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Structure-based virtual screening of bioherbicide candidates for weeds in sugarcane plantation using *in silico* approaches

Penapisan virtual berdasarkan struktur kandidat bioherbisida untuk gulma pada perkebunan tebu menggunakan pendekatan in silico

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Abstrak

Gulma pada perkebunan tebu berdampak negatif pada produktivitas tebu. Beberapa pendekatan telah dilakukan untuk menangani gulma, termasuk penggunaan diuron sebagai herbisida sintetik. Bagaimanapun, penggunaan diuron dalam jangka panjang berefek buruk dan menyebabkan produksi 3,4-Dichloroaniline yang akumulasinya dapat menyebabkan pengikisan hara tanah. Oleh sebab itu, penelitian ini bertujuan untuk mencari herbisida alami. Dengan meniru mekanisme diuron yang menghambat proses fotosintesis pada protein fotosistem II D1 (psbA) yang terdapat pada gulma, empat belas senyawa sebagai kandidat potensial bioherbisida ditambatkan secara virtual menggunakan program PyRx v.0.9.5 pada situs yang spesifik. Tiga spesies gulma utama yang dipilih adalah Eleusine indica, Praxelis clematidea, *dan* Momordica charantia. *Skor ikatan afinitas selanjutnya dikalkulasi dan diperingkat untuk penapisan enam senyawa terbaik sebagai kandidat bioherbisida. Interaksi setiap kompleks dan prediksi aktivitas biologis kemudian dilakukan dengan program Discovery Studio dan PASS server, secara berurutan. Aurachin P, Aurachin A, dan Cyanobacterin muncul pada peringkat teratas dengan skor afinitas yang tinggi pada psbA yakni -6 hingga -9 kkal mol^{-1}. Interaksi asam amino yang terlibat pada kompleks menunjukkan 50-90% kesamaan pada kompleks kontrol, yakni psbA dan diuron. Di samping itu, prediksi aktivitas biologis Aurachin P, Aurachin A, dan Cyanobacterin menunjukkan istilah yang terkait dengan inhibisi proses fotosintesis melalui jalur enzimatis. Maka, senyawa aktif tersebut kemungkinan memiliki aksi penghambatan proses fotosintesis dan mengendalikan gulma pada perkebunan tebu.*

[Kata kunci: diuron, penambatan molekul, penghambatan fotosintesis]

Abstract

Weeds in sugarcane have negatively affected the sugar yield rate. Several approaches have been carried out to overcome the weeds, including the usage of diuron as synthetic herbicide. However, the long-term usage of diuron is known to have a negative effect leads to the production of 3,4-Dichloroaniline responsible for soil leach and bioaccumulation. Therefore, this study aimed to find a potential natural herbicide. By mimicking the diuron's mode of action which inhibits the process of photosynthesis through blocking the Photosystem II protein D1 (psbA) of the weeds, fourteen compounds as potential candidate bioherbicides were virtually docked by PyRx v.0.9.5 software to the specific site. Three important species of the weeds were chosen including *Eleusine indica*, *Praxelis clematidea*, and *Momordica charantia*. The binding affinity score was further calculated and ranked to screen the top six compounds as bioherbicide candidates. Interaction of each complex and the biological activity prediction were then performed by Discovery Studio software and PASS server, respectively. Aurachin P, Aurachin A, and Cyanobacterin were placed in the top ranked compounds with high binding affinity score around -6 to -9 kcal mol^{-1} toward the psbA. The amino acid interaction involved in the complex shows 50-90% similar to the control, psbA and diuron complex. Besides, the biological activity prediction of Aurachin P, Aurachin A, and Cyanobacterin exhibits the terms related to the inhibition of photosynthesis process via enzymatic pathway. Thus, the active compounds might have inhibition action in the photosynthesis process and control the weeds in sugarcane.

[Keywords: diuron, molecular docking, photosynthesis inhibition]

Introduction

Controlling weeds in sugarcane is a challenge that farmers should face. The growth of weeds itself is faster than the sugarcane (*Saccharum officinalis* L.), mainly in the early stages of crop growth. Yields of sugarcane reported decrease around 24 to 93% as a result of nutrient loss by competing with crops for water, nutrients, and sunlight (Singh & Kumar, 2013). Singh *et al.* (2011) highlighted a significant increase of the sugarcane weeds in the plantation as farmers have

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limited expertise and knowledge to improve the weed management.

The utilization of herbicides is a common practice in weed management of plant crops. Previous research reported the high efficacy of herbicides (90-99%) in killing weeds (Wakabayashi & Boger, 2002; Délye *et al*., 2013). In the USA, herbicides have been widely used (95%) in cotton, soybean, maize, and sugar beet (Gianessi, 2005). Based on the *Sistem Informasi Pestisida* database in 2020, the usage of herbicides in Indonesia is targeted to many commodities including acacia, orchid, grapes, apple, corn mill, onion, and shallot. However, chemical herbicides usage has been banned due to the negative effects in soil health, aquatic environments, and the atmosphere. The substance also has negative effects to human health and ecosystem sustainability (Morales *et al*., 2013; Huovinen *et al*., 2015; Velki *et al*., 2019).

Diuron is one of the most familiar synthetic herbicides used by farmers to control weeds in their sugar plantation (Peng, 2012). The substance belongs to the phenyl amide family and acts as a photosynthesis inhibitor by preventing oxygen production (Wessels & Veen, 1956). In addition, it blocks the electron transfer in the photosystem II (PSII). The D1 protein is the center of the PSII reaction and C-terminal processing of the precursor D1 protein is important (Teixeira & Elzbieta, 2013). Some reports mentioned the substance effectivity in killing the annual and perennial grassy weeds. It has been widely used in plant crops such as cotton, sugarcane, alfalfa, and wheat. However, the usage of diuron has been reported to cause environmental problems. Diuron has been detected in 28% of river samples in the USA National Canal System. Besides, the presence of diuron produces the intermediate substances, leading to the formation of 3,4-Dichloroaniline (3,4-DCA) which causes soil leaching and bioaccumulation (Giacomazzi & Cochet, 2004). While in Indonesia, the diuron usage mainly for sugarcane is still allowed. The regulation in Indonesia allowing the diuron used as an active compound for ten brands of pesticides, including Amrocon 80 WP, Bioron 80 WP, Gonzales 80 WP, Gulmaron 500 SC, Gulmaron 80 WP, Maron 80 WP, Ronindo 500 SC, Ronindo 80 WP, Sidaron 80 WP, and Viaron 500 SC (Sistem Informasi Pestisida, 2020). If this condition continues to happen, it might affect soil health in the environment. This finding leads to the classification of diuron as a harmful substance causing the suppression of its utilization within 20 years based on the Directive 2000/60/CE. To overcome this condition, researchers tried to develop herbicides derived from the secondary metabolite of plant species and microbes to minimize the environmental effects and creating safer and non-toxic compounds (Nusrat *et al*., 2018; Radhakrishnan *et al*., 2018). Dayan & Duke (2014) have reviewed the varieties of next-generation herbicides from natural compounds and the detailed mechanisms of action. This article was used as a database of bioherbicides source in this study. This study provides insight into finding the bioherbicides virtually using the structural bioinformatics approach that incorporates a molecular docking method to find better performance and safer bioherbicides than diuron. The analysis was based on its mechanism of action to block the photosynthesis process in sugarcane. Targeting the protein and predicting the binding affinity using the molecular docking approach have been used years for the drug discovery process (Meng *et al*., 2011; Pinzi & Rastelli, 2019) due to its accuracy and effectivity to screen the candidates. The approaches were then adopted to find the candidates of bioherbicides targeting a specific protein in plants.

Material and Methods

Samples retrieval of bioherbicide candidates

The candidate of the bioherbicides list was retrieved from the review paper of Dayan & Duke (2014). This previous study provided the list of the mechanism of action (MOA) which is targeting PSII electron transport and its sources for bioherbicides. The compounds classified as natural phytotoxin isolates from various organisms (*Sorghum bicolor*, *Syctonema hofmanni*, *Fischerella muscicola*, and *Stigmatella aurantica*). The 3D structure of the candidate compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) with the unique ID (Table 1).

Protein sequences retrieval, modeling, and 3D structure analysis

Three types of weeds: *Eleusine indica*, *Praxelis clematidea*, and *Momordica charantia* were chosen based on the field observation. The sequences of Photosystem II protein D1 (psbA) from *E. indica* (ID K9MXP5), *P. clematidea* (ID W8RMZ2), and *M. Charantia* (ID A0A2I6BZY2) were retrieved from the Uniprot database (https://uniprot.org). The protein target was further modeled utilizing I-TASSER software (https://zhanglab.ccmb. medumich.edu/I-TASSER/) (Yang & Zhang, 2015) with a unique template having high similarity with the structure of amino acid sequences. The considerations were made to choose the proper model protein from I-TASSER including (1) The rank of proteins, which is based on TM-score of the structural alignment between the query structure and known structures in the PDB library; (2) The lowest Root Mean Square Deviation (RMSD[a]) score, represents the smallest RMSD value between residues that are structurally aligned by TM-align; (3) The highest IDEN[a], represents the highest percentage sequence identity in the structurally aligned

Table 1. Candidates for natural compounds of bioherbicides from PubChem database
Tabel 1. Kandidat bioherbisida asal senyawa alami dari database PubChem

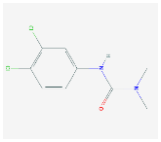
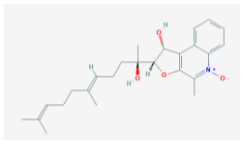
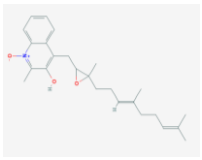
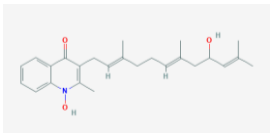
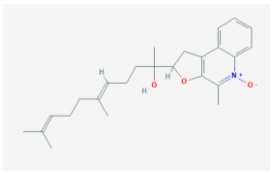
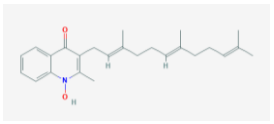
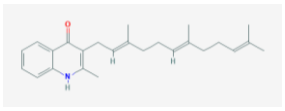
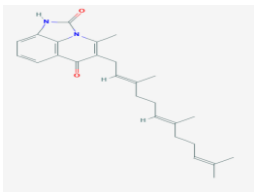
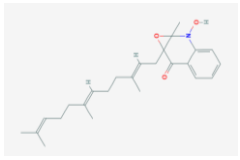
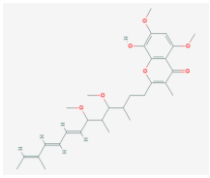
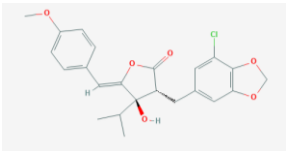
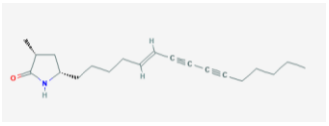
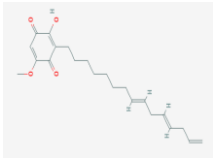
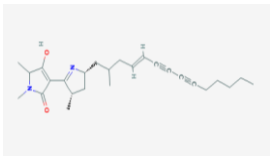
CID *Nomor identitas*	Chemical formula *Rumus kimia*	Chemical structure *Struktur kimia*	Molecular weight ($g\ mol^{-1}$) *Berat molekul* ($g\ mol^{-1}$)	Compound name *Nama senyawa*
3120	$C_9H_{10}Cl_2N_2O$		233.09	Diuron (control)
25157870	$C_{25}H_{33}NO_4$		411.5	Aurachin P
122706081	$C_{25}H_{33}NO_3$		395.5	Aurachin B epoxide
25188767	$C_{25}H_{33}NO_3$		395.5	Aurachin Re
6439172	$C_{25}H_{33}NO_3$		395.5	Aurachin A
6439171	$C_{25}H_{33}NO_2$		379.5	Aurachin C
6124753	$C_{25}H_{33}NO$		363.5	Aurachin D
13746957	$C_{26}H_{32}N_2O_2$		404.5	Aurachin E
6439168	$C_{25}H_{33}NO_2$		379.5	Aurachin B
90657799	$C_{25}H_{33}NO_3$		395.5	Aurachin-C epoxide

Table 1. (continue)

CID *Nomor identitas*	Chemical formula *Rumus kimia*	Chemical structure *Struktur kimia*	Molecular weight (g mol^{-1}) *Berat molekul (g mol^{-1})*	Compound name *Nama senyawa*
5353970	$C_{30}H_{42}O_7$		514.6	Stigmatellin
6437843	$C_{23}H_{23}ClO_6$		430.9	Cyanobacterin
70684692	$C_{20}H_{29}NO$		299.4	Fischerellin B
14427830	$C_{22}H_{30}O_4$		358.5	Sorgoleone 358
135476235	$C_{26}H_{36}N_2O_2$		408.6	Fischerellin A

region; and (4) The highest value of Cov score represents the highest coverage of the alignment by TM-align and is equal to the number of structurally aligned residues divided by the length of the query protein. To compare the psbA structure between three weeds, the amino acid sequences were aligned using MUSCLE in BioEdit software (Hall, 2011). The RMSD of each 3D structure protein was calculated to reveal the structure differences using PyMol (Schrödinger, USA) (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC).

Molecular docking analysis and visualization

In order to understand the molecular interaction and affinity binding between bioherbicide candidate compounds and psbA, virtual screening in molecular docking was carried out using PyRx 0.9.5 software developed by Dallakyan & Olson (2015) and has been widely used (de Sousa *et al*., 2020; Kulkarni *et al*., 2020; Venkateshan *et al*., 2020). The research design simply looked for the inhibitor of the psbA and found a better natural compound candidate than diuron. The grid used for docking analysis was center X: 65368, Y: 81888, Z: 91545, dimensions (Å) X: 7168, Y: 7358, and Z: 10405. The docking was done specifically at diuron's binding site as a control. The docking complex and amino acid interaction were visualized using Discovery Studio R2017 (Dassault Systèmes BIOVIA, BIOVIA Visualizer, Release 2017).

Biological activity prediction

In order to explore the biological activity of candidate compounds, prediction analysis was carried out using the PASS server website (http://www.pharmaexpert.ru/passonline/index.php). This web server calculated the biological activity based on the structural similarity in the database. It scored the prediction range from 0 to 1. The score above 0.7 represents the prediction accuracy of the laboratory test (Filimonov *et al*., 2018).

Results and Discussion

Amino acid sequence alignment, protein modeling, and RMSD analysis

Amino acids sequences were aligned using MUSCLE to identify the possibility of structure variation of psbA among three weeds. The alignment showed the amino acid differences among 353 amino acids between psbA in three weeds of *E. indica*, *P. Clematidea*, and

M. charantia (Figure 1). Three amino acids differences appeared in the psbA of *P. Clematidea* including residue pairs number 11 (T → E); 346 (L → I); and 351 (L → T). While in *M. charantia*, six differences were found in residue pairs 11 (T → E); 238 (K → R); 346 (L → V); 348 (A → V); 349 (P → T); and 351 (L → I) compared to the *E. indica* protein sequences.

The protein structure was modelled to visualize the 3D structure of psbA from those three weeds. The template 4YUUA was selected by the I-TASSER algorithm among thousands of proteins in LOMET database (Figure 2A-C). The superimpose analysis was carried out by calculating the RMSD scores representing the atomic distances to identify the structure differences (Figure 2D). The psbA of *E. indica* and *P. clematidea* has RMSD score of 1.016, whilst the *E. indica* and *M. charantia; M. charantia* and *P. clematidea* showed approximately similar RMSD scores, around 0.9 (Table 2). The data confirmed that structure variation among those three proteins was found.

The distance-based measurement by RMSD analysis of each D1 protein from *E. indica*, *P. clematidea* and *M. charantia* exhibited deviation score, ~1 Å. A report from Eyal *et al.* (2005) stated the limit of accuracy of protein modeling is ~8 Å, which leads to the conclusion that the D1 protein has no meaningful differences in terms of structure and function. The RMSD value was calculated based in the pairs of atoms and the distance between two atoms (Kufareva & Abagyan, 2012). Slight differences were spotted in the 3D structure of D1 protein from each weed species when visualized using superimpose approaches, which supports the RMSD values (Figure 2D, red circle).

Figure 1. Protein sequence alignment of psbA in *E. indica*, *P. clematidea* and *M. charantia*. The amino acids sequences were aligned using MUSCLE with parameters BLOSUM98 matrix. The red box shows differences of amino acid sequences from three psbA

Gambar 1. Pensejajaran sekuen psbA dari spesies E. indica, P. clematidea *dan* M. charantia. *Pensejajaran dilakukan menggunakan MUSCLE dengan parameter matriks BLOSUM98. Kotak merah menunjukkan perbedaan sekuen asam amino dari tiga psbA*

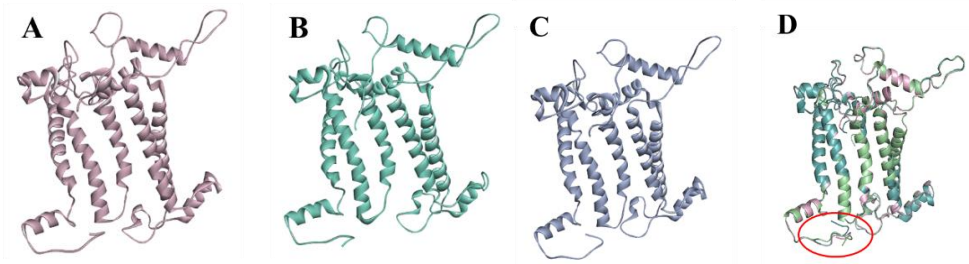


Figure 2. Protein modelling of psbA A) *E. indica*, B) *P. clematidea*, C) *M. charantia*, D) Superimposed visualization of three compared proteins. The superimpose was visualized in PyMOL to calculate the RMSD values for each comparison. Red circle shows the gap differences among models

Gambar 2. Pemodelan protein D1 Photosystem II dari A) E. indica, *B)* P. clematidea, *C)* M. charantia, *D) visualisasi dari superimpose tiga protein D1 photosystem II dari masing-masing spesies gulma menggunakan PyMOL untuk kalkulasi nilai RMSD. Lingkaran merah menunjukkan perbedaan rentang nilai RMSD antar model*

Virtual screening of molecular docking analysis of bioherbicide compound candidates

The molecular interaction of each psbA from each weed was observed to the compound candidates. Fourteen candidates of bioherbicide were selected based on Dayan & Duke (2014) and docked into the psbA, compared to the diuron as the control. The virtual screening approach could examine a target and a subset of compounds in order to reduce the number of compounds to test in the laboratory. It predicts ligand binding modes by specific algorithms in computational technique. The scoring results represent the global minimum of the energy needed for the interaction (Salmaso & Moro, 2018).

The top six compounds were ranked from each complex based on the binding affinity score. Aurachin P, Aurachin A, and Cyanobacterin appeared in the top six of each protein complexes from different psbA weeds species. Cyanobacterin showed the highest potential as a candidate compound to block D1 protein of *E. indica*, followed by Aurachin P and Aurachin A, with affinity score -6.7, -6.2, and -6.2 kcal mol^{-1}, respectively. While in complex with psbA of *P. clematidea*, Aurachin A exhibited high affinity, followed by Aurachin P and Cyanobacterin. Aurachin P was first among other compounds in the interaction with psbA of *M. charantia*, followed by Aurachin A and Cyanobacterin (Table 3). Those three compounds exhibited a higher binding affinity than the diuron as control.

Table 2. RMSD analysis of psbA of *E. indica*, *M. charantia*, and *P. clematidea*
Tabel 2. Analisis RMSD psbA dari spesies E. indica, M. charantia, *dan* P. clematidea

Species 1 *Spesies 1*	Species 2 *Spesies 2*	RMSD value (Å) *Nilai RMSD (Å)*
E. indica	*M. charantia*	0.912
E. indica	*P. clematidea*	1.016
M. charantia	*P. clematidea*	0.931

Table 3. Binding affinity score of psbA and candidate compounds of bioherbicide. Virtual screening in molecular docking was carried out using PyRx 0.9.5. Yellow highlight shows the top 6 score of docking

Tabel 3. Skor afinitas ikatan dari psbA dan kandidat bioherbisida. Skrining virtual dilakukan dengan penambatan molekular menggunakan software PyRx 0.9.5. Tanda kuning menunjukkan 6 skor penambatan teratas

Receptor *Reseptor*	Ligand *Ligan*	Binding Affinity (kcal/mol) *Afinitas ikatan (kkal/mol)*	Receptor *Reseptor*	Ligand *Ligan*	Binding Affinity (kcal/mol) *Afinitas ikatan (kkal/mol)*	Receptor *Reseptor*	Ligand *Ligan*	Binding Affinity (kcal/mol) *Afinitas ikatan (kkal/mol)*
Eleusine indica	Diuron (control)	-5.4	*Praxelis clematidea*	Diuron (control)	-6	*Momordica charantia*	Diuron (control)	-6.3
	Cyanobacterin	-6.7		Aurachin B epoxide	-7.6		Aurachin P	-9
	Aurachin E	-6.4		Aurachin Re	-7.5		Aurachin A	-8.9
	Aurachin B	-6.3		Aurachin A	-7.5		Aurachin D	-8
	Aurachin P	-6.2		Aurachin P	-7.4		Aurachin-C epoxide	-8
	Aurachin A	-6.2		Aurachin E	-7.2		Aurachin B epoxide	-7.9
	Aurachin D	-6.1		Cyanobacterin	-6.8		Cyanobacterin	-7.9
	Aurachin B epoxide	-6		Aurachin B	-6.8		Aurachin B	-7.9
	Aurachin C	-6		Aurachin-C epoxide	-6.8		Aurachin E	-7.8
	Aurachin-C epoxide	-5.8		Aurachin D	-6.6		Aurachin C	-7.8
	Aurachin Re	-5.7		Sorgoleone 358	-6.5		Aurachin Re	-7.7
	Sorgoleone 358	-5.3		Aurachin C	-6.5		Sorgoleone 358	-6.5
	Stigmatellin	-4.9		Fischerellin B	-6.2		Fischerellin B	-6.3
	Fischerellin B	-4.8		Stigmatellin	-5.7		Stigmatellin	-5.7
	Fischerellin A	-0.9		Fischerellin A	-0.9		Fischerellin A	-0.8

The complex interaction was analysed to find out the type of the involved amino acid (Figure 3). Mostly, the complex shows van der Waals interaction between molecules (Table 4, green bubble). The analysis showed 50%-90% of amino acids responsible in the interaction between Aurachin A, Aurachin P, and Cyanobacterin were similar to the complex psbA and diuron for each weed species (Table 4). The high similarity of the binding site represents the resemblance of function candidate compounds as D1 protein blocker. Some of them bind into specific amino acids which has an important function. Based on the UniProt database, the amino acid involved in the interaction has several important roles. Amino acid (AA) number 161 has a function in tyrosine radical intermediate, AA number 170 and 333 playing role in the calcium-manganese-oxide [Ca-4Mn-5O]; manganese 1 and 4.

Biological potential activity of Aurachin P, Aurachin P and Cyanobacterin

In order to explore the potential of Aurachin P, Aurachin A, and Cyanobacterin, biological activity prediction was conducted (Table 5). The data explaining the potential activity of Aurachin P and Aurachin A was similar, mostly related to the inhibitory activity of prenyl-diphosphatase, undecaprenyl-phosphate mannosyl transferase, and plastoquinol-plastocyanin reductase inhibitor. Those terms related to the inhibition of enzyme and catalytic activity playing an important role in photosynthesis. In contrast, Cyanobacterin acts as a 1-Acylglycerol-3-phosphate O-acyltransferase inhibitor, related to the negative regulation of phosphatic acid biosynthesis. The activity of candidate bioherbicides supports the prediction to inhibit the photosynthesis process in weeds.

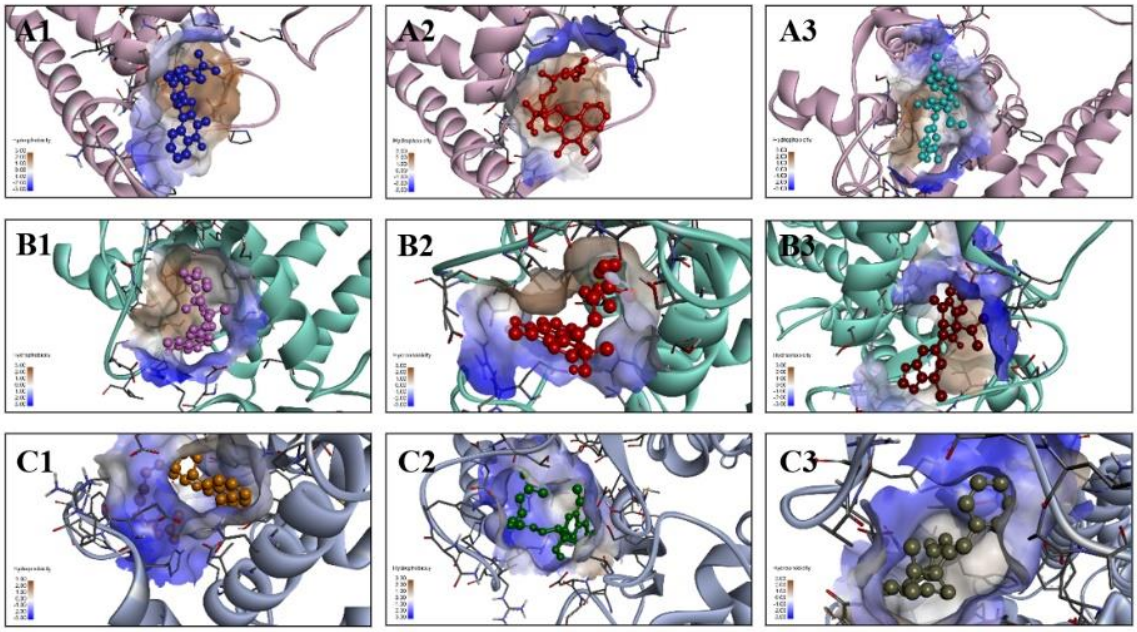


Figure 3. Molecular docking interaction between psbA (cartoon) and bioherbicide candidates (ball and sticks): (A1) D1 protein of *E. indica* and Aurachin P, (A2) D1 protein of *E. indica* and Aurachin A, (A3) D1 protein of *E. indica* and Cyanobacterin; (B1) D1 protein of *P. clematidea* and Aurachin P, (B2) D1 protein of *P. clematidea* and Aurachin A, (B3) D1 protein of *P. clematidea* and Cyanobacterin; (C1) D1 protein of *M. charantia* and Aurachin P, (C2) D1 protein of *M. charantia* and Aurachin A, (C3) D1 protein of *M. charantia* and Cyanobacterin. The docking complexes were visualized using Discovery Studio R2017

Gambar 3. Interaksi molekular docking antara psbA (kartun) dan kandidat bioherbisida (garis dan bola): (A1) Protein psbA E. indica dan *Aurachin P, (A2) Protein psbA* E. indica dan *Aurachin A, (A3) Protein psbA* E. indica dan *Cyanobacterin; (B1) Protein psbA* P. clematidea dan *Aurachin P, (B2) Protein psbA* P. clematidea *dan Aurachin A, (B3) Protein psbA* P. clematidea *dan Cyanobacterin; (C1) Protein psbA* M. charantia dan *Aurachin P, (C2) Protein psbA* M. charantia dan *Aurachin A, (C3) Protein psbA* M. charantia dan *Cyanobacterin. Kompleks hasil penambatan divisualisasikan menggunakan Discovery Studio R2017*

Table 4. Amino acid interaction between psbA of each weed species and potential candidate bioherbicides Aurachin P, Aurachin A, and Cyanobacterin. The bold font emphasizes the amino acid that also found in the diuron (control)

Tabel 4. Interaksi asam amino antara kompleks psbA dari masing-masing spesies gulma dan kandidat bioherbisida Aurachin P, Aurachin A, dan Cyanobacterin. Huruf tebal menunjukkan asam amino yang ditemukan juga pada diuron (kontrol)

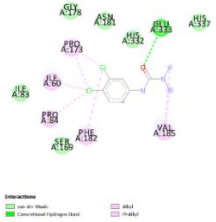
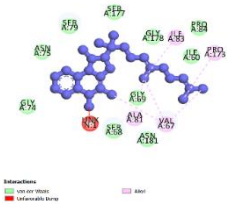
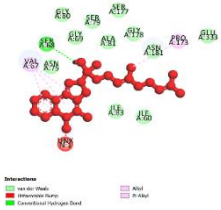
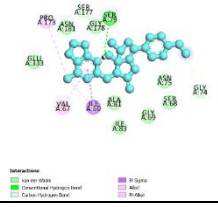
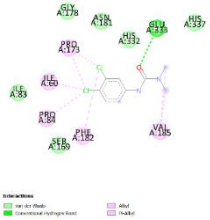
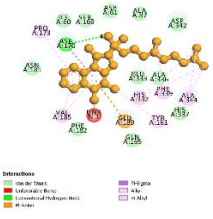
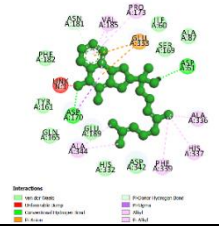
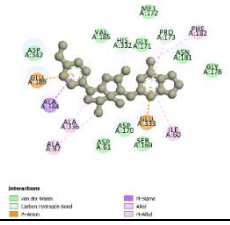
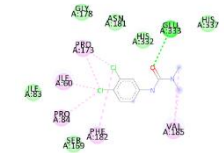
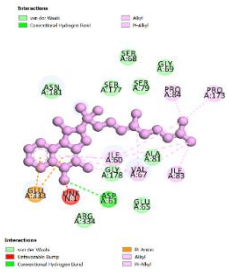
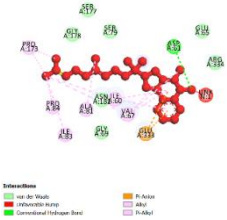
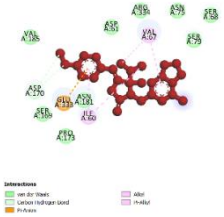
Receptor *Reseptor*	Ligand *Ligan*	Interaction *Interaksi*	Visualization *Visualisasi*
E. indica	Diuron	**ILE60, VAL67, SER68, GLY69, ASN75, SER79, ALA81, ILE83, PRO84, PRO173**	
	Aurachin P	**ILE60, VAL67, SER68, GLY69, GLY74**, **ASN75, SER79, ALA81, ILE83, PRO84, PRO173,** SER177, GLY178, ASN181	
	Aurachin A	**ILE60, VAL67, SER68, GLY69, ASN75, SER79, GLY80, ALA81, ILE83, PRO173,** SER177, GLY178, ASN181	
	Cyanobacterin	**ILE60, VAL67, SER68, GLY69,** GLY74, **ASN75, SER79, ALA81, ILE83, PRO173,** SER177, GLY178, ASN181, GLU333	
M. charantia	Diuron	**ILE60, ASP61, ALA87, TYR161, SER169, ASP170, PRO173, PHE182, VAL185, PHE186, HIS332, GLU333**	
	Aurachin P	**ILE60, ASP61, ALA87, TYR161**, GLN165, **SER169, ASP170, PRO173,** ASN181, **PHE182, VAL185**, GLU189, **HIS332, GLU333,** ALA336, HIS337, PHE339, ALA344	
	Aurachin A	**ILE60, ASP61, ALA87, TYR161,** GLN165, **SER169, ASP170, PRO173,** ASN181, **PHE182,** VAL185, GLU189, **HIS332, GLU333,** ALA336, HIS337, PHE339, ASP342, ALA344	

Table 4. (continue)

Receptor *Reseptor*	Ligand *Ligan*	Interaction *Interaksi*	Visualization *Visualisasi*
P. clematidea	Cyanobacterin	**ILE60, ASP61, ALA87, SER169, ASP170,** GLY171, MET172, **PRO173,** GLY178, ASN181, **PHE182, VAL185,** GU189, **HIS332, GLU333**, ALA336, ASP342, ALA344	
	Diuron	**ILE60, VAL67, GLY69, ALA81, ILE83, PRO84, SER169, PRO173, SER177, GLY178, ASN181, GLU333**	
	Aurachin P	**ILE60,** ASP61, GLU65, **GLY69,** SER89, **ALA81, ILE83, PRO84, PRO173, SER177, GLY178, ASN181, GLU333**, ARG334	
	Aurachin A	**ILE60**, ASP61, GLU65, **VAL67, GLY69**, SER79, **ALA81, ILE83, PRO84, PRO173, SER177, GLY178**, ASN182, **GLU333, ARG334**	
	Cyanobacterin	**ILE60,** ASP61, **VAL67,** SER68, ASN75, SER79, SER169, ASP170, **PRO173, ASN181**, VAL185, **GLU333**, ARG334	

Biological activity of Aurachin P and Aurachin A was predicted to be related to the terms as an inhibitor in plastoquinol-plastocyanin reductase. This enzyme playing a role in the linear electron transfer chain that contributes to oxygenic photosynthesis in the chloroplast. Linear electron chain is responsible to oxidize water into molecular oxygen and reducing the NADP+ to NADPH. This condition makes the environment of transmembrane proton gradient which will be converted by ATP synthase into chemical energy (ATP). The plastoquinone enzyme catalyzes the electrotransfer between Photosystem II and I, which is the photosynthetic reaction centers of oxygenic photosynthesis (Gao *et al*., 2018). When this process was inhibited by Aurachin P and Aurachin A, the photosynthesis process will not happen.

Cyanobacterin was predicted to have 1-Acylglycerol-3-phosphate O-acyltransferase inhibitor activity. This enzyme playing roles in phosphatic acid biosynthesis. It may regulate neutral lipid accumulation and participate in lipid turnover regulation. The phospholipid is well known to play crucial roles in the development and signal transduction. It also regulates the homeostasis in growth and development stages under stress conditions. Phosphatic acid was proven to act as a key for thylakoid lipid biosynthesis in the chloroplast (Yao & Xue, 2018). If this process were inhibited by Cyanobacterin, the growth of weeds will be terminated.

Table 5. Biological activity prediction of Aurachin P, Aurachin A, and Cyanobacterin using the PASS server. Pa: Probability of activity

Tabel 5. Prediksi aktivitas biologis dari Aurachin P, Aurachin A, dan Cyanobacterin menggunakan PASS Server. Pa: prediksi aktivitas

Aurachin A		Aurachin P		Cyanobacterin	
Pa score *Skor Pa*	Activity *Aktivitas*	Pa score *Skor Pa*	Activity *Aktivitas*	Pa score *Skor Pa*	Activity *Aktivitas*
0.752	Lipid peroxidase inhibitor	0.750	Prenyl-diphosphatase inhibitor	0.800	Membrane integrity agonist
0.748	Prenyl-diphosphatase inhibitor	0.738	Undecaprenyl-phosphate mannosyltransferase inhibitor	0.800	Aspulvinone dimethylallyltransferase inhibitor
0.734	Undecaprenyl-phosphate mannosyltransferase inhibitor	0.707	Plastoquinol-plastocyanin reductase inhibitor	0.800	Carminative
0.721	Plastoquinol-plastocyanin reductase inhibitor	0.726	Antineoplastic	0.700	CYP2H substrate
0.741	Ubiquinol-cytochrome-c reductase inhibitor	0.723	Ubiquinol-cytochrome-c reductase inhibitor	0.600	1-Acylglycerol-3-phosphate O-acyltransferase inhibitor

Conclusion

This study provided insight into the bioherbicide candidate compounds which has shown potentially better affinity than synthetic herbicide diuron. It is indicated that the Aurachin P, Aurachin A, and Cyanobacterin were the best blocker candidate compounds for Photosystem II D1 protein to inhibit the growth of selected sugarcane weeds. However, efficacy tests are required to confirm the potential effectivity of the compounds found in this research.

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