Asia-Pacific Journal of Science and Technology :Volume :30 Issue :04 Article ID: APST-30-04-15 Research Article

APST

Asia-Pacific Journal of Science and Technology

https//:www.tci-thaijo.org/index.php/APST/index

Published by Research and Innovation Department,
Khon Kaen University, Thailand

# The effect of methanolic extract of *Peronema canescens* Jack. on HCT116 colorectal cancer cell line: In silico and in vitro approaches

Rian Ka Praja[1], Rizki Rachmad Saputra[2], Muhammad Priyadi[3*], Reny Rosalina[2], Decenly[4], Dwi Hermayantiningsih[2]

[1]Department of Microbiology, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[3]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[4]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Palangka Raya, Palangka Raya, Central Kalimantan, Indonesia
[*]Corresponding author: muhammad.priyadi@mipa.upr.ac.id

Received 20 December 2023
Revised 19 November 2024
Accepted 11 March 2025

**Abstract**

*Peronema canescens* Jack. as a plant native to the forests of Kalimantan is empirically used by the Dayak community as a traditional medicine to treat various diseases, including its potential as an anticancer agent. Colorectal cancer, one of the cancers that many people suffer from, has the potential to be treated using herbal plants. In various phytochemical studies, the content of secondary metabolite compounds and pharmacological activity of *P. canescens* have been known in previous research, such as cytotoxic activity against cancer cells. The molecular target involved in the development of cancer is the epidermal growth factor receptor (EGFR), a protein responsible for the process of cancer. This study aimed to investigate the cytotoxic activity of *P. canescens* leaves on HCT116 colorectal cancer cells and molecular studies on EGFR. The methods used included cytotoxic tests using the neutral red (NR) test and molecular docking tests using Autodock Vina with the target protein EGFR. The results of the cytotoxic test of *P. canescens* leaf extract on HCT116 cells of 48-hour incubation had an $IC_{50}$ value of 578.36 µg/mL and *P. canescens* leaf extract with 72-hour incubation was 400.88 µg/mL. The results of the molecular docking test showed that bioactive compound derivatives of *P. canescens*, pregnan20-one,3-(acetyloxy)-5,6:16,17-diepoxy-(3α,5α,6α,16α) and resibufogenin, have a high binding affinity to EGFR, with values of -7.9 and -7.8 kcal/mol each, comparable to osimertinib (-8.3 kcal/mol).

**Keywords:** Cytotoxicity, *Peronema canescens*, HCT116, EGFR

## 1. Introduction

Colorectal cancer is a malignant tumor and is the third most common cancer worldwide which is expected to increase 60% to >2.2 million new cases and 1.1 million deaths in 2030 [1]. Colorectal colon cancer is a serious threat to public health because it is difficult to operate and metastasizes easily [1,2]. Several factors can cause colorectal cancer, such as environment, lifestyle (including lack of exercise, which can lead to obesity), and eating habits (excessive intake of saturated fats and low intake of vegetables and fruit) [3,4]. Current colorectal cancer treatment primarily involves surgery, coupled with chemotherapy and an improved diet. Early diagnosis by endoscopy is the main goal in diagnosing and treating colon cancer. Therefore, chemotherapy has an important role in the clinical treatment of colon cancer. Various types of cancer therapy and complementary agents have been developed for its treatment. Efforts to search for alternatives to treat cancer are still ongoing, but alternative drug candidates for this disease are still very few [5].

Among the various molecular targets involved in cancer development, the epidermal growth factor receptor (EGFR) stands out as an important regulator of the processes of cell proliferation, survival, and metastasis. Aberrant EGFR signaling has been observed in various types of cancer, making it an attractive target for anticancer therapies such as colorectal. Although synthetic EGFR inhibitors have shown some success, challenges such as resistance and off-target effects have spurred interest in exploring natural sources for EGFR-targeted drug candidates [6].

EGFR, a receptor tyrosine kinase within the ErbB protein family, necessitates ligand binding for the activation of its tyrosine kinase domain. This activation triggers signaling pathways responsible for cell proliferation, angiogenesis, migration, survival, and adhesion. Given the crucial role of these pathways in cancer cell survival, targeting EGFR is essential in treating colorectal carcinoma metastases [7,8]. Presently, numerous therapeutic drugs are available for colorectal cancers, including monoclonal antibodies like cetuximab and panitumumab. These antibodies function similarly by binding to EGFR's extracellular domain, inhibiting ligand interaction, and inducing tyrosine kinase internalization and destruction. Consequently, apoptosis is promoted by blocking downstream EGFR pathways [9]. However, cetuximab and panitumumab come with various adverse effects, such as rash, itching, skin changes, headache, gastrointestinal issues, and infections [2]. Another drug, osimertinib, serves as an irreversible EGFR/HER2 inhibitor and has proven effective in cancer cells with EGFR gene mutations [10,11]. Common adverse reactions to osimertinib include rash, diarrhea, stomatitis, and fatigue [12]. Therefore, the discovery of new bioactive compounds to inhibit EGFR from natural products with less adverse effects is essential.

One of the plants that have the potential to be explored and developed as a raw material for colorectal anticancer drugs is the sungkai plant (*Peronema canecens* Jack). *P. canescens*, a native plant species with a rich history of traditional medicinal use, has significant potential as a valuable resource in the field of natural products research. Sungkai acetone extract has seven peronemins compounds such as peronemin A2, A3, B1, B2, B3, C1, and D1 [13]. In addition, it has been reported that peronemin compounds have anticancer activity [14]. Previous study reported that the natural compound of methanol extracts of *P. canescens* was pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-,(3α,5α,6α,16α)-, resibufogenin, methyl stearate, butyl 4,7,10,13,16,19-docosahexaenoate, and hexadecenoic acid [15].

Based on the description above, it is necessary to conduct research on the colorectal anticancer activity of the methanol extract of *P. canescens* through the in-silico test of its secondary metabolite compounds on the EGFR protein and cytotoxic test using HCT116 colorectal cancer cells.

## 2. Materials and methods

### *2.1 In silico molecular docking*

The experiment used Autodock Vina 4, a molecular docking program, from The Scripps Institute in the United States. PyMol used to see the binding pocket of the receptors. With BIOVIA Discovery Studio, which was available at https://discover.3ds.com, the receptor and its binding site were created. Installed on a desktop running Windows 10 Pro, OpenBabel 2.3.2 ACD/ChemSketch (freeware version 10.00) was driven by an AMD A8-7410 APU (4 CPUs, 2.2 GHz, 4 GB Memory).

The main target of colorectal cancer (CRC) was paired with *P. canescens* leaf extract compounds. A total of 5 natural compounds and 1 reference compounds were scrutinized for their anticancer potential. Downloading the CRC protein structure was done via the protein databank EGFR (PDB ID: 6Z4B) (http://www.rcsb.org/). Autodock Vina software was used to prepare ligands and proteins for molecular docking. The target protein was processed by removing water molecules, hydrogenation, optimizing energy, and adjusting position parameters. The grid box and grid center were adjusted for specific docking as follows, EGFR (25 x 25 x 25; 44.547 x -14.093 x -4.719). In this study, the exhaustiveness was set at 64, the number of modes was 10, and the grid spacing was 0.375. To start with the simulation, the native ligand of each receptor was attached to the receptor protein. The validity of the docking procedure was determined by whether the native ligand could return to its original position with an RMSD value of less than 2 Å [16]. Following the validation step, the docking process was carried out using ligands from the substance *P. canescens*. The purpose of these parameters was to increase molecular bonding precision. The docked structure with the lowest energy was the optimal ligand-receptor structure. The visualization tool BIOVIA Discovery Visualizer was used to present the results.

### *2.2 Preparation of plant material and extraction*

The material used was *P. canescens* leaves obtained from Palangka Raya City, Central Kalimantan Province. Fresh *P. canescens* leaves were cleaned of dirt, washed with running water until clean, drained, then weighed as wet weight, cut, then air-dried until the weight was stable (no significant change in weight after weighing for several days in a row), then the dried simplicial was powdered with a blender and stored in a dry plastic bag,

labeled then stored in a place protected from sunlight. *P. canescens* leaf simplicial was soaked in 2 liters of methanol for 1 week. The filtrate was collected and filtered using filter paper and concentrated using an evaporator until the extract was thick.

*2.3 Preparation of cell culture and anti-cancer assay*

The cytotoxicity of *P. canescens* leaf extract against HCT116 cells was evaluated using the neutral red (NR) test. Cells were harvested and cultured into 96-well plates at a density of $4 \times 10^5$ cells/ml. After 24 hours incubation in an incubator with 5% $CO_2$. Cells were treated with various concentrations of *P. canescens* leaf extract samples and cisplatin as a positive control and then incubated for 48 hours and 72 hours. After that, the medium was replaced with 100 µL of 50 µg/ml neutral red (NR) solution. The solution mixture was incubated at 37 °C in an incubator with 5% $CO_2$ for 2 h. After incubation, the cells were pelleted by centrifugation at 2500 rpm, 10 min, and 25°C then discarded the NR and medium. Cells were rinsed with 100 µl PBS (pH 7.4) then 150 µl 0.33% HCl in isopropanol solution was added to each well. The absorbance of the solution was measured at 540 and 660 nm [17]. The absorbance data obtained is used to calculate the percent cell viability using the following formula:

$$\%\ cell\ viability = \frac{Sample\ absorbance - Blank\ sample\ absorbance}{Control\ absobance - Blank\ control} \times 100\%$$

*2.4 Data analysis*

Data analysis of the $IC_{50}$ cytotoxic test results was classified into activity categories and molecular studies using energy affinity compared to native ligands.

**3. Results and discussion**

*3.1 Molecular docking*

Data was generated by redocking between native ligands toward each receptor protein. Redocking toward EGFR produced a binding energy value of -8.3 kcal $mol^{-1}$. Using an in silico structure-based methodology, we have investigated five compounds from *P. canescens* leave to produce several different potent receptor inhibitors. Based on the previous findings [15], the natural compound of methanol extracts was pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-,(3α,5α,6α,16α)-, resibufogenin, methyl stearate, butyl 4,7,10,13,16,19-docosahexaenoate, and hexadecenoic acid. Our research revealed that about five chemical constituents of *P. canescens* leave mentioned before, had higher binding energies when bound to EGFR (Table 1 and Table 2) compared to osimertinib as the original ligand. Based on in silico analysis, five possible compounds were selected for further investigation.

**Table 1** Binding energy values of *P. canescens* chemical compounds toward EGFR, ERK1/2, and AKT1.

| No | Compound | Binding Energy (kcal/mol) |
|---|---|---|
| | | EGFR |
| 1. | *Native Ligand* | *-8.3* |
| 2. | *Common Drug* | *-8.3* |
| 3. | Pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3α,5α,6α,16α)- | -7.9 |
| 4. | Resibufogenin | -7.8 |
| 5. | Methyl stearate | -6.2 |
| 6. | Butyl 4,7,10,13,16,19-docosahexaenoate | -5.6 |
| 7. | Hexadecanoic acid | -4.4 |

The molecular docking results demonstrated that bioactive compounds from *P. canescens* leaves, such as pregnan-20-one and resibufogenin, exhibited binding energy values of -7.9 and -7.8 kcal/mol, respectively, against EGFR, slightly higher than the reference drug osimertinib (-8.3 kcal/mol). In molecular docking, higher (less negative) binding energy indicates a weaker interaction between the ligand and the receptor, implying that these compounds may have a lower inhibitory potential compared to osimertinib [18]. A strong ligand-receptor interaction (reflected by a more negative binding energy) often translates into enhanced binding stability and greater inhibition of protein function [19]. However, it is important to note that binding energy alone is not always predictive of therapeutic efficacy; other factors, such as bioavailability, cellular uptake, and metabolic stability, also play critical roles [20].

Despite the relatively weaker binding energy, the identified compounds are still valuable leads in drug discovery. Natural products often offer unique chemical scaffolds that can be optimized through structural

modifications to improve binding affinity [11]. Additionally, compounds with moderate binding strength may result in reduced side effects. Osimertinib, for example, is known to cause adverse effects such as rash, diarrhea, and fatigue due to its strong, irreversible inhibition of EGFR [10]. In contrast, a less potent but selective inhibitor might provide therapeutic benefits while minimizing toxicity—a key consideration in developing long-term cancer therapies [9].

Another significant advantage of natural compounds is their potential to overcome drug resistance. Resistance to EGFR inhibitors such as osimertinib is a major clinical challenge, often emerging due to secondary mutations in the EGFR protein or activation of compensatory pathways [7]. Natural products like those from *P. canescens* may interact differently with EGFR, offering new modes of inhibition that can circumvent resistance mechanisms [21]. Furthermore, the presence of multiple active compounds in a plant extract could provide synergistic effects, targeting various pathways simultaneously, which may further contribute to anticancer efficacy [2].

Overall, while the current docking results suggest that the binding of *P. canescens* compounds to EGFR is weaker than osimertinib, these natural derivatives provide promising scaffolds for further optimization. Future studies could focus on chemical modifications to enhance their binding affinity and selectivity. Moreover, in vitro and in vivo evaluations will be necessary to determine their pharmacokinetic properties, toxicity profiles, and anticancer efficacy in colorectal cancer models [22].

According to the in vitro test toward HCT116, the sungkai's methanol extract has no inhibition activity toward cell culture was confirmed. Nevertheless, the binding energy toward that receptor is supported by the comprehensive study of colorectal cancer (CRC). The content that is being presented highlights the critical functions the EGFR plays in the course of CRC and highlights the potential of these proteins as therapeutic targets [21,23,24]. Since EGFR is overexpressed in CRC, it is a desirable target for therapeutic approaches. Inhibitors that interfere with EGFR signaling have been found by molecular docking studies, which is consistent with the effectiveness of targeted treatments for CRC such as osimertinib, ulicertinib, and capivasertib [20]. Similarly, CRC is frequently associated with deregulation of the ERK1/2 pathway, which is critical for cell signaling and promotes tumor growth. The inhibition of ERK1/2 has become a popular therapeutic approach [25]. Molecular docking studies have demonstrated the inhibitory potential of peronemin derivatives against ERK1/2, indicating the importance of targeting this pathway in the treatment of colorectal cancer.

**Table 2** Binding energy, binding site, and binding similarity of sungkai compound derivatives.

| Compound | Binding Energy (Kcal/mol) | Binding Site | | Binding Similarity % |
|---|---|---|---|---|
| | | Hydrogen Bonding | Hydrophobic Interaction | |
| Osimertinib (CD) | -8.3 | Asp855 | Asp800, Leu799, Arg803, Cys797, Lys745, Gly719, Leu858, Leu844, Gly796, Met793, Leu792, Val726, Leu718, Ala722, Gly721, Asn842, Asp837, Ser720, Arg841 | 100 |
| pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3α,5α,6α,16α)- | -7.9 | | Gly719, Cys797, Leu718, Arg841, Leu844, Ala743, Met790, Ile744, Leu788, Lys745, Asp855, Val726, Thr854, Asn842, Ser720 | 52.63 |
| Resibufogenin | 7.8 | | Ser720, Gly719, Arg841, Leu718, Gln791, Ala743, Met793, Leu844, Leu792, Met790, Lys745, Val726, Gly796, Asp855, Cys797, Gly721, Asp800 | 73.68 |
| methyl stearate | -6.2 | | Thr854, Leu718, Gly796, Val726, Leu792, Ala743, Met790, Asp855, Leu844, Met793, Lys745, Leu858, Leu777, Met766, Leu788, Leu861, Leu862, Ala763, Leu747, Val786, Ile759 | 47.36 |
| butyl 4,7,10,13,16,19-docosahexaenoate | -5.6 | | Lys728, Leu792, Met790, Ala743, Val726, Leu844, Thr854, Asp855, Gly721, Asn842, Gly719, Arg841, Lys745, Cys797, Gly796, Leu718, Met793, Pro794, | 68.42 |
| hexadecanoic acid | -4.4 | | Ala743, met790, met793, gly796, leu718, thr854, leu844, val726, arg841, leu792, arg841, cys797, asn842, lys745, asp855, gly721 | 63.15 |

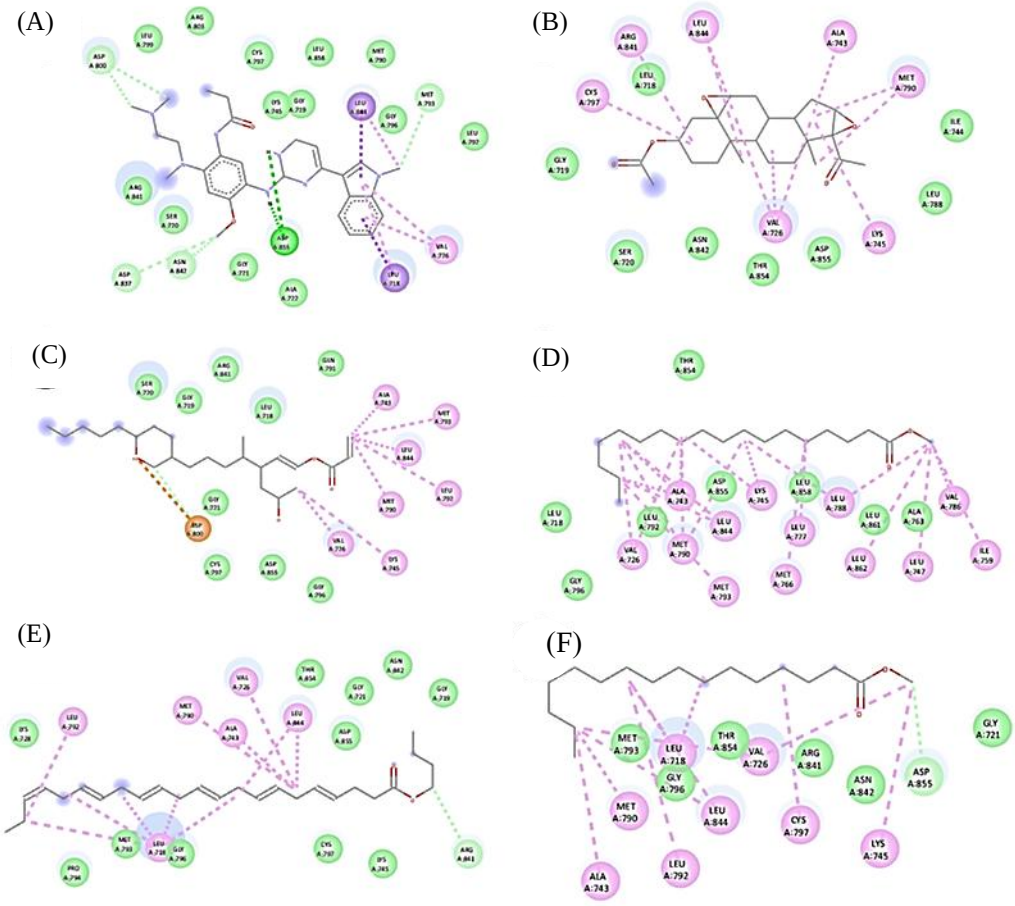


**Figure 1** 2D visualization of peronemin derivatives toward EGFR: ( A) Osimertinib, ( B) pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3α,5α,6α,16α)-,(C)resibufogenin,(D) methyl stearate, (E) butyl 4,7,10,13,16,19-docosahexaenoate, and ( F) hexadecenoic acid. Then, using Biovia Discovery Visualizer Studio (Figure 1), potential ligands for use as EGFR inhibitors were identified based on the type of chemical bond interaction and location of amino acid residues on the target protein; Figure 1 illustrates this interaction in two dimensions. Redocking analysis showed that osimertinib interacted with H-Bonding to Asp855 residues Asp800, Leu799, Arg803, Cys797, Lys745, Gly719, Leu858, Leu844, Gly796, Met793, Leu792, Val726, Leu718, Ala722, Gly721, Asn842, Asp837, Ser720, Arg841 through hydrophobic interactions.

The main topic discussed in the interaction simulation between five significant compounds from *P. canescens* leaf extract is pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3α,5α,6α,16α)-, resibufogenin, methyl stearate, butyl 4,7,10,13,16,19-docosahexaenoate, and hexadecenoic acid because they have binding energies that are slightly lower than the control molecules. By comparing the interactions between native ligands and derived compounds with EGFR, binding similarities were analyzed. The results showed remarkable binding similarity (47-73%) of the derived chemicals (Table 2). It is important to understand the similarity of ligand binding to the native ligand in the domain of molecular biology and drug design. This highlights how important it is that a ligand – usually a drug or small molecule – interacts in a manner comparable to the native ligand with a receptor or enzyme in a biological system. This similarity in binding is very important because it allows the ligand to have the desired effect on the target receptor or enzyme. A correlation consistent with similar inhibitory mechanisms showed that the mode of action of the ligands is similar to the original ligands based on their affinity for each other. These associations are very helpful in the drug design process because they explain the interactions between new chemicals and biological targets. When a new ligand binds similarly to the original ligand, it shows promise for therapeutic use or further scientific investigation due to the alignment in binding and mode of action [18,19].

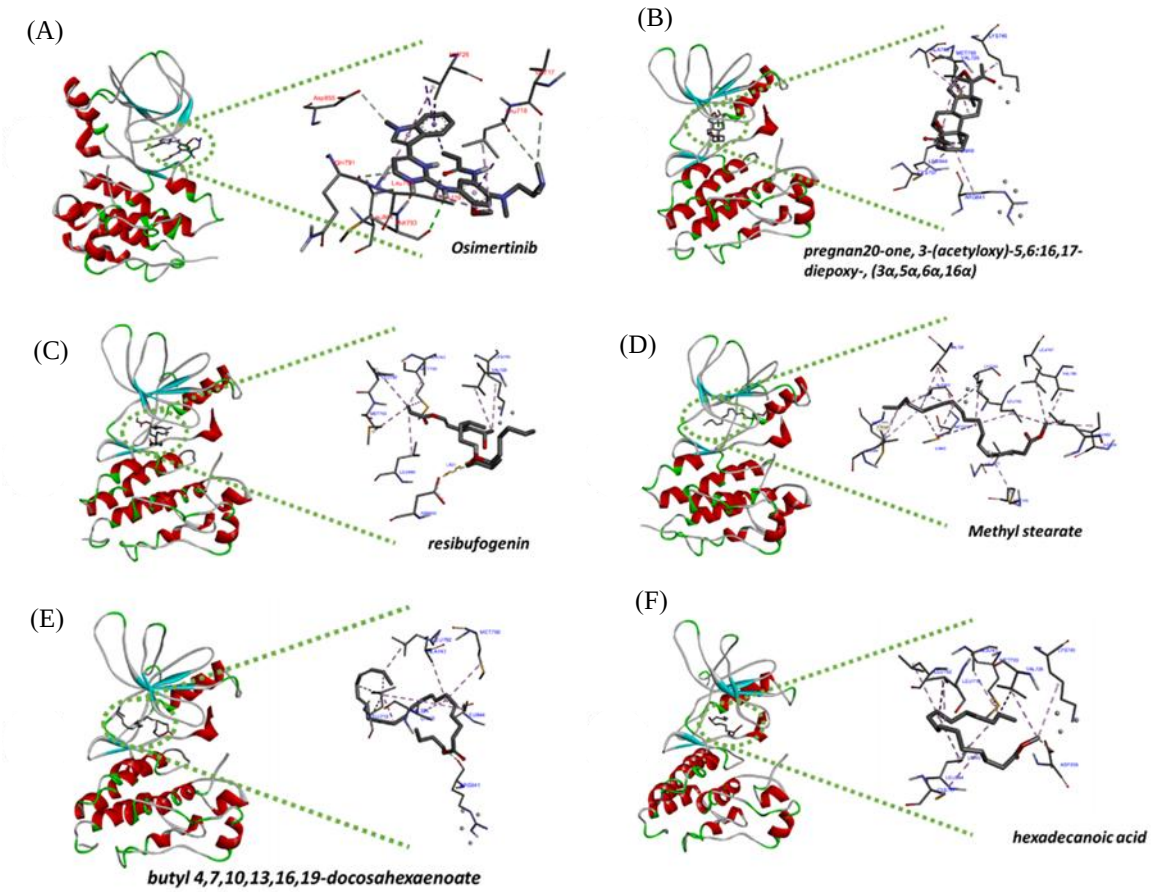


**Figure 2** 3D visualization of peronemin derivatives toward EGFR: (A) Osimertinib, (B) pregnan20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-,(3α,5α,6α,16α)-,(C)resibufogenin, (D) methyl stearate, (E) butyl 4,7,10,13,16,19-docosahexaenoate, and (F) hexadecenoic acid.

*3.2 Anticancer Activity*

Anticancer activity was determined through cytotoxic tests on HCT 116 cells using the MTT assay method. Samples added to HCT 116 cells were incubated for 48 hours and 72 hours.

**Table 3** Value of % live cells from HCT116 cells for 48 hours.

| Group | Viable Cells (%) |
|---|---|
| Negative Control | 100 |
| Sungkai Leaf Extract 1.0 µg/mL | 98.11 |
| Sungkai Leaf Extract 10.0 µg/mL | 94.00 |
| Sungkai Leaf Extract 100 µg/mL | 82.24 |
| Sungkai Leaf Extract 250 µg/mL | 75.23 |
| Sungkai Leaf Extract 500 µg/mL | 57.84 |
| Positive Control (Cisplatin 100 µg/mL) | 49.86 |

The results of the calculation of live cells in Table 3 and Table 4 show that the higher the concentration of the extract, the percentage of HCT116 cell life decreased until it approached the value of 50%. This supported that there was a decrease in cell proliferation rate with increasing doses.

**Table 4** Value of % live cells from HCT116 cells for 72 hours.

| Group | Viable Cells (%) |
|---|---|
| Negative Control | 104.34 |
| Sungkai Leaf Extract 1.0 µg/mL | 102.08 |
| Sungkai Leaf Extract 10.0 µg/mL | 84.96 |
| Sungkai Leaf Extract 100 µg/mL | 73.85 |
| Sungkai Leaf Extract 250 µg/mL | 54.43 |
| Sungkai Leaf Extract 500 µg/mL | 46.63 |
| Positive Control (Cisplatin 100 µg/mL) | 29.15 |

The calculation results showed the effect of each concentration of each sample on the % viable cells number. After that, the $IC_{50}$ value was calculated by carrying out a linear regression between log concentration vs % viable cells to obtain the equation Y = Bx + A where Y is the probit number and X is the log concentration.

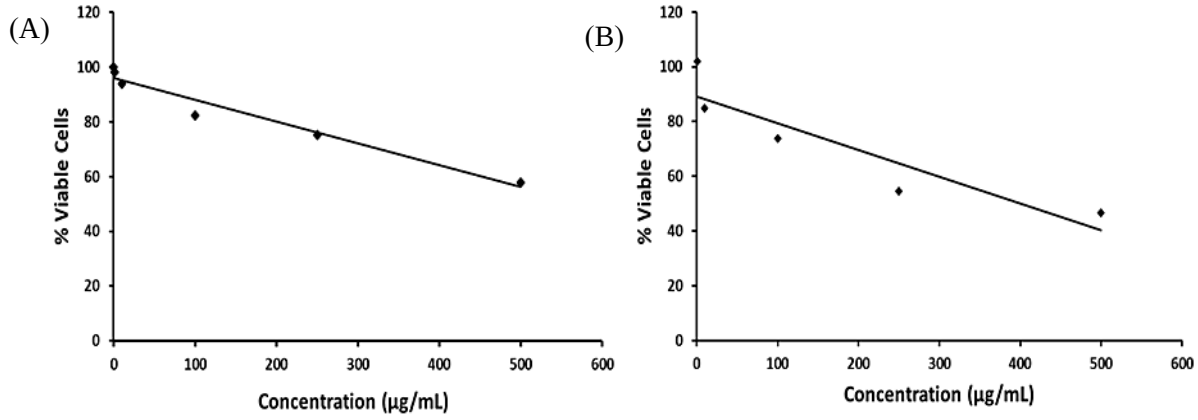


**Figure 3** Cytotoxic profile of the extract against HCT 116 cells 48 hours incubation (A) and 72 hours incubation (B).

The value of 50% of viable cells is entered into the regression equation as a Y value so that an X value can be obtained and the antilog result of the X value is referred to as inhibitory concentration ($IC_{50}$ )(Figure 3).

**Table 5** $IC_{50}$ Value of sungkai leaf extract on HCT116 cells.

| Group | $IC_{50}$ value (µg/mL) | Category |
|---|---|---|
| Sungkai Leaf Extract incubation 48 hours | 578.36 | Not Active |
| Sungkai Leaf Extract incubation 72 hours | 400.88 | Not Active |

*National Cancer Institute Category (Vijayarathna & Sasidharan, 2012; Çoruh & Özdoğan, 2017):
Active ($IC_{50}$< 30 µg/mL)
Moderate ($IC_{50}$30-100 µg/mL)
Not active ($IC_{50}$ > 100 µg/mL)

The anticancer activity of *P. canescens* leaf extract was tested using a cytotoxic test on HCT116 colon cancer cells to obtain the $IC_{50}$ value. The $IC_{50}$ value is defined as the minimum concentration of a cytotoxic compound that can inhibit the growth of cancer cells so that cell growth capacity is reduced by 50% [22]. Based on the National Cancer Institute [26,27], cytotoxic tests in Table 5, showed that the $IC_{50}$ value of *P. canescens* extract was greater than the positive control cisplatin so the cytotoxic activity of *P. canescens* leaf extract was known to be weaker than cisplatin. This is because the lower the $IC_{50}$ value, the stronger the cytotoxic activity of a compound. *P. canescens* leaf extract incubated for 48 hours had an $IC_{50}$ value of 578.36 µg/mL in the inactive category ($IC_{50}$ > 100 µg/mL). Meanwhile, *P. canescens* leaf extract incubated for 72 hours had an $IC_{50}$ value of 400.88 µg/mL in the inactive category ($IC_{50}$ > 100 µg/mL).

From various literature and ethnomedicinal studies, it is known that the most frequently used part of the *P. canescens* plant is the leaves. For generations, people have used *P. canescens* leave to treat various diseases, from fever to increasing immunity. The compound content in *P. canescens* are responsible for its pharmacological activity.

The solvent in the extraction process will affect the content of secondary metabolite compounds in the extract. The use of methanol as a solvent is related to studies on several plants that have anticancer activity on HCT116 colon cancer cells such as *Saurauia vulcani*, *Curcuma longa*, and *Grewia obtusa* [22,28,29]. Simultaneously, the use of n-hexane solvent is effective in attracting non-polar compounds such as steroids and terpenoids so it is hoped that it can attract the terpenoid compound group which is cytotoxic. Several terpene compounds are known to have activity on various types of colon cancer cells [30,31].

The weak cytotoxic activity of *P. canescens* leaf extract also depends on the type of cancer cells used so the anticancer activity of the *P. canescens* plant can also be seen in several other types of colon cancer cells such as HT-29 or WiDr. This is due to differences in the characteristics of cancer cells, sample concentration, and mutations in colon cancer cells. The chloroform subfraction of methanol extract from *P. canescens* leaves has strong cytotoxic activity on HT-29 colon cells [32]. Therefore, the colon anticancer potential of the *P. canescens* plant is still very broad to test its activity in various colon cancer cells and other cancer cells considering that several metabolite compounds in *P. canescens* have the potential to be anticancer compounds.

## 4. Conclusions

Based on the results of the cytotoxic test with the $IC_{50}$ value, it can be concluded that the methanol extract of *P. canescens* has an inactive category against HCT116 colorectal cancer cells for incubation for 48 hours and 72 hours. In addition, molecular docking tests show that five derivative compounds from *P. canescens* leaves exhibited weaker binding affinity to EGFR compared to a common drug of EGFR inhibitor.

## 5. Acknowledgements

This study was financially supported through a Junior Lecturer Research Grant (Penelitian Dosen Pemula) 2023 by the Institute for Research and Community Services/Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) of Universitas Palangka Raya.

## 6. Conflict of interest

The authors declare no conflict of interest.

## 7. References

[1] Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683–691.

[2] Janani B, Vijayakumar M, Priya K, Kim JH, Prabakaran DS, Shahid M, et al. EGFR-based targeted therapy for colorectal cancer-promises and challenges. Vaccines. 2022;10(4):1–15.

[3] Giftson JS, Jayanthi S, Nalini N. Chemopreventive efficacy of gallic acid, an antioxidant and anticarcinogenic polyphenol, against 1,2-dimethyl hydrazine induced rat colon carcinogenesis. Invest New Drugs. 2010;28(3):251–259.

[4] Minker C, Duban L, Karas D, Järvinen P, Lobstein A, Muller CD. Impact of procyanidins from different berries on caspase 8 activation in colon cancer. Oxid Med Cell Longev. 2015;2015.

[5] Grothey A, Venook AP. Optimizing adjuvant therapy for localized colon cancer and treatment selection in advanced colorectal cancer. J Natl Compr Cancer Netw. 2018;16(5S):611–15.

[6] Lin X, Wang G, Liu P, Han L, Wang T, Chen K, et al. Gallic acid suppresses colon cancer proliferation by inhibiting SRC and EGFR phosphorylation. Exp Ther Med. 2021;21(6):1–11.

[7] Martinelli E, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP, et al. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. Ann Oncol. 2020;31(1):30–40.

[8] Piawah S, Venook AP. Targeted therapy for colorectal cancer metastases: a review of current methods of molecularly targeted therapy and the use of tumor biomarkers in the treatment of metastatic colorectal cancer. Cancer. 2019;125(23):4139–4147.

[9] Saletti P, Molinari F, Dosso S De, Frattini M. EGFR signaling in colorectal cancer: a clinical perspective. Gastrointest Cancer Targets Ther. 2015;5:21–38.
[10] Guo L, Huang S, Wang X. PUMA mediates the anti-cancer effect of osimertinib in colon cancer cells. Onco Targets Ther. 2017;10:5281–5288.
[11] Troiani T, Napolitano S, Della Corte CM, Martini G, Martinelli E, Morgillo F, et al. Therapeutic value of EGFR inhibition in CRC and NSCLC: 15 years of clinical evidence. ESMO Open. 2016;1(5):1–14.
[12] Jiang Y, Shi Y, Wang Z, Li H, Wang Z, He K, et al. Analysis of adverse drug reactions of Osimertinib in the 2nd-line treatment of EGFR mutant advanced nonsmall cell lung cancer. Precis Med Sci. 2022;11(2):69–81.
[13] Latief M, Fisesa AT, Sari PM, Tarigan IL. Aktivitas antiinflamasi ekstrak etanol daun sungkai (*Peronema Canescens* Jack) pada mencit terinduksi karagenan. J Farm Sains dan Prakt. 2021;7(2):2579–4558.
[14] Fikriansyah M, Nelson N, Latief M, Tarigan IL. Anticancer activities of seven peronemins (A2, A3, B1, B2, B3, C1, and D1) from *Peronema canescens* Jack: A prediction studies. Chempublish J. 2023;7(1):54–63.
[15] Amalia ID. Profil metabolit pada tingkat perkembangan daun sungkai (*Peronema canescens* Jack.) .Skripsi. Sriwijaya University; 2022.
[16] Prahasti AE, Yuanita T, Rahayu RP. Computer aided drug discovery utilization in conservative dentistry. J Int Dent Med Res. 2022;15(2):899–903.
[17] Ezhilarasan D, Ali D, Varghese R. Sesamol induces cytotoxicity via mitochondrial apoptosis in SCC-25 cells. Hum Exp Toxicol. 2021;40(12_suppl):S423–S433.
[18] Copeland RA. The drug-target residence time model: A 10-year retrospective. Nat Rev Drug Discov. 2016;15(2):87–95.
[19] Shultz MD. Two decades under the influence of the rule of five and the changing properties of approved oral drugs. J Med Chem. 2019;62(4):1701–1714.
[20] Doan NQH, Truong TN, Nguyen PTV. Molecular docking studies of glycyrrhetinic acid derivatives as anti-colorectal cancer agents. Curr Comput Aided Drug Des. 2020;17(3):429–44.
[21] Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. New Engl J Med Rev.2008;358(11):1160–1174.
[22] Pasaribu G, Hudiyono S, Budianto E, Cahyana AH, Pari G. In-vitro anticancer activity of *Saurauia vulcani* leaf extracts against human colon WiDr and HCT-116 cancer cells. Rasayan J Chem. 2021;2021(Special Issue):165–174.
[23] Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007;26(22):3291–3310.
[24] Manning BD, Cantley LC. AKT/PKB signaling: Navigating downstream. Cell. 2007;129(7):1261–74.
[25] Chan G, Gu S, Neel BG. Erk1 and Erk2 are required for maintenance of hematopoietic stem cells and adult hematopoiesis. Blood. 2013;121(18):3594–3598.
[26] Vijayarathna S, Sasidharan S. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. Asian Pac J Trop Biomed. 2012;2(10):826–829.
[27] Çoruh N, Özdoğan N. Wild-growing *Rosa heckeliana* Tratt .: Phenolic constituents with cytotoxic and antioxidative properties. Turkish J Biol. 2017;41:195–212.
[28] Madhuri BS, Bhaskar D, Sathyamurthy B. Invitro studies on the effect of *Curcuma longa* methanol extracts in colorectal HCT - 116 cell lines. Indo Am J Pharm Sci. 2017;4(9):3015–3023.
[29] Abdelaziz MAA, Azger Dusthackeer VN, Peraman R. Anticancer activity of *Grewia obtusa* fruit on HCT-116, MCF-7, and HeLa tumour cells besides antitubercular activity. Indian J Pharm Educ Res. 2022;56(3):765–771.
[30] Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. Nat Prod Commun. 2020;15(3): 1–13
[31] Kamran S, Sinniah A, Abdulghani MAM, Alshawsh MA. Therapeutic potential of certain terpenoids as anticancer agents: a scoping review. Cancers (Basel). 2022;14(5):1100.
[32] Ibrahim A, Siswandono, Bambang Prajogo EW. Cytotoxic activity of *Peronema canescens* Jack leaves on human cells: HT-29 and primary adenocarcinoma colon cancer. Pharmacogn J. 2021;13(6):1389–1396.