

The Exploration of Vetiver (*Vetiveria zizanioides*) as Co-Chemotherapy of Lung Cancer Selectively Targets AKR1C1: Bioinformatics Approach

Lucky Octavianus Saputra¹, Salsabila Yusfita Fawzy¹, Ratih Kurnia Wardani¹, Endang Lukitaningsih^{2*}

¹Bachelor of Pharmacy Study Program, Faculty of Pharmacy, Universitas Gadjah Mada, North Sekip, Yogyakarta 55281, Indonesia

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, North Sekip, Yogyakarta 55281, Indonesia

Abstract

Reactive Oxygen Species (ROS) is one of the cancer-causing agents, one of which is lung cancer. In addition to being carcinogenic, ROS can also be used to kill cancer cells themselves, by increasing their levels to the threshold of apoptosis. Therefore, it is necessary to inhibit certain antioxidant enzymes that are highly expressed in lung cancer. One of them is AKR1C1 which plays a role in the eradication of intracellular ROS. However, AKR1C1 has a high structural similarity to AKR1C2, so it can inhibit therapy causing selectivity problems. Vetiver (Vetiveria zizanioides) has potential as an anticancer. This study was conducted to explore vetiver as a co-chemotherapeutic agent for lung cancer targeting AKR1C1 selectively. The method used is distillation, identification of vetiver compounds using GCMS, and through bioinformatics studies. Predictive analysis with KNIME was carried out to determine the activity of the test compound. All tested vetiver compounds had a predictive value of 1 (active) on AKR1C1 and 0 (inactive) on AKR1C2. Through GCMS obtained 354 compounds were identified. These compounds are used to filter the compounds predicted by KNIME. The molecular docking results showed that of the 10 tested vetiver compounds, there was 1 compound that had the strongest bond in interacting with AKR1C1, namely beta vetispirene compound with an S-score of -15.12 kcal/mol, and stronger than native ligand and aspirin. Based on the research data, it can be concluded that the beta vetispirene compound in vetiver can be a potential co-chemotherapy agent for lung cancer in targeting AKR1C1 selectively. However, further research is needed to prove its activity on lung cancer cells.

Keywords: ROS (Reactive Oxygen Species), Lung Cancer, AKR1C1, selectivity, vetiver (Vetiveria zizanioides).

INTRODUCTION

Reactive Oxygen Species (ROS) is an oxidative molecule that can damage cell components, one of which is DNA. ROS can be generated from various sources, from inside the body to external

Submitted: October 25, 2021 Revised: September 21, 2022 Accepted: September 23, 2022 Published online: November 30, 2022

*Corresponding author: lukitaningsih_end@ugm.ac.id



exposure. Pollutants are the main contributor to the formation of ROS. Although humans naturally have a ROS elimination mechanism, if the intracellular ROS is too high, the elimination process will be disrupted. The accumulated ROS will trigger damage and lead to carcinogenesis (Gašparović, 2020).

Lung cancer is one type of cancer that can be triggered by ROS. Since 1985, lung cancer has become a common and frequent occurrence. Lung cancer has become the largest contributor to the diagnosis of cancer, accounting for 1,350,000 new cases and 12.4% of the total cases. The survival ability of patients with lung cancer in the United States is approximately 5 years and causes 1,180,000 or 17.6% of the total cancer deaths (Cruz, et al., 2011).

Chemotherapy is commonly known as cancer therapy by relying on drugs. However, most chemotherapy is less effective and not selective in attacking cancer cells, some are even toxic to normal body tissues. Doxorubicin is one of the chemotherapy that is still used, but in some cases it shows side effects such as myocardial damage, rapid weight gain, and chest pain. Currently, several therapies use a combination of drugs in the treatment of cancer patients. Combination chemotherapy provides more effective results than single agents. Therefore, we need a chemotherapy companion (co-chemotherapy) that has selective activity and synergistic effect to be able to reduce the dose of chemotherapy that was previously said to be toxic (Meiyanto and Jenie, 2007).

Protein Aldo Keto Reductase Family 1 Member C1 (AKR1C1) is a member of the AKR1C enzyme family. AKR1C1 plays a role in reducing the dominant 20-ketosteroid in humans and plays an important role in the reductive inactivation of progesterone to 20α -DHP. In addition, AKR1C1 also plays a role in reducing the amount of 4-hydroxy-2-nonenal (HNE) which is a pro-oxidant compound (Briozic, *et al.*, 2006). AKR1C1 has a complex role in carcinogenesis. Although the exact mechanism in

carcinogenesis is not known, AKR1C1 is involved in cancer cell proliferation due to its association with MMP2, MMP9, EGFR, AKT, and P-ERK proteins (Tian, *et al.*, 2016). There is evidence of a strong correlation between AKR1C1 expression levels and malignant transformation, as well as resistance to cancer therapy (Wenners, *et al.*, 2016). Decreased AKR1C1 expression has also been shown to decrease cancer cell progression (Tian, *et al.*, 2016).

Based on the previous description, it is known that AKR1C1 is an enzyme that metabolizes ROS or pro-oxidant compounds. Inhibition of AKR1C1 is thought to affect intracellular ROS levels. In cancer therapy, the level of ROS in cells is modulated to reach a limit that forces cancer cells to initiate a suicide program or apoptosis (Larasati, et al., 2018). In addition to triggering genotoxicity and apoptosis, AKR1C1 inhibition can reduce the progression, proliferation, migration of cancer cells, and chemotherapy resistance (Tian, et al., 2016). However, the AKR1C1 protein has a high structural similarity to the AKR1C family of enzymes, making it difficult for selective therapy targeting AKR1C1. The highest structural similarity is in AKR1C2, where the difference in the active site is only found in amino acid number 54 so that the selectivity is low. To be able to target AKR1C1 selectively, the compound must be able to interact with the Leu54 amino acid in AKR1C1 (Yang, et al., 2017).

Vetiver (*Vetiveria zizanioides*) is a plant that belongs to the Poaceae family. This plant, which is famous for its fragrant aroma, grows in various plains of Indonesia. Vetiver essential oil contains various compounds, especially the sesquiterpene group with the typical compound cameroonian-7-alpha-ol (13.57%), caryophyllene (4.65%), humulene (3.22%), beta-vetispirene (1, 6-4.5%), chusimol (3.4-13.7%), vetiselinenol (1.3-7.8%), and beta-vetivone (2.5-6.3%). However, so far, essential oils have only been used as raw materials for perfumes, food preservatives, tranquilizers, anti-inflammatory, spasmolytic, and as local anesthetics.



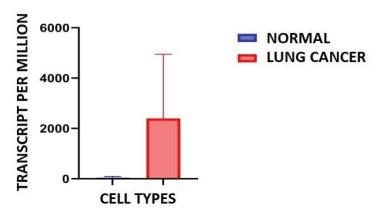


Figure 1. Graph of AKR1C1 expression level in normal cells and lung cancer cells.

Whereas vetiver extract has been shown to have strong cytotoxic activity against MCF-7 breast cancer cells (Chitra, *et al.*, 2014).

Based on the potency of vetiver and AKR1C1, therapy targeting AKR1C1 selectively can be a promising solution for lung cancer. By looking at the lack of chemotherapy which has many side effects, a co-chemotherapy agent is needed that is able to reduce the side effects of the current chemotherapy. Therefore, vetiver will be explored to be directed as a potential co-chemotherapeutic agent targeting AKR1C1 selectively.

METHODS AND MATERIALS

Tools and Materials

Tools: Apparatus Clevenger, 1L distillation flask, glassware, Gas Chromatoghrapy Mass Spectrofotometry (GCMS) QP-2010 Shimadzu Japan with RTx1-MS Column, laptop with specifications in the form of an Intel Core I3 kitchen and 4GB RAM, software in the form of Google Chrome, KNIME 4.6.1, MOE 2010, GraphPad, and platforms online in the form of UALCAN (http://ualcan.path.uab.edu/analysis.html), RCSB PDB (https://www.rcsb.org), GeneCards (https://www.genecards.org), and PROTEIN BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Materials: 3 kg vetiver, 10 L water, vetiver essential oil, helium gas, AKR1C1 protein data on

cancer cells obtained from the database UALCAN, AKR1C1 homologous protein data obtained from the database BLAST, data on active compounds of vetiver obtained from the results of GCMS, data on natural AKR1C1 inhibitors, and data on AKR1C1 inhibitors that have been found from GeneCards.

Vetiver (Vetiveria zizanioides) Preparation

Determination was carried out on vetiver and the results of the determination confirmed that the species is *Vetiveria zizanioides*. The determination was made at the Department of Pharmaceutical Biology, Faculty of Pharmacy UGM. A total of 3 kg of vetiver was distilled by steam and 10 L of water for 4-6 h. The essential oil from steam distillation and water is collected and the yield is calculated.

Gas Chromatoghrapy Mass Spectrofotometry (GCMS) Analysis

The vetiver essential oil obtained from the distillation was collected in a separating funnel and then analyzed for its metabolomic profile using GCMS. GCMS analysis uses an ionization energy system of 70 eV. Identification and determination of the structure is carried out based on the relative mass of the molecule and its fractions, in order to obtain a chromatogram of the vetiver volatile oil compound. The GCMS that used has the following column and tool conditions: Column HP-5MS UI;



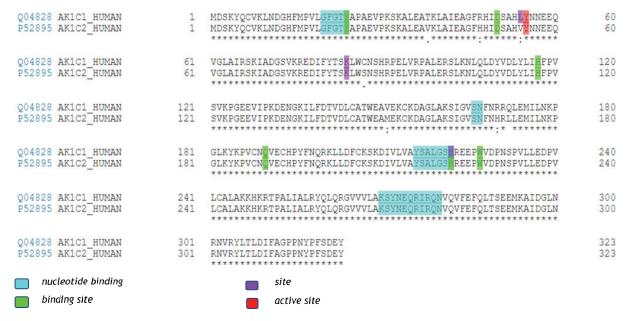


Figure 2. Comparison of amino acid sequences in AKR1C1 and AKR1C2.

Length 30m; I. D. 0,25mm; Film 0,25µm; Max Temperature 325/350°C; Carrier Gas Helium UHP (He); Injector Temperature 260°C; Split flow 50 ml/min; Split ratio 50; Front Inlet Flow 1,00 ml/min; MS Transfer Line Temperature 250°C; Ion Source Temperature 200°C; Purge Flow 3 ml/min; Gas Saver Flow 5 ml/min; Gas Saver Time 5 min.

Identification of AKR1C1 Expression Levels with UALCAN

The UALCAN database was used to obtain AKR1C1 protein expression data in normal cells and cancer cells. How to use the database is to access the website http://ualcan.path.uab.edu/analysis.html on Google Chrome and write the keyword "AKR1C1" in the search field. Then, select lung cancer.

Table 1. AKR1C1 inhibitor compounds in GeneCards database.

| Name | Status |
|----------------|----------|
| Aspirin | Approved |
| Salicylic acid | Approved |
| NADH | Approved |
| Fenofibrate | Approved |
| Lumateperone | Approved |

Identification of AKR1C1 Protein Homology with BLAST

The BLAST database was used to search for AKR1C1 protein homology which had high similarity. How to use the database is to access the website https://blast.ncbi.nlm.nih.gov/Blast.cgi on Google Chrome and perform a search filter only for Homo sapiens organisms. After finding the results of protein homology, one protein with the highest similarity was selected, then compared with the amino acid composition. This step is used to prove the similarity of AKR1C1 with AKR1C2.

AKR1C1 Inhibitor Exploration with GeneCards

AKR1C1 inhibitor exploration was carried out using the GeneCards database. How to use the database is to access the website https://www.genecards.org on Google Chrome and write the keyword "AKR1C1" in the search field. Then, select "Drugs" to get AKR1C1 inhibitor data. Drugs or compounds that have the status of "Approved" means that they have been tested and proven to be able to inhibit AKR1C1.



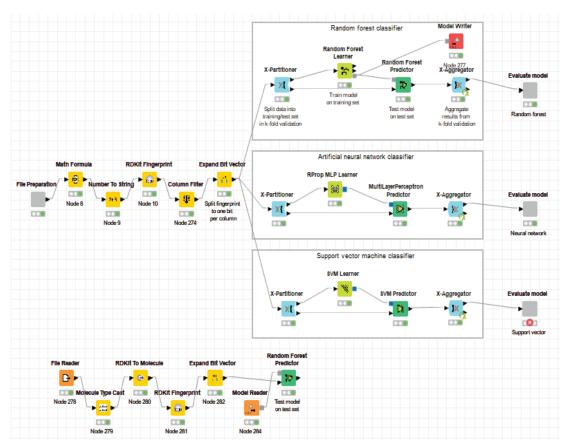


Figure 3. Machine learning compound activity test in KNIME.

Activity Test of Vetiver Compounds with KNIME

KNIME was used to identify the activity of vetiver compounds that selectively reactive to AKR1C1 (inactive on AKR1C2). The data used are IC₅₀ inhibitor and Smiles Code values for active compounds in vetiver. A machine learning program was made in KNIME with inhibitor data AKR1C1 and AKR1C2 to see the selectivity and set it with a cut off =7.0. IC_{50} values and Smiles Code active compounds of vetiver were used as parameters. These parameters were compared for each type of active compound in vetiver and produced a readable output to determine the potential active compound in vetiver. After the program is run, the results will appear in the form of numbers 0 and 1. If the predicted value obtained is 1, then the compound is active on the protein being tested, whereas if the

predicted value obtained is 0, then vice versa. For validation, it can be seen from the P Classification value (P>0.95) and the overall accuracy obtained (>70%).

Identification of Vetiver Compounds Using Extraction and Gas Chromatoghrapy Mass Spectrofotometry (GCMS)

A total of 25 g of vetiver simplicia was put into a 1L distillation flask and mixed with 50 mL of distilled water. A total of 1 mL of hexane was added to dissolve the essential oil for 8 h in the extraction process. Furthermore, the oil phase is collected and hexane evaporation is carried out so that the amount of oil can be evaluated according to the function during distillation. The essential oil extracted from vetiver was then analyzed for its compound content using GCMS.



Identification of vetiver compounds using GCMS was carried out at LPPT UGM. The extracted essential oil was prepared and put into the GCMS apparatus. The compounds in the vetiver essential oil will be separated based on the elution rate or the time it takes for the compounds to be identified. After the identification process is complete, the GC results chromatogram and a list of the names of the compounds identified are obtained.

Molecular Docking with MOE 2010

The molecular docking process was carried out using the MOE 2010 application. Molecular docking was used to see the strength of the bonds between proteins and vetiver compounds. The initial step in using the 2010 MOE application is to prepare protein according to the protein code obtained from the Protein Data Bank (PDB). Then, self-docking was carried out with the Triangle Matcher, London dG, and forcefield protocols as the settings for placement, rescoring, and refinement. After that, the test compound was prepared by minimizing energy and selecting the MMFF94x force field setting. Furthermore, the docking process was carried out with the test compound and the results were obtained in the form of an S-score. Then, a

visualization process is carried out to determine the amino acids that play a role and their interactions. The results obtained from the analysis process are S-score, Root Mean Square Deviation (RMSD), type of bond, docking pose, and interacting amino acids. The S-score value represents the strength of the bond between the protein and the test compound. The smaller the S-Score value, the greater the bond strength. The S-score value can be rejected if the RMSD value is >2. A good bond strength is if the S-score of the test compound is smaller than the S-score of the native ligand.

RESULTS

Expression Level of AKR1C1 in Normal Cells and Lung Cancer Cells

Based on Figure 1, AKR1C1 has an overexpression in lung cancer cells, with a significance value of 1.65*10 -12 (<0.05), which means the difference is significant. These results indicate that the AKR1C1 protein plays an important role in lung cancer defense mechanisms by maintaining ROS levels in cancer cells so that they do not reach the threshold of apoptosis.

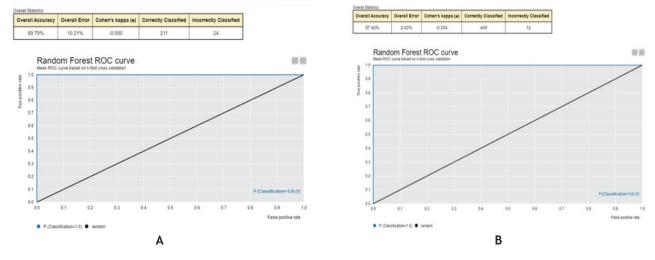


Figure 4. Validity of machine learning AKR1C1 (A) and machine learning AKR1C2 (B) in KNIME.



Table 2. Activities of vetiver compounds on AKR1C1 and AKR1C2.

| No | Compounds | Prediction (Classification) AKRICI | Prediction (Classification) AKRIC2 |
|----|---------------------------|---------------------------------------|---------------------------------------|
| I | beta cadinene | 1.0 | 0.0 |
| 2 | beta guaiene | 1.0 | 0.0 |
| 3 | cadina-1,4-diene | 1.0 | 0.0 |
| 4 | delta guaiene | 1.0 | 0.0 |
| 5 | alpha calacorene | 1.0 | 0.0 |
| 6 | zierone | 1.0 | 0.0 |
| 7 | cadina-4,9-diene | 1.0 | 0.0 |
| 8 | Isoledene | 1.0 | 0.0 |
| 9 | alpha gurjunene | 1.0 | 0.0 |
| 10 | beta humulene | 1.0 | 0.0 |
| П | dehydroaromadendrene | 1.0 | 0.0 |
| 12 | alpha curcumene | 1.0 | 0.0 |
| 13 | beta vatirenene | 1.0 | 0.0 |
| 14 | beta vetivenene | 1.0 | 0.0 |
| 15 | zingiberene | 1.0 | 0.0 |
| 16 | valencene | 1.0 | 0.0 |
| 17 | delta Cadinene | 1.0 | 0.0 |
| 18 | alloaromadendrene epoxide | 1.0 | 0.0 |
| 19 | beta nootkatol | 1.0 | 0.0 |
| 20 | cedr-8-en-13-ol | 1.0 | 0.0 |
| 21 | Nootkatone | 1.0 | 0.0 |
| 22 | alpha amorphene | 1.0 | 0.0 |
| 23 | cis-eudesma-6, I I-diene | 1.0 | 0.0 |
| 24 | alpha muurolene | 1.0 | 0.0 |
| 25 | valerenol | 1.0 | 0.0 |
| 26 | valerenal | 1.0 | 0.0 |
| 27 | zizanoic acid | 1.0 | 0.0 |
| 28 | khusimene | 1.0 | 0.0 |



| No | Compounds | Prediction (Classification) AKRICI | Prediction (Classification) AKRIC2 |
|----|-----------------------|------------------------------------|------------------------------------|
| 29 | gamma muurolene | 1.0 | 0.0 |
| 30 | gamma himachalene | 1.0 | 0.0 |
| 31 | a Ipha-Selinene | 1.0 | 0.0 |
| 32 | gamma cadinene | 1.0 | 0.0 |
| 33 | cycloisolongifolene | 1.0 | 0.0 |
| 34 | a lpha ylangene | 1.0 | 0.0 |
| 35 | alpha copaene | 1.0 | 0.0 |
| 36 | beta patchoulene | 1.0 | 0.0 |
| 37 | isolongifolene | 1.0 | 0.0 |
| 38 | sativene | 1.0 | 0.0 |
| 39 | longifolene | 1.0 | 0.0 |
| 40 | thujopsene | 1.0 | 0.0 |
| 41 | longifolenaldehyde | 1.0 | 0.0 |
| 42 | prezizaene | 1.0 | 0.0 |
| 43 | beta selinene | 1.0 | 0.0 |
| 44 | (+) delta selinene | 1.0 | 0.0 |
| 45 | 10-epi gamma eudesmol | 1.0 | 0.0 |
| 46 | beta vetispirene | 1.0 | 0.0 |
| 47 | cubenol | 1.0 | 0.0 |

Protein Homology of AKR1C1

The degree of similarity between AKR1C1 and AKR1C2 protein was measured using BLAST method. Based on the analysis carried out, homology value of the similarity of the two proteins was 97.8% with the ratio of amino acid sequences as shown in Figure 2. Based on Figure 2, almost all of the active sites and the binding of the two proteins are

the same. This high similarity can cause selectivity problems in AKR1C1-targeted therapies.

AKR1C1 Inhibitor Exploration

AKR1C1 inhibitors from exploration results using the GeneCards database are shown in Table 1. Drugs or compounds that have an "Approved" status mean that they have been



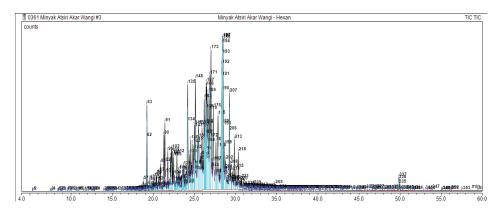


Figure 5. Chromatogram of GC-MS results of essential oils from fragrant roots.

tested and proven to be able to inhibit AKR1C1. Furthermore, these inhibitors were compared with their interactions with the active compounds of vetiver at the molecular docking stage.

Activity Analysis of Vetiver

KNIME-based machine learning analysis was carried out to search for active compounds of vetiver that have activity on target proteins. In this stage, 2 types of machine learning were used to see the selectivity of the vetiver compound. These compounds were tested for their activity on AKR1C1 and AKR1C2 proteins. Machine learning that has been created must be validated first. A program is said to be valid if the *P* classification value >0.95 and Overall Accuracy > 70% (Beisken, *et al.*, 2013).

Based on Figure 4, machine learning AKR1C1 has a *P* classification value of 1 and an Overall Accuracy of 89.79%. Then, in machine learning AKR1C2 has a *P* classification value of 1 and an Overall Accuracy of 97.40%. Both results are in accordance with the requirements so that the machine learning used can be declared valid.

Based on Table 2, 47 active compounds of vetiver that were tested had activity against AKR1C1 but no activity on AKR1C2, so it can be concluded that all active compounds of vetiver were selective in inhibiting AKR1C1.

Identification of Vetiver Compounds

Vetiver essential oil was obtained by distillation and its content was analyzed by GCMS.

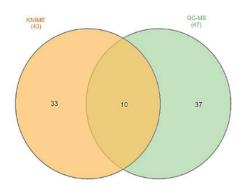


Figure 6. Venn diagram of slice of fragrant root compounds result of KNIME and GC-MS analysis.



Table 3. Results of slices of fragrant root compounds.

| No | Compounds | Smiles Code |
|----|---------------------------|---------------------------------|
| I | alloaromadendrene epoxide | CC1CCC2C1C3C(C3(C)C)CCC24CO4 |
| 2 | alpha gurjunene | CCICCC2C(C2(C)C)C3=C(CCC13)C |
| 3 | beta guaiene | CCICCC(=C(C)C)CC2=CICCC2C |
| 4 | (+) delta selinene | CCI=C2C=C(CCC2(CCCI)C)C(C)C |
| 5 | beta vetispirene | CCICC=CC(=C)CI2CCC(=C(C)C)C2 |
| 6 | Cubenol | CCICCC(C2CI(CCC(=C2)C)O)C(C)C |
| 7 | alpha amorphene | CCI=CC2C(CCI)C(=CCC2C(C)C)C |
| 8 | alpha calacorene | CCI=CCC(C2=CIC=CC(=C2)C)C(C)C |
| 9 | Nootkatone | CCICC(=O)C=C2CI(CC(CC2)C(=C)C)C |
| 10 | alpha ylangene | CC1=CCC2C3C1C2(CCC3C(C)C)C |

Based on Figure 5, 354 compounds were identified with 100 compounds having the highest abundance. The compound with the highest abundance was then searched for the Smiles Code. After knowing the Smiles Code, proceed to the filtering stage for the active compound of vetiver from the analysis using KNIME.

InteractiveVenn was used to find the intersection between vetiver compounds from KNIME analysis and GCMS analysis. Based on Figure 6, of the 47 compounds from the KNIME analysis, only 43 were read. Then, of the 100 compounds resulting from the GCMS analysis, only 47 were read. This can happen because several compounds have the same Smiles Code. After that, the slices were obtained in the form of 10 vetiver compounds which can be seen in Table 3.

Molecular Docking of Test Compounds with AKR1C1

Molecular docking is a simulation test used to analyze the interaction of the compound molecule from vetiver oil with the target protein AKR1C1. The protein model used in molecular

docking comes from RCSB PDB with code 6a7a which is AKR1C1 complexed with a new inhibitor with novel scaffold. Based on Table 4, all the results obtained are valid because the RMSD value <2. Beta vetispirene compound has the strongest bond among the 10 compounds tested with an S-score of -15.12 kcal/mol, and is stronger than the inhibitors that have been found, namely native ligand and aspirin with an S-score of -11.74 kcal/ moles and -8.37 kcal/mol.

Based on the visualization results in Table 5, beta vetispirene is able to interact with the amino acid Leu54, which is visualised by red circle. Beta vetispirene interactions with AKR1C1 are dominated by strong hydrophobic bonds between some amino acids, namely Tyr24, His222, Glu224, Leu54, Trp227, Tyr66, Ile129, Trp86, Leu308, Leu306, and His117.

DISCUSSION

In cancer therapy, the level of ROS in cells is modulated to reach a limit that forces cancer cells to initiate a suicide program or apoptosis. Inhibition



Table 4. Molecular docking results of fragrant root compounds against AKR1C1.

| No | Compounds | S-score (kcal/mol) | RMSD (Å) |
|----|---------------------------|--------------------|----------|
| ı | native ligand | -11,74 | 0,74 |
| 2 | alloaromadendrene epoxide | -10,38 | 1,31 |
| 3 | alpha gurjunene | -8,78 | 0,46 |
| 4 | beta guaiene | -9,06 | 1,18 |
| 5 | (+) delta selinene | -9,16 | 1,55 |
| 6 | beta vetispirene | -15,12 | 1,93 |
| 7 | cubenol | -10,53 | 1,36 |
| 8 | alpha amorphene | -9,24 | 0,53 |
| 9 | alpha calacorene | - 8,79 | 1,71 |
| 10 | nootkatone | -10,73 | 0,71 |
| П | alpha ylangene | -8,78 | 1,38 |
| 12 | aspirin | - 8,37 | 0,88 |

through AKR1C1 will affect intracellular ROS levels (Larasati, *et al.*, 2018). Inhibition of AKR1C1 can play a good role in suppressing cancer growth. Therefore, this study was conducted to explore vetiver as a co-chemotherapeutic agent for lung cancer targeting AKR1C1 selectively. To determine the expression of AKR1C1 in lung cancer, the identification was carried out using the UALCAN database. Based on Figure 1. result, AKR1C1 has significantly overexpressed in lung cancer cells, with a significance value of 1.65*10 -12 (<0.05). Therefore, it can be proved that AKR1C1 inhibition will be very promising in killing cancer cells.

Inhibition of AKR1C1 poses a selectivity problem because the protein has a high similarity to AKR1C2, which is its isoform (Yang, *et al.*, 2017). Based on BLAST analysis, the degree of similarity between AKR1C1 and AKR1C2 protein is 97.8% with the ratio of amino acid sequences as shown

in Figure 2. This can cause selectivity problems in AKR1C1-targeted therapies. Therefore, we need a compound capable of selectively targeting AKR1C1.

KNIME-based machine learning analysis was carried out to search for active compounds of vetiver that have activity on target proteins. Machine learning that has been created must be validated first. A program is said to be valid if the *P* classification value >0.95 and Overall Accuracy >70% (Beisken, *et al.*, 2013). After it was proven that the machine learning used was valid according to Figure 4., it was continued by analyzing the active compounds of vetiver essential oil obtained from Deng, *et al.* (2019). Based on Table 2, 47 active compounds of vetiver that were tested show a value of 1 on AKR1C1 and a value of 0 on AKR1C2, which means the active compound has activity against AKR1C1 but no activity on AKR1C2. So it can



Table 5. Visualization of molecular docking of vetiver test compounds against AKR1C1.

| Visualisasi | Native Ligand | Beta Vetispirene | Aspirin |
|-------------|--------------------------|-------------------------------|---|
| 2D | | | San |
| 3D | | | |
| | Val228, Trp227, Glu224, | Tyr24, His222, Glu224, Leu54, | His222, Asn167, Leu305, |
| | Tyr55, His222, His117, | Trp227, Tyr66, Ile129, Trp86, | His117, Tyr55, Tyr216, |
| Amino | Leu306, Leu308, Tyr24, | Leu308, Leu306, and His117 | Asp50, Leu266, Gln150. |
| Acids | Leu54, Phe311, Trp86, | | Tyr24 |
| | lle129, lle310 , Phe118. | | |
| | Asn167 | | |

Visualization is made in 2D and 3D. In the 2D visualization, the molecular structure of the ligands and the interacting amino acids can be seen. In the 3D visualization, the ligands are depicted in the shape of sticks and balls and the active protein sites are depicted with pink ribbons.

be concluded that all active compounds of vetiver were selective in inhibiting AKR1C1. The results of the bioinformatics study were proven by testing the analysis of vetiver compounds using GCMS. InteractiveVenn was used to find the intersection between vetiver compounds from KNIME analysis and GCMS analysis. Smiles Code of the 100 compounds with the highest abundance of GCMS results compared to compounds from KNIME

analysis. 354 compounds were identified with 100 compounds having the highest abundance. Based on Table 3, the slices were obtained in the form of 10 vetiver compounds, they were alloaromadendrene epoxide, alpha gurjunene, beta guaiene, (+) delta selinene, beta vetispirene, cubenol, alpha amorphene, alpha calacorene, nootkatone, and alpha ylangene. These 10 final compounds will be continued in the molecular docking process.



Molecular docking is a simulation test used to analyze the interaction of the compound molecule from vetiver oil with the target protein AKR1C1 compared to native ligand and known inhibitor (aspirin). Aspirin was used as a comparison compound because based on Table 1, aspirin has been shown to be able to inhibit the activity of AKR1C1. On the other side, the selectivity of AKR1C1 was confirmed through the KNIME analysis data, the sample was proven to have no interaction with AKR1C2. Therefore, molecular docking of AKR1C2 is not required. This molecular docking analysis is valid because the RMSD value <2. Based on Table 4, the results of the S-scores from native ligand alloaromadendrene epoxide, alpha gurjunene, beta guaiene, (+) delta selinene, beta vetispirene, cubenol, alpha amorphene, alpha calacorene, nootkatone, alpha ylangene, and aspirinare were -11,74; -10,38; -8,78; -9,06; -9,16; -15,12; -10,53; -9,24; -8,79; -10,73; -8,78; and -8,37 respectively. Beta vetispirene compound has the strongest bond among the 10 compounds tested with an S-score of -15.12 kcal/mol, and is stronger than the inhibitors that have been found, namely native ligand and aspirin with an S-score of -11.74 kcal/ moles and -8.37 kcal/mol. From these results, it can be concluded that beta vetispirene is a potential vetiver root compound as an inhibitor of AKR1C1.

After that, visualization was carried out to see the types of bonds and interactions that occurred to strengthen the results obtained. The compound must be able to interact with the Leu54 amino acid on AKR1C1 to be able to target AKR1C1 selectively (Yang, *et al.*, 2017). Based on Table 5, beta vetispirene is able to interact with the amino acid Leu54. Therefore, this interaction causes beta vetispirene to have the strongest bond among 9 other vetiver compounds, even stronger than native ligands and aspirin. Beta vetispirene can interact with AKR1C1 even though it does not have hydrogen bonds, but the interactions are dominated by strong hydrophobic bonds between some amino acids, namely Tyr24, His222, Glu224, Leu54,

Trp227, Tyr66, Ile129, Trp86, Leu308, Leu306, and His117.

CONCLUSION

ROS are radical compounds which if the levels are too high in the body will trigger the emergence of cancers, lung cancer is one of them. However, ROS can also be used as agents to inhibit cancer progression by increasing ROS levels to exceed the threshold of apoptosis so that cancer cells will die by themselves. Therefore, it is necessary to inhibit certain antioxidant enzymes that are highly expressed in lung cancer cells. From the results of the study, AKR1C1 was shown as one of antioxidant enzyme that have overexpressed in lung cancer, so that the inhibition of AKR1C1 would be very promising. However, this protein was shown to have a high similarity structure to AKR1C2, causing selectivity problems. After exploring the vetiver compounds through GCMS and monitoring the activity of the compounds through a structural approach, beta vetispirene derived from vetiver oil has the strongest and most selective interaction with AKR1C1. The selectivity based on the presence of binding interactions with the amino acid Leu54, which is the amino acid that differentiates between the two proteins. Therefore, it can be concluded that beta vetispirene has the potential to be further tested to be used as a co-chemotherapeutic agent for lung cancer because it is able to selectively inhibit AKR1C1.

ACKNOWLEDGMENT

Thank you to the Direktorat Pembelajaran dan Kemahasiswaan, Direktorat Jenderal Pendidikan Tinggi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi Republik Indonesia, also Pusat Prestasi Nasional for funding our research. We also thank Universitas Gadjah Mada and Cancer Chemoprevention Research Center (CCRC), Faculty Pharmacy, Universitas Gadjah Mada for facilitating this research.



REFERENCES

- Brozic, P., Smuc, T., Gobec, S., and Rizner, T.L., 2006, Phytoestrogens as inhibitors of the human progesterone metabolizing enzyme AKR1C1, *Mol. Cell. Endocrinol.*, **259**(1-2), 30-42.
- Beisken, S., Meinl, T., Wiswedel, B., De Figueiredo, L. F., Berthold, M., and Steinbeck, C., 2013, KNIME-CDK: Workflow-driven Cheminformatics, BMC Bioinformatics., 14, 257.
- Chitra, T., Jayashee, S., and Rathinamala, J., 2014, Evaluation of anticancer activity of Vetiveria Zizanioides against human breast cancer cell line, *International Journal of Pharmacy and Pharmaceitocal Sciences.*, **6**(1), 164-166.
- Cima, F., and Ballarin, L.. 2021, Genotoxicity and Immunotoxicity of Organotins, *Biochemical and Biological Effects of Organotins.*, **15**, 97-111.
- Cruz, C.S.D., Tanoue, L.T., and Matthay, R.A., 2011, Lung Cancer: Epidemiology, Etiology, and Prevention, *Clinics in chest medicine*, **32**(4), 605-644.
- Deng, G., David, A., Wang, F., Sun, X., Li, H., Lin, J., and Li, P., 2019, Chemical Composition, Antioxidant, and Antimicrobial Activities of *Vetiveria zizanoides* (L.) Nash Essential Oil Extracted by Carbon Dioxide Expanded Ethanol, *Molecules.*, 24(10), 1897.
- Gašparović, A., 2020, Free Radical Research in Cancer, *Antioxidants.*, **9**(2), 157.
- Issaravanich, S., Palanuvej, C., Tunsaringkarn, T.,

- Rungsiyothin, A., Vipunngeun, A., Chuthaputti, A., and Ruangrungsi, N., 2008, Pharmacognosic specification of Vetiveria zizaniodes roots in Thailand, *J Health Res.*, **22**(1), 9-14.
- Larasati, Y.A., Kato, N.Y., Nakamar, I., Yokoyama, T., Meiyanto, E., and Kato, J.Y., 2018, Curcumin targets multiple enzymes involved in the ROS metabolic pathway to suppress tumor cell growth, *Scientific Reports.*, 8(1), 2039.
- Meiyanto, E., and Jenie, R.I., 2007, Ko-kemoterapi ekstrak etanolik daun sambung nyawa (*Gynura procumbens* (Lour.) Merr.) dan Doxorubicin pada sel kanker payudara, *Majalah Farmasi Indonesia*, **18**(2), 81-87.
- Tian, H., Li, X., Jiang, W., Lv, C., Sun, W., Huang, C., and Chen, R., 2016, High expression of AKR1C1 is associated with proliferation and migration of small-cell lung cancer cells, *Lung Cancer: Targets and Therapy.*, **7**, 53-61.
- Wenners, A., Hartmann, F., Jochens, A., Roemer, A.M., Alkatout, I., Klapper, W., Mackelenbergh, M. V., Mundhenke, C., Jonat, W., and Bauer, M., 2016, Stromal markers AKR1C1 and AKR1C2 are prognostic factors in primary human breast cancer, *Int. J. Clin. Oncol.*, **21**, 548-556.
- Zeng, C.M., Chang, L.L., Ying, M.D., Cao, J., He, Q.J., Zhu, H. and Yang, B., 2017, Aldo-Keto Reductase AKR1C1-AKR1C4: Functions, Regulation, and Intervention for Anti-cancer Therapy, *Frontiers in Pharmacology*, **8**, 119.