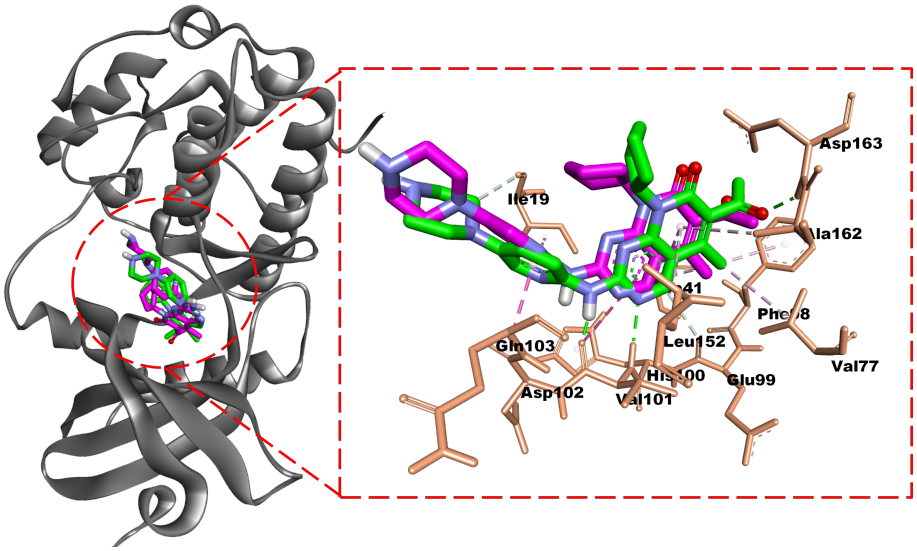



**Fig. 2**. Superimpose and the interaction between the co-crystal palbociclib (green) and the docked pose (pink) in CDK-6.

Based on the docking simulation results, palbociclib exhibited the highest affinity towards CDK-6 with a binding energy of -9.3 kcal/mol. In this regard, this compound's carboxyl and pyrimidine groups formed hydrogen bonds with the residues Val101, Asp163, Ile19, and Glu99 at the active site of CDK-6 (Figure 1). Additionally, the benzene ring of the pyrimidine group contributed to the formation of hydrophobic interactions with the residues Ala41, Val77, Phe98, His100, Asp102, Leu152, and Ala162. Meanwhile, a total of 7 compounds from fraction B were found to have affinities within the binding energy range of -8.9 to -6.4 kcal/mol (Table 1). Two compounds from the B fraction of *Lobophytum* sp., Ar-Abietatriene, and 3-Iso-ajmalicine, exhibited the best affinities among the other compounds, with binding energies of -8.9 kcal/mol and -8.8 kcal/mol, respectively.

**Table 4**. Summary of the docking result and interactions of all identified compounds in Lobophytum sp.'s fraction B against CDK-6

| Compounds | Binding Energies (kcal/mol) | Hydrogen bonds | Hydrophobic Interactions |
|---|---|---|---|
| Palbociclib | -9.3 | Val101, Asp163, Ile19, Glu99 | Ala41, Val77, Phe98, His100, Asp102, Leu152, Ala162 |
| Ar-Abietatriene | -8.9 | - | Ile19, Val27, Ala41, Val77, Phe98, Leu152, Ala162 |
| 3-Iso-ajmalicine | -8.8 | His100, Val101, Glu99 | Ile19, Val27, Asp102, Leu152 |
| 19β-glucosyl-14-deoxy-11,12-dedihydroandrogaphoside | -7.8 | Gln149, Asn150, Asp163 | Ile19, Val27, Ala41, His100, Val101, Leu152 |
| Saurufuran B | -7.7 | Lys147, Val180, Thr182, Glu99 | Val27, Ala41, Val77, Phe98, Leu152, Ala162 |
| Neociwujiaphenol | -7.4 | Asn150, Asp163 | Ile19, Val27, Ala41, Leu152, Ala162 |
| Oxyphyllacinol | -7.2 | Val101 | Ile19, Val27, Ala41, Lys43, Leu152, Ala162 |
| Arachidonic Acid | -6.4 | - | Tyr24, Val27 |

The molecular interactions of Ar-Abietatriene were observed with the methyl group and its benzene ring, forming hydrophobic contacts with Ile19, Val27, Ala41, Val77, Phe98, Leu152, and Ala162 (Figure 2A). Interestingly, although this compound did not exhibit hydrogen bond, its affinity was superior to other compounds. We speculate that interactions with the residues Ala41, Val77, and Phe98 are crucial in their affinity [22]. On the other hand, compound 3-Iso-ajmalicine revealed hydrogen bonding with the residues His100, Val101, and Glu99, facilitated by this compound's carbonyl and pyrrole groups. Additionally, its quinolizine ring contributed to hydrophobic interactions with the residues Ile19, Val27, Asp102, and Leu152 at the CDK-6 active site (Figure 3B).

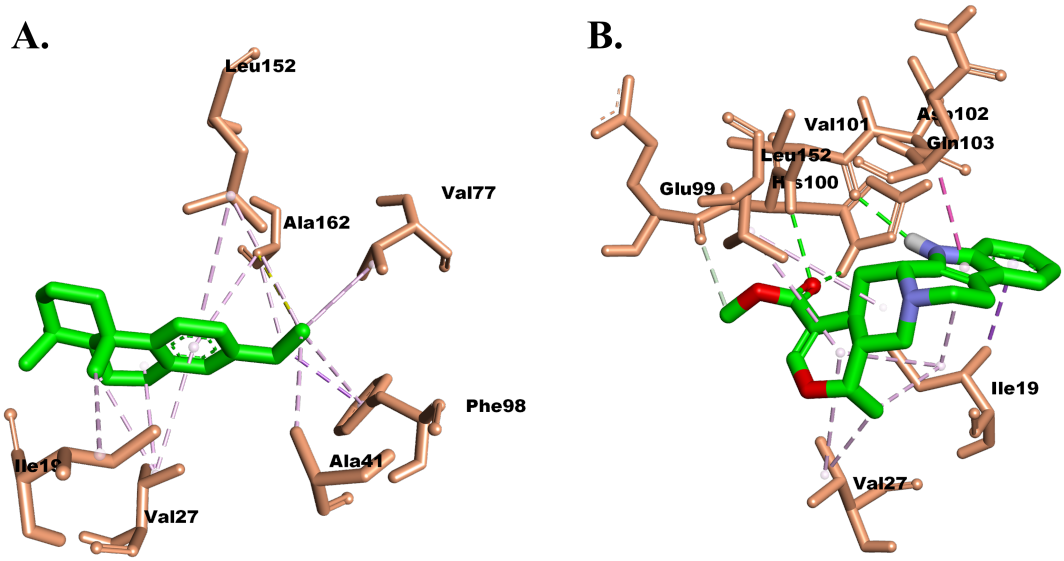
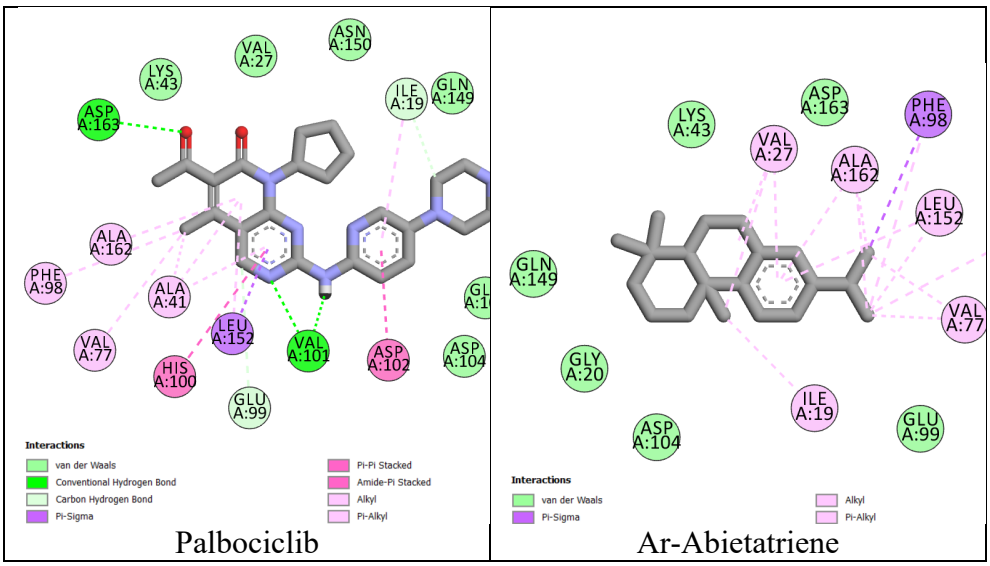
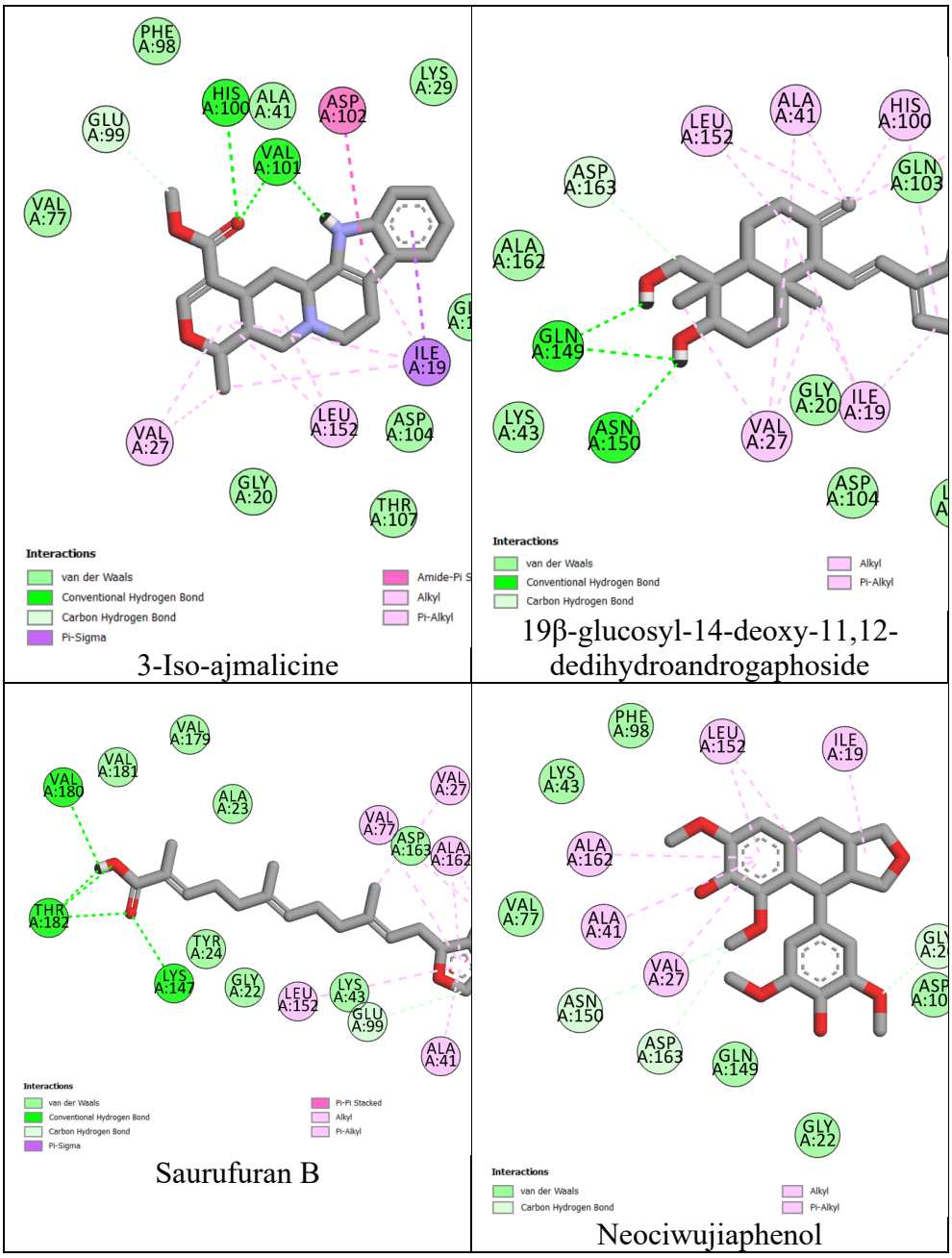



**Fig. 3**. Molecular interactions of (A) Ar-Abietatriene and (B) 3-Iso-ajmalicine against CDK-6

Overall, all three compounds were able to interact hydrophobically with residues in the active site of ERα, such as Ile19, Val27, Ala41, Leu152, and Ala162. Additionally, they exhibited varying hydrogen bonding compared to palbociclib and the two best compounds (Figure 3). These residues in CDK-6 play crucial roles in its structure and function. Ile19 and Ala47 are located near the active site of CDK-6. It is involved in forming hydrophobic interactions with ligands that bind to CDK-6. These residues contribute to stabilizing ligand binding and help maintain the structural integrity of the active site. In addition, Ala162, situated near the ATP binding site of CDK-6, plays a crucial role in establishing a cavity within the active site of CDK-6, facilitating its interaction with the ligand.




PHE A:98
LYS A:29
HIS A:100
ALA A:41
ASP A:102
GLU A:99
VAL A:101
VAL A:77
ILE A:19
LEU A:152
ASP A:104
VAL A:27
GLY A:20
THR A:107
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Amide-Pi S
Alkyl
Pi-Alkyl
3-Iso-ajmalicine
LEU A:152
ALA A:41
HIS A:100
ASP A:163
GLN A:103
ALA A:162
GLN A:149
GLY A:20
ILE A:19
LYS A:43
ASN A:150
VAL A:27
ASP A:104
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl
19β-glucosyl-14-deoxy-11,12-dedihydroandrogaphoside
VAL A:179
VAL A:181
VAL A:180
ALA A:23
VAL A:27
VAL A:77
ASP A:163
ALA A:162
THR A:182
TYR A:24
LYS A:147
GLY A:22
LEU A:152
LYS A:43
GLU A:99
ALA A:41
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Pi Stacked
Alkyl
Pi-Alkyl
Saurufuran B
PHE A:98
LEU A:152
ILE A:19
LYS A:43
ALA A:162
VAL A:77
ALA A:41
VAL A:27
ASN A:150
ASP A:163
GLN A:149
GLY A:22
Interactions
van der Waals
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl
Neociwujiaphenol

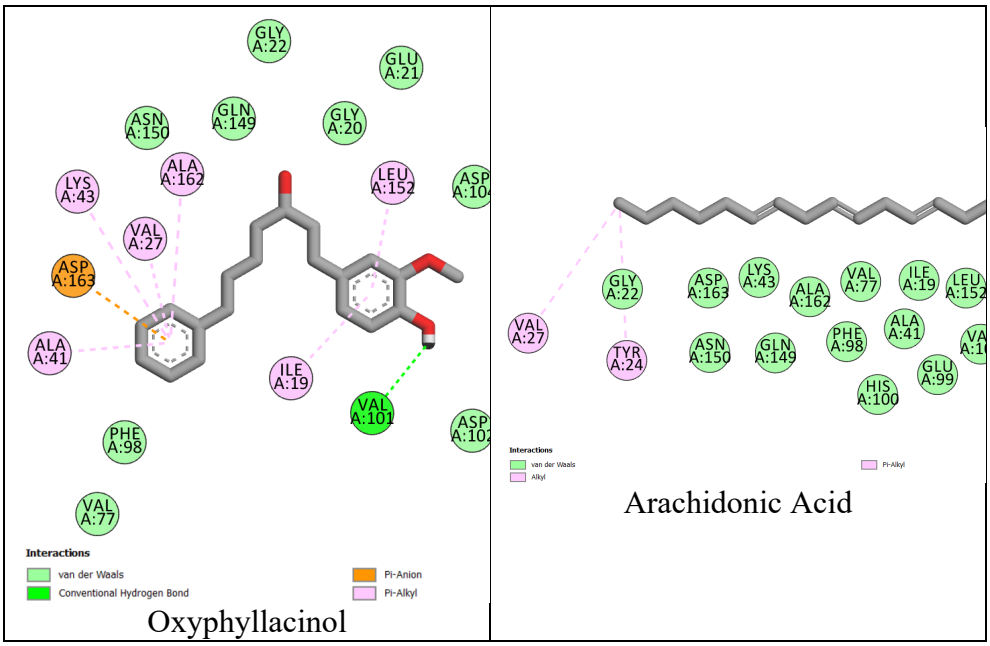



**Fig. 4**. Molecular interactions of all identified compounds in *Lobophytum* sp.'s fraction B against CDK-6

Through this computational study, two potential compounds from Fraction B have been identified: Ar-Abietatriene and 3-Iso-ajmalicine. These compounds are projected to have a crucial role in anticancer activity. Their promising characteristics suggest they hold significant potential as candidates for cancer treatment. The substantial evidence supporting this potential lies in the remarkable affinity exhibited by these three compounds towards CDK-6. CDK-6, which is critical in driving cell cycle progression and proliferation, is frequently deregulated and overly active in various cancer types. Targeting CDK-6 with specific inhibitors has emerged as a promising strategy to curtail tumor growth and enhance the overall outcomes of cancer treatments. The strong interaction of these compounds with CDK-6 highlights their potential, offering a new avenue to improve cancer treatments.

## 5 Conclusion

Fractionation of ethylacetate extract of *Lobophytum* sp. produced seven fractions (A-G). The Fraction B (9.7% w/w) is the most toxic toward *Artemia salina* brine shrimp. LC-MS/MS data indicated that the fraction contains 19b-glukocyl-14-deoxy-11,12-didehydrographoside, 3-isoazmalicine, abietraticine, arachidonic acid, neociwujiaphenol, oxyphyliacinol, saurufuran B and some unidentified compounds with molecular formulas $C_{37}H_{46}O_7$, $C_{35}H_{44}O_5$, and $C_{20}H_{26}O_2$. Further studies on the potential of fractions and compounds that have an important role are carried out in in-silico cancer cells. Ar-Abietatriene and 3-Iso-ajmalicine play vital roles in the anti-cancer attributes of *Lobophytum* sp.'s fraction B. Their interactions with important cancer-related receptors show their potential to inhibit cell cycle progression and cell proliferation. Additional investigations and studies are needed to comprehensively delve into these compounds' therapeutic capabilities in cancer treatment.

## Acknowledgments

The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia supported the project with a research grant under scheme of the Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2023, contract No. 28/UN29.20/PG/2023.

## References

1. I. Sahidin, C.W. Sabandar, Wahyuni, R.Hamsidi, M.H. Malaka, B. Sadarun, L.O. Aslan. MJAS, **22** (3): 375-82. (2018).
2. I. Sahidin, C.W. Sabandar, Wahyuni, R. Hamsidi, S.A. Mardikasari, W.O.S. Zubaydah, B. Sadarun, W.O.S. Musnina, A. Darmawan, A. Sundowo. The Nat. Prod. J., **10** (3): 312-321 (2020)
3. Wahyuni, A. Fristiohady, M.H. Malaka, F. Malik, M.I Yusuf, M. Leorita, B. Sadarun, A. Saleh, W.O.S. Musnina, C.W. Sabandar, I. Sahidin. JAPS, **9**(10): 008 (2019).
4. A. Fristiohady, Wahyuni, F. Malik, L.O.M.J. Purnama, I. Sahidin. AJPCR, **12**(12): 97-100 (2019).
5. A. Fristiohady, B. Sadrun, Wahyuni, M.H. Malaka, F. Ahmad, F. Malik, L.O.M.J. Purnama, I. Sahidin, JAPS**, 10**(6):081-089 (2020).
6. B. Sadarun, N.S. Rahmatika, A.W.M. Yodha, A. Fristiohady, A. Sundowo, S.N. Baharum, I. Sahidin,, Indonesian Journal of Marine Sciences, **27**(1): 29-36 (2022)
7. E. Wanda, B. Sadarun, Rahmadani, *Sapa Laut*, **3**(1): 9-15 (2018)
8. T.A. Mohamed, A.I. Elshamy, T.A. Hussein, J.H. Su, J.H. Sheu, M.E.F. Hegazi, Journal of Asian Natural Products Research, **19**(3), 201-207 (2017)
9. N.P. Thao, B.T.T. Luyen, N.T.T. Ngan, S.B. Shong, N.X. Chuong, N.H. Nam, P.V. Kiem, Y.H. Kim, C.V. Minh. Bioorganic & Med. Chem. Let. **24**: 228-232 (2014)
10. F.Z. Yin., Y.G. Yao, Z.Y. Zhang, Tetrahedron, **90**:132204 (2021)
11. T.H. Quang, T.T. Ha, C.V. Minh, P.V. Kiem, D.T.T. Thuy., Bioorganic & medicinal Chemistry, **19**: 2625-2632 (2011)
12. C.P. Mint, P.V. Kiem, N.X. Niem, C.V. Minh. Bioorganic & medicinal Chemistry Letters, **21**: 2155-2159 (2011)
13. Q. Zhang, X.W. Li, L.G. Yao, B. Wu, Y.W. Guo, Fitoterapia, **133**: 70–74 (2019)
14. S.Y. Cheng, Z.H. Wen, S.F. Chiou, C.Y. Duh., Tetrahedron, **64**: 9698–9704 (2008)
15. Q. Zhang, L.F. Liang, Z.H. Miao, B. Wu, Y.W. Guo, Steroids, **141**: 76-80 (2019)
16. P.K. Roy, S. Roy, K. Ueda. Fitoterapia, **136** ; 104162 (2019).
17. K. Pedoja, L. Husson, L. Bezos, A.W. Pastier,. Quaternary Science Reviews, **188**: 37e57 (2018)
18. Y.H. Tapilatu, HK-ICONS 2014, Procedia Chemistry, **14**; 484–492 (2015)
19. M.Y. Putra, T. Munarsih, I.S. Arlyza, Asia Pac J. trop Biomed **6**(11): 909-913 (2016)
20. E. Fattorusso, A. Romano, O. Taglialatela-Scafati, M.J. Achmad, Tetrahedron Letters, **49;** 2189–2192 (2008)
21. S. Pisutthanan, P. Plianbangchang, N. Pisutthanan, O. Muanrit. *Naresuan University Journal*, **12** (2), 13-18 (2004)
22. P. Chen, N.V. Lee, B.W. Murray,, 2016, *Mol. Cancer Ther.*, 15 (10), 2273–2281 (2016)
23. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, *J. Comput. Chem.*, 30 (16), 2785–2791(2009)
24. I. Sahidin, Nohong, M.A. Manggau, N.I. Muktiarni, 2023, *Indones. J. Sci. Technol.*, 8 (2), 217–242 (2023)

25 T. Oleg, and J.O. Arthur, *J. Comput. Chem.*, 31 (2), 455–461 (2010).
26. A. Arfan, R. Muliadi, A. Asnawi, *J. Kartika Kim.*, 5 (2), 145–151 (2022)
27. T. Subarmaniam, R.N.M. Rusli, K.V. Perumal, Z.A. Zakaria. Int J Mol Sci. 9;24(6):522 4 (2023)
28. C. Wang, N. Zhang, Z. Wang, J. Liu, Molecules, 22(8), 1280. (2017)
29. Y.H. Li, F. Chen, J.F. Wang, T. Guo, Chem Cent J. 2;7:134 (2013)
30. B.Y. Hwang, J.H. Lee, J.B. Nam, J.J. Lee, J Nat Prod., 65(4):616-7 (2002)
31. J.D. Phillipson, and S.R. Hemingway, Phytochemistry, 14(8), 1855–1863 (1975)
32. S.S. Bhar SS, and M.M. Ramana J Org Chem. 10;69(25):8935-7 (2004)
33. Q. Bian, W. Wang, N. Wang, R. Dai, PLOS ONE, 11(11), e0166777 (2016)
34. S. Nur, A.N. Aisyah, E. Lukitaningsih, A.S. Hajar, JAPS, **11**(08):054-061 (2021).
35. S. Nebenfuehr, K. Kollmann, V. Sexl, 2020, *Int. J. Cancer*, **147** (11), 2988–2995 (2020).
36. J.L. Kohlmeyer, D.J, Gordon, M.R. Tanas, V. Monga, D.E. Quelle, D. E., *Int. J. Mol. Sci.*, **21** (8) (2020)
37. C.L. Braal, E.M. Jongbloed, S.M. Wilting, SR.H.J.Mathijssen, R. H. J., Koolen, A. Jager, A., *Drugs*, **81** (3), 317–331 (2021)