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An *In silico* Approach to Identify a Potential Phyto-Herbicide Candidate against 5-Enolpyruvyl Shikimate-3-Phosphate (EPSP) Synthase

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ABSTRACT

Crop contamination with chemical herbicide residues is one of the major problems on a global scale. Bioherbicides have been accepted as a promising material to be used in weed control. This study aims to find a potential phyto-herbicide candidate using an *in silico* approach. A 2D similarity search was used to find the natural compounds having a chemical structure similar to that of glyphosate against natural compound databases. Then, phyto-herbicide candidates were confirmed via molecular docking and property screening. 2-Phosphoglycerate, C00000123, was selected as the potential phyto-herbicide candidate based on the lowest binding energy (-6.45 kcal/mol) similar to that of the reference glyphosate (-7.42 kcal/mol), and it was not a substrate of EPSP synthase. The binding pattern between 2-Phosphoglycerate and EPSP synthase was similar to that of glyphosate binding via Lys22, Lys411, Gly96, Arg124, Arg344, and Arg386. Moreover, 2-Phosphoglycerate had a herbicide-likeness property and was an enzyme inhibitor. It was produced in *Oryza sativa* L. Therefore, 2-Phosphoglycerate displayed an effective inhibition. However, further wet-lab experiments must be performed to validate the herbicide effectiveness of 2-Phosphoglycerate, and its role as an effective phyto-herbicide inhibitor.

Keywords: 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase; Molecular docking; *Oryza sativa* L.; Phyto-herbicide; Two-dimensional (2D) similarity search

1. Introduction

The requirement for food has been increasing rapidly due to population growth. However, the quality of materials and ingredients is falling into a crisis because of toxic chemical herbicide contamination. These

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chemical herbicide residues are not only dangerous to the surrounding ecosystem, including soil and water supply, but also have diverse effects on human health and living organisms [1].

Among chemical herbicides, glyphosate is one of the widely applications to control weed [2-4]. Glyphosate is a phosphonomethyl derivative of the amino acid glycine, which was synthesized in 1950. It is a nonvolatile compound, a white odorless crystalline solid and stable in air. It does not undergo photochemical degradation, and it has a basic amino group and three ionizable acidic sites [2]. Glyphosate is a non-selective post-emergent herbicide for controlling the growth of various weed species (over 150 species), including mono and dicotyledonous plants [2].

There is much scientific evidence showing that glyphosate can control weeds by inhibiting an enzyme 5-enolpyruvyl shikimate-3-phosphate synthase [3, 4]. EPSP synthase (EC 2.5.1.19) is the central enzyme on the sixth step of the shikimate pathway. It synthesizes the essential aromatic amino acids such as phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp), in fungi, plants and prokaryotes [3]. These aromatic amino acids are not only crucial components of protein biosynthesis; but also serve as precursors for diverse secondary metabolites, which are important for plant growth. In addition, the shikimate pathway is a major link between primary and secondary metabolism in higher plants [5].

Unfortunately, glyphosate is harmful to the ecosystem and human health. It can interfere with the metabolic pathways of microorganisms, negatively influencing their growth. It has been linked to neurological and endocrine issues in both humans and animals, and behavioral modification on specific bioindicators. Moreover, it is classified as a probable human carcinogen under Group 2 [2].

Thus, farmers should avoid or at least reduce their usage of chemical herbicides. The next important question is that if chemical herbicides are not used, then what should be used instead? This question challenged the author to find the potential phyto-herbicide candidate. It is the natural product of plants, which has structure and binding energy similar to glyphosate. Moreover, it passes the criteria of an herbicide and an enzyme inhibitor property.

Bioherbicides are “products of natural origin for weed control”. They have diverse advantages, i.e., a short-lived environmental fate, multiple modes of action reducing the risk of herbicide resistance, and low toxicity [6]. Therefore, they are expected to be more environmentally friendly. Currently, there are 13 commercial bioherbicides. Only the Beloukha® product is derived from rapeseed oil, *Brassica napus* L. as a phyto-herbicide [7]. Therefore, searching for a natural phyto-herbicide is still very challenging.

Several approaches have been carried out to find a phyto-herbicide. Historically, traditional methods have been used for crude extracts on weed control under laboratory and field experiments. Then, high-throughput screening and structural optimization have been used for bioherbicide discovery [8]. In another approach, researchers have been trying to develop herbicides derived from the secondary metabolites of plant species and microbes to minimize the environmental effects and create safer, and non-toxic compounds [9]. These techniques are costly and time-consuming. Therefore, a computational strategy is a very interesting alternative way due to its low cost and time.

Currently, two-dimensional (2D) similarity search combined with molecular docking is performed in the field of herbicide discovery [10-12]. This combination approach is complementary, and it would improve the performance of *in silico* screening [12]. 2D similarity search is the most common method for ligand-based virtual screening. It assumes that similar molecules are more likely to have similar biological or physicochemical properties than dissimilar ones [13]. It involves the retrieval of molecules from a database that are structurally similar to reference

compounds [12]. Chemical structure similarity of compounds at 2D represents the fingerprints encoding fragment substructures based on a bit-string. The bit-string is prepared by indicating 1 or 0 [14]. If the fragment of glyphosate or reference compound matches with the fragment of natural product present in the database, it is given the value of "1"as ON bit. If the fragment is absent, it is given the value of "0" as OFF bit.

The most popular method of quantifying the degree of similarity between molecules is the Tanimoto coefficient (τ) [13, 15]. It is determined by comparing two molecules as bit – string fingerprints. It is calculated as follows:

$$\tau = N12 / N1 + N2 - N12,$$

where $N1$ is number of features (ON bits) in molecule 1, $N2$ is the number of features (ON bits) in molecule 2, and $N12$ is the number of features (ON bits) common to both molecule 1 and 2 [14]. In Kumar [14]'s example calculation, if molecule 1 has 1 1 0 1 1, and molecule 2 has 0 1 1 1 0, the Tanimoto coefficient equals 0.4, given by 2/(4+3-2).

Molecular docking (Fig. 1) is one of the techniques most commonly used in medicinal chemistry and drug design due to its accuracy and effectiveness in screening the candidates [9, 15]. It is applied to investigate a specific binding mode of a ligand with a protein and evaluate the binding affinity [15, 16].

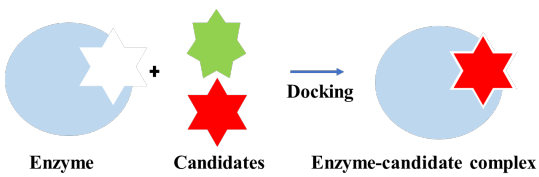


Fig. 1. Enzyme-ligand docking.

Interestingly, based on the author's acknowledgment, the 2D similarity search-molecular docking combination is rarely used in the field of herbicide discovery.

In order to increase the likelihood of success, the best phyto-herbicide candidates having the structure and binding energy similar to those of glyphosate should always check the physicochemical properties by using the Molinspiration Cheminformatics database [17]. The herbicide-likeness property is analyzed according to Tice's rule [18]. The molecular descriptors acting as the criteria to identify the herbicide property are molecular weight, hydrophobicity (MLogP), number of H-bond acceptors (HBA) and donors (HBD), and number of rotatable bonds [18]. Furthermore, the Molinspiration database can predict the bioactivity such as GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and enzyme inhibitors [17].

Therefore, the objectives of this study were (1) to find the best phyto-herbicide candidate using the 2D similarity search-molecular docking combination approach, and (2) to investigate the herbicide-likeness property, and bioactivity prediction of an enzyme inhibitor via the Molinspiration database.

2. Materials and Methods

2.1 Computer system

All the computational studies were conducted on a PC with an Intel Core i3 processor, 6 GB of memory, and a 64-bit operating system.

2.2 Structural preparation

The overall methodology is shown in Fig. 2. In this study, a SMILES format of glyphosate, obtained from the PubChem database [19] was used as the query structure. The query structure was added to the SIMCOMP (SIMilar COMPound) search tool [20] implemented in the KEGG database [21]. In this tool, KNApSAcK [22] was selected as the target database, and global search was chosen as the search option. The other parameters were used according to the default value of the tool. Only compounds belonging to a plant were selected as phyto-herbicide candidates.

The structures of EPSP synthase binding with glyphosate were investigated from the RCSB Protein Data Bank [23]. PDB IDs: 1G6S and 2GGA were of interest based on a wild-type structure. The resolutions of X-ray structure of 1G6S and 2GGA were 1.50 Å and 1.70 Å, respectively [24, 25]. In this study, the 1G6S structure was selected based on its high resolution acting as an enzyme receptor.

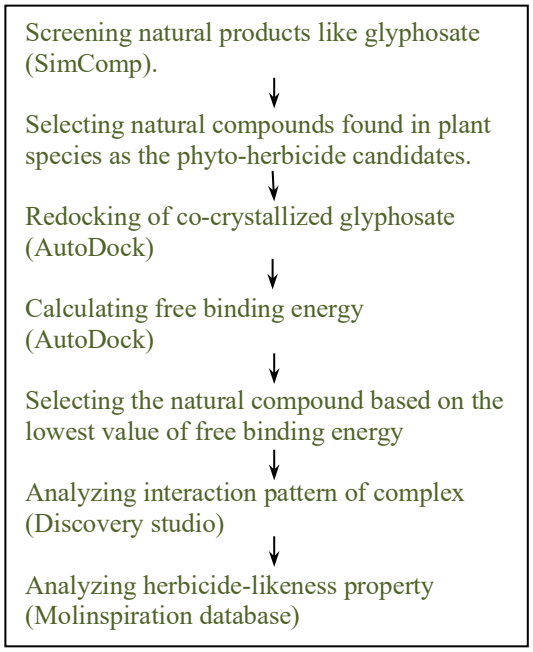


Fig. 2. Overall computational methodology of this study.

2.3 Molecular docking

To know the accuracy of molecular docking, redocking was performed prior to the phyto-herbicide candidate investigation. The co-crystallized glyphosate was extracted from the EPSP synthase structure (PDB ID: 1G6S). Then it was redocked again into its active site. Typically, a root mean square deviation (RMSD) is used as a quantitative measure of accuracy. If the RMSD value is less than 2 Å, it indicates a valid docking technique and successful docking results [26]. These redocked parameters can be used for docking phyto-herbicide candidates.

In order to understand the affinity binding between phyto-herbicide candidates and EPSP synthase receptor, the free binding energy was calculated using AutoDock version 4.2.6 [27]. The suitable grid box for docking was 40 x 40 x 40 Å in grid size with a center of X: 60.526, Y: 9.077, Z: 29.519, and a grid spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was used as a search engine with the default parameters. LGA is the best search algorithm of AutoDock using Solis-Wets local search after each generation of genetic algorithm for energy minimization [28]. The genetic algorithm begins by creating a random population of individuals (number of individuals in population: 300). Furthermore, a new random number generator has been introduced. The creation of the random initial population is followed by a loop over generations, repeating until the maximum number of generations (27000) or the maximum number of energy evaluations (2500000) is reached. A generation consists of five stages: mapping and fitness evaluation, selection, crossover (crossover rate: 0.8), mutation (mutation rate: 0.02), and elitist selection (elitism: 1) [29]. The values in parentheses were set to dock in this study. The result of the local search is used to update the fitness value and its representation associated with an individual. Even though the Solis-Wets local search operator searches through the genotypic space, it can still be qualified as Lamarckian, because any environmental adaptations of the ligand acquired during the local search will be inherited by its offspring [28]. The genetic algorithm was set to 50 runs. Glyphosate was docked in the same receptor as a reference inhibitor. The phyto-herbicide candidate with the lowest free energy of binding closest to that of glyphosate was selected as the best phyto-herbicide candidate.

The estimated free energy of binding (E_{bind}) is calculated as follows:

$$E_{bind} \text{ (kcal/mol)} = (1) + (2) + (3) – (4),$$

where (1), (2), (3), and (4) refer to final intermolecular energy, final total internal energy, torsional free energy, and unbound system's energy (kcal/mol), respectively. Intermolecular energy is the sum of van der Waals (vdW), hydrogen bonds (Hbond), desolvation (desolv) energy and electrostatic energy [27, 29].

2.4 Property screening

The hydrogen bonds between the docked complex of the best phyto-herbicide candidate and the receptor were analyzed using Discovery Studio Visualizer software [30]. Physicochemical calculation and bioactivity prediction of the best phyto-herbicide candidate and glyphosate were calculated using the Molinspiration database [17]. Herein, Tice' s rule was used as the criteria to determine the herbicide-likeness property.

2.5 Statistical analysis

An independent *t*-test was analyzed to evaluate whether or not the binding energy of phyto-herbicide candidates differs from those of glyphosate at the confidence level of 95%. The top 3 binding energies of each compound were used to calculate the *t*-test using SPSS program version 25 for Windows [31].

3. Results and Discussion

3.1 Phyto-herbicide candidate

According to the 2D similarity search, a total of 16 natural compounds having the similarity scores in the range of 0.61-0.40 were obtained (unpublished). The organism sources were bacteria and plants. However, due to the requirement of a large amount of crude extract, the bacterial compounds were not suitable for this study. The remaining compounds were phyto-herbicide candidate as shown in Table 1. Although the results showed a low similarity score, there are many studies showing that dissimilar ligands can also result in similar bioactivities [32].

Arabidopsis thaliana (L.) Heynh. is a small rosette plant that is a popular application as a model organism [33]. It is not native, and not of major agronomic significance to Thailand. Hence, phyto-herbicide candidates produced in *A. thaliana* were not suitable for this study. The remaining phyto-herbicide candidates were chosen to calculate the free binding of energy.

3.2 Free binding of energy

The image of co-crystallized glyphosate overlays on the redocked glyphosate is shown in Fig. 3. They showed the same orientation. RMSD value of the redocking process was 0.326 Å indicating the valid docking protocol.

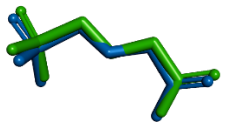


Fig. 3. Redocked glyphosate (blue ligand) overlay with co-crystallized glyphosate (green ligand) by Discovery Studio Visualizer [30].

Table 1. Phyto-herbicide candidates searching from SIMCOP search tool and plant species.

No.	Candidate_ID	Name	Similarity Score	Plant species
1	C00007287	3-Phosphoserine	0.56	*Arabidopsis thaliana*
2	C00007385	O-Phospho-L-homoserine	0.52	*Arabidopsis thaliana*
3	C00007471	L-4-Aspartyl phosphate	0.48	*Arabidopsis thaliana*
4	C00000798	Phosphoenolpyruvic acid	0.48	*Oryza sativa*
5	C00007286	3-Phosphoglycerate	0.45	*Arabidopsis thaliana*
6	C00000123	2-Phosphoglycerate	0.45	*Oryza sativa*
7	C00001361	Glycine	0.43	*Spondias mangifera* *Caesalpinia sappan* *Vigna unguiculata* *Mirabilis jalpa* *Solanum lycopersicum*
8	C00007291	Betaine	0.43	*Avicennia marina* *Hylocereus ocamponis*
9	C00001343	L-Azetidine-2-carboxylic acid	0.42	*Delonix regia*

Phyto-herbicide candidates were docked in an EPSP synthase receptor for calculating the lowest free energy values of binding. The lowest free energy values of binding were in the range of -4.90 to -7.51 kcal/mol as shown in Table 2. The more negative the free binding energy value, the better and stronger the binding between enzyme and ligand [34]. Although the free energy of binding (-7.44 kcal/mol) of C00000798 (Phosphoenolpyruvic acid) differed insignificantly ($p > 0.05$) from that of glyphosate (-7.36 kcal/mol), it could not be selected because it is the substrate of EPSP synthase [24, 25]. In spite of free binding energy value (-6.12 kcal/mol) of C00000123 (2-Phosphoglycerate) significant difference ($p \leq 0.05$) from that of glyphosate -7.36 kcal/mol), but it was selected as the best phyto-herbicide candidate due to its the lowest free energy of binding similar to that of glyphosate than the remaining compounds.

Table 2. The lowest free energy of binding between EPSP synthase receptor and phyto-herbicide candidates including glyphosate.

Candidates	The free binding of energy (kcal/mol)	*p*-value
Glyphosate	-7.42(-7.36)	-
C00000123	-6.45(-6.12)*	0.002
C00000798	-7.51(-7.44)	0.286
C00001361	-5.54(-5.54)*	0.000
C00001343	-5.69(-5.68)*	0.000
C00007291	-4.90(-4.89)*	0.000

Asterisks (*) mark *p*-values ≤ 0.05, indicating a significant difference. The values in parentheses are the average value ($n = 3$) of free binding energy.

Remarkably, the plant species that produced 2-Phosphoglycerate is *Oryza sativa* L., which is widely grown in Thailand. Moreover, rice is considered an allelopathic plant, which is a powerful tool for biological and eco-friendly weed management [35]. This is consistent with other research according to the review by Rahaman et al. [35] Supportively, Chung et al. [36] indicate that the allelopathic compounds in rice (*Oryza sativa* L.) straw serve as natural herbicides by inhibiting seed germination and the growth of weeds. Gu et al. [37] found that compounds extracted from rice hulls could produce plenty of secondary metabolites to defend against weeds and pathogens. Moreover, the rice husks and the rice straw are agricultural wastes.

Molecular docking is an important component of the structure-based drug design process, predicting protein-ligand binding orientation [38]. AutoDock is a free program for calculating the estimated free energy of binding of protein-ligand interaction [27]. The interaction pattern of 2-Phosphoglycerate inside the binding pocket of the EPSP synthase receptor is displayed in Fig. 4A, compared to those of glyphosate as shown in Fig. 4B. 2-Phosphoglycerate formed hydrogen bonds with Lys22, Gly96, Arg124, Arg344, Glu341, Arg386, and Lys411. Glyphosate formed hydrogen bonds with Lys22, Gly96, Arg124, Gln171, Arg344, Glu341, Arg386, and Lys411. These binding results are consistent with previous work; Schönbrunn et al. [24] show the residues in the EPSP synthasez-S3P-glyphosate complex, including Lys22, Gly96, Asn94, Arg124, Gln171, Glu341, Arg344, Arg386, and Lys411. The glyphosate binding site is dominated by charged residues, including Lys22, Arg124, and Lys411 [24]. The results of the binding pattern indicate that 2-Phosphoglycerate can inhibit the activity of EPSP synthase receptor.

3.3 Property of phyto-herbicide candidate

The herbicide-likeness property and the bioactivity score of 2-Phosphoglycerate and glyphosate, are shown in Table 3. In the field of herbicide discovery, the physiochemical properties have been analyzed based on Tice's rule (molecular weight in range of 150-500 Dalton, partition coefficient ≤ 3.5 (logP), the number of hydrogen bond donors ≤ 3 and acceptors 2-12, and the number of rotatable bonds < 12) [18]. According to Tice's rule, 2-phosphoglycerate passed almost all molecular

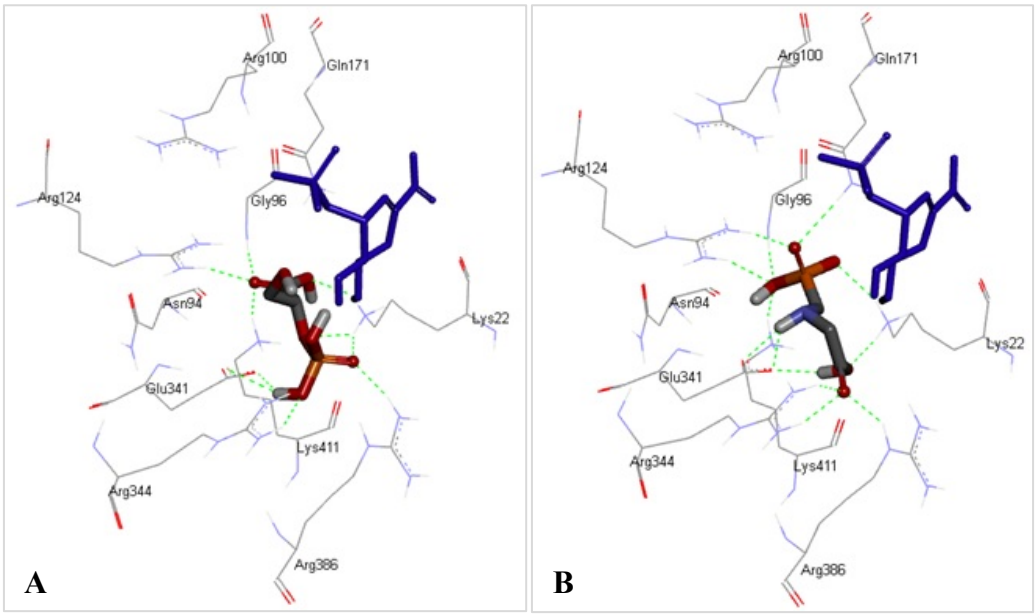
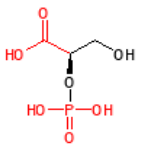
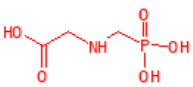


Fig. 4. Binding mode of compounds docked in binding pocket of receptor: (A) 2-Phosphoglycerate; (B) glyphosate. Green dashed lines indicate hydorgen bonds. S3P molecule is shown as blue stick model. Protein residues are shown as line model. 2-Phosphoglycerate and glyphosate are displayed as stick model. Figures were created using the Discovery Studio Visualizer [30].

Table 3. Properties of the best phyto-herbicide candidate (2-Phosphoglycerate).

Molecular descriptor	2-Phosphoglycerate's value	Glyphosate's value
Molecular weight	186.06	169.07
MLogP	-2.38	-2.84
Hydrogen bond acceptor	7	6
Hydrogen bond donor	4	4
Rotatable bonds	4	4
Bioactivity prediction		
Enzyme inhibitor	1.26	1.03
Structure		
Formula	$C_3H_7O_7P$	$C_3H_8NO_5P$
2D structure		

descriptors criteria, except the number of hydrogen donors. However, compared to glyphosate, it showed an equal value. This indicates that the 2-Phosphoglycerate could be a potential phyto-herbicide candidate like a glyphosate. From the bioactivity predicted result, 2-Phosphoglycerate had the enzyme inhibitor bioactivity score of 1.26, which is similar to glyphosate's score of 1.03. The maximum score is 2. The larger the value of the score, the higher the probability that the particular molecule will be active [17]. Yang et al. [38] reported that a bioactivity score of 5.0-0.0 is considered moderately active, and a score of ≥ 0 is considered active. Finally, it can be concluded that 2-Phosphoglycerate could be an active enzyme inhibitor. This is consistent with previous study, N-1H-indazol-5-yl-2-(6-methylpyridin-2-yl) quinazolin-4-amine (score 0.38) is an active enzyme inhibitor [38].

4. Conclusion

This study provided insight into the finding of phyto-herbicide candidates. It is also revealed that 2-Phosphoglycerate could be the potential phyto-herbicide candidate acting as an enzyme inhibitor via EPSP synthase inhibition. Therefore, 2-Phosphoglycerate may serve as an important phyto-herbicide. Moreover, it can be found in *Oryza sativa* L., which is the major agronomic plant of Thailand. Rice fields generate huge amounts of agricultural waste. This may be the alternative way to change agricultural wastes to be an economic value material. The other benefits could reduce environmental contamination with chemical herbicide residues, increase the safety for farmers and consumers, and reduce costs. In this study, no biological experiments have been carried out. Therefore, further wet-lab experiments should be performed to examine its herbicide effectiveness.

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References

[1] Paola Balderrama-Carmona A, Patricia Silva-Beltrán N, Alberto Zamora Alvarez L, Patricia Adan Bante N, Felipe Moran Palacio E. Consequences of herbicide use in rural environments and their effect on agricultural workers. IntechOpen 2020.

[2] Ferrante M, Rapisarda P, Grasso A, Favara C, Conti GO. Glyphosate and environmental toxicity with "One Health" approach, a review. Environ Res 2023;235:116678.

[3] Tall T, Puigbó P. The Glyphosate Target Enzyme5-Enolpyruvyl Shikimate 3-Phosphate Synthase (EPSPS) Contains Several EPSPS-Associated Domains in Fungi. Proceedings 2021;76:6.

[4] Zulet-González A, Barco-Antoñanzas M, Gil-Monreal M, Royuela M, Zabalza A. Increased Glyphosate-Induced Gene Expression in the Shikimate Pathway Is Abolished in the Presence of Aromatic Amino Acids and Mimicked by Shikimate. Front Plant Sci 2020;11:459.

[5] Averesch NJH, Krömer JO. Metabolic engineering of the shikimate pathway for production of aromatics and derived compounds-present and future strain construction strategies. Front Bioeng Biotechnol 2018;6(32):1-32.

[6] Bailey KL. The bioherbicide approach to weed control using plant pathogens. In: Abrol DP, editor. Integrated Pest Management. Academic Press, San Diego. 2014;245-66.

[7] Cordeau S, Triolet M, Wayman S, Steinberg C, Guillemin JP. Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Prot 2016;87:44-9.

[8] Dayan FE, Duke SO. Discovery for new herbicide sites of action by quantification of plant primary metabolite and enzyme pools. Engineering 2020;6(5):509-14.

[9] Permatasari GW, Putranto RA, Widiastuti H. Structure-based virtual screening of bioherbicide candidates for weeds in sugarcane plantation using *in silico* approaches. Menara Perkebunan 2020; 88(2):100-10.

[10] Antony A, Karuppasamy R. Searching of novel herbicides for paddy field weed management-A case study with Acetyl-CoA Carboxylase. Agronomy 2022;12:1635.

[11] Kumar N, Rani P, Agarwal S, Singh DV. 6-Ethoxy-4- N-(2-morpholin-4-ylethyl) -2-N-propan-2-yl-1,3, 5-triazine-2, 4-diamine endows herbicidal activity against Phalaris minor a weed of wheat crop field: An in-silico and experimental approaches of herbicide discovery. J Mol Model 2022; 28(4):77.

[12] Szilágyi K, Flachner B, Hajdú I, Szaszkó M, Dobi K, L″orincz Z, Cseh S, Dormán G. Rapid identification of potential drug candidates from multi-million compounds'

repositories. combination of 2D similarity search with 3D ligand/structure based methods and *in vitro* screening. Molecules 2021;26:5593.

[13] Skinnider MA, Dejong CA, Franczak BC. et al. Comparative analysis of chemical similarity methods for modular natural products with a hypothetical structure enumeration algorithm. J Cheminform 2017;9:46.

[14] Kumar A. Chemical similarity methods: A tutorial review. The Chem. educator, 2011;16:46-50.

[15] Almalki FA, Shawky AM, Abdalla AN, Gouda AM. Icotinib, Almonertinib and Olmutinib. A 2D Similarity/Docking-based study to predict the potential binding modes and interactions into EGFR, Molecules 2021;26(21):6423.

[16] Leng XY, Gao S, Ma YF, Zhao LX, Wang M, Ye F, Fu Y. Discovery of novel HPPD inhibitors: Virtual screening, molecular design, structure modification and biological evaluation. Pestic Biochem Physiol 2023;192:105390.

[17] Molinspiration Cheminformatics free web services, https://www.molinspiration.com, Slovensky Grob, Slovakia Republic

[18] Avram S, Funar-Timofei S, Borota A, Chennamaneni SR, Manchala AK, Muresan S. Quantitative estimation of pesticide-likeness for agrochemical discovery. J Cheminform 2014; 6(42).

[19] Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. PubChem 2023 update. Nucleic Acids Res 2023;51(D1):D1373-80.

[20] Hattori M, Tanaka N, Kanehisa M, Goto S. SIMCOMP/SUBCOMP: Chemical structure search servers for network analyses. Nucleic Acids Res 2010;38:W652-6.

[21] Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 2000;28:27-30.

[22] Afendi FM, Okada T, Yamazaki M, Hirai-Morita A, Nakamura Y, Nakamura K, Ikeda S, Takahashi H, Md. Altaf-Ul-Amin Darusman LK, Saito K, Kanaya S. KNApSAcK family databases: Integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol 2012;53:e1(1-12).

[23] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000;28:235-42.

[24] Schönbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci USA 2001;98(4):1376-80.

[25] Funke T, Han H, Healy-Fried ML, Fischer M, Schönbrunn E. Molecular basis for the herbicide resistance of Roundup ready crops. PNAS 2006;103(35):13010-5.

[26] Pratama MRF, Poerwono H, Siswodihardjo S. Introducing a two-dimensional graph of docking score difference vs. similarity of ligand-receptor interactions. IJBiotech 2021;26(1):54-60.

[27] Morris GM, Huey R, Lindstorm W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTool4: Automated docking with selective flexible. J Comput Chem 2009;30(16):2785-91.

[28] Atilgan E, Hu J. Improving protein docking using sustainable genetic algorithms. IJCISIM, 2011;3;248-55.

[29] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding

Free Energy Function. J Comput Chem, 1998;19;1639-62.

[30] D.S. Biovia, "Discovery Studio Visualizer, 19.1", San Diego: Dassault Systèmes. 2019.

[31] IBM Corp. Released 2017. IBM SPSS statistics for Windows, version 25.0. Armonk, NY: IBM Corp.

[32] Xu X, Zou X. Dissimilar ligands bind in a similar fashion: A guide to ligand binding-mode prediction with application to CELPP Studies. Int J Mol Sci 2021;22,12320.

[33] Krämer U. The natural history of model organisms: Planting molecular functions in an ecological context with *Arabidopsis thaliana*. eLife 2015;4:e06100.

[34] Jain AS, Sushma P, Dharmashekar C, Beelagi MS, Prasad SK, Shivamallu C, Prasad A, Syed A, Marraiki N, Prasad KS, *In silico* evaluation of flavonoids as effective antiviral agents on the spike glycoprotein of SARS-CoV-2. Saudi J Biol Sci 2021;28(1):1040-51.

[35] Rahaman F, Shukor Juraimi A, Rafii MY, Uddin K, Hassan L, Chowdhury AK, Karim SMR, Yusuf Rini B, Yusuff O, Bashar HMK, Hossain A. Allelopathic potential in rice - a biochemical tool for plant defense against weeds. Front Plant Sci 2022;13: 1072723.

[36] Chung IM, Ahn JK, Yun SJ. Identification of allelopathic compounds from rice (*Oryza sativa* L.) straw and their biological activity. Can J Plant Sci 2001;81:815-9.

[37] Gu CZ, Xia XM, Lv J, Tan JW, Baerson SR, Pan ZQ, Song YY, Zeng RS. Diterpenoids with herbicidal and antifungal activities from hulls of rice (*Oryza sativa*). Fitoterapia 2019;136:104183.

[38] Yang X, Xing X, Liu Y, Zheng Y. Screening of potential inhibitors targeting the main protease structure of SARS-CoV-2 via molecular docking. Front Pharmacol 2022;13(6):962863.