v. 3, n. 6, 2023

International Journal of Biological and Natural Sciences

MOLECULAR DOCKING STUDY OF NICOTINE AND NORNICOTINE WITH THE ENZYME 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE

Román Adrián González-Cruz
Laboratorio de Fitoquímica, Unidad de Biotecnología y Prototipos, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla de Baz, Estado de México, México.

Israel Valencia
Laboratorio de Fitoquímica, Unidad de Biotecnología y Prototipos, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla de Baz, Estado de México, México.

Zyanya Elena Gómez-Vázquez
Laboratorio de Fitoquímica, Unidad de Biotecnología y Prototipos, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla de Baz, Estado de México, México.

Abstract: Glyphosate is a polar organic acid consisting of a glycine molecule and a phosphonomethyl molecule, used as a broad-spectrum herbicide as it inhibits the shikimate pathway, preventing the synthesis of aromatic amino acids. However, WHO has classified it as extremely toxic. In this study, a molecular docking analysis of the inhibitory activity of nicotine, nornicotine, and glyphosate on the binding site of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme was carried out. Glyphosate presented the most stable model, while the nicotine and nornicotine models exhibited higher binding energy and lower efficiency. Nicotine and nornicotine showed low affinity to the enzyme compared to glyphosate according to K_i values. Glyphosate interacted with 18 residues, nornicotine interacted with 17, and nicotine interacted with 12. Only glyphosate and nornicotine interacted with residues belonging to the enzyme's binding site. The presence of a methyl group in nicotine and its absence in nornicotine affects the binding stability and affinity of the models, as well as interactions with the binding site residues.

Keywords: Glyphosate, Nicotine, Nornicotine, Shikimate, Molecular Docking, 5-enolpyruvylshikimate-3-phosphate Synthase.

INTRODUCTION

Herbicides are chemical substances with varying levels of toxicity, in some cases very high toxicity (Burger and Fernandez, 2004), which interfere with the biochemical processes of plants. They are classified into three categories depending on their mechanism of action: systemic, which are absorbed by the plant and affect the production of growth hormones; sterilizers, which kill organisms that assist plant growth; and contact, which kill the leaves and interfere with the photosynthesis process (Cruz, 2013). The chemical composition of herbicides that confers toxicity against pests can also affect other organisms, including humans, as well as harm the environment by migrating from the plant root tissues to the soil. Here, it can mobilize thanks to competition with phosphorus, reaching non-target plants (Salazar and Aldana, 2011).

In the Mexican regulatory herbicide catalog, glyphosate is classified as a grade IV phosphonomethylglycine herbicide (slightly toxic) while the WHO has listed it as a strong irritant and classified it as extremely toxic in class I (COFEPRIS, 2009). Glyphosate is a polar organic acid composed of a glycine molecule and a phosphonomethyl molecule. It appears as a crystalline powder that is somewhat rain-resistant and is used as a broad-spectrum, post-emergence, non-selective herbicide. It enters through the leaves and travels to storage organs where it inhibits the shikimate pathway by binding to the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, thereby preventing the synthesis of aromatic amino acids (Devine *et al*., 1993; Williams *et al*., 2000; Cruz, 2013). See Figures 1 and 2.

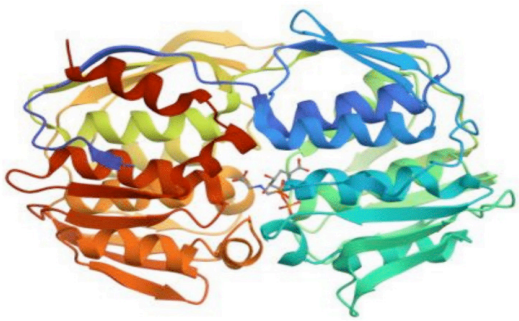

Figure 1. 3D image of the enzyme 5-enolpyruvylshikimate-3-P synthase. The enzyme is a transferase composed of 427 residues. The binding site is made up of residues Arg 25, Arg 120, Lys 339, Arg 343 and Arg 385. Within the active site, the Asp 312 residue functions as an electron acceptor, while the Glu 340 residue functions as an electron donor (Park *et al*., 2003).

Even though glyphosate is considered slightly toxic in Mexico, SEMARNAT (the Secretariat of Environment and Natural Resources) has established a total ban on its use by 2024 due to reported evidence of environmental and health damage (Forbes, 2020). Therefore, it is necessary to find safe alternatives to replace glyphosate to prevent afflictions in the agricultural sector. One alternative is plant secondary metabolites such as nicotine and nornicotine, which are alkaloid compounds considered toxic and produced to prevent herbivory. Nicotine and nornicotine are the most abundant compounds found in plants of the *Nicotiana* genus, with nicotine being the first to be synthesized and the most abundant. Nornicotine, on the other hand, is synthesized from the demethylation of nicotine and has greater insecticidal activity (Figure 3) (Nuñez, 1963; Botte *et al*., 1997; Alcántar, 2005).

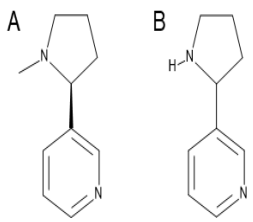

Figure 3. Chemical structure of A: nicotine and B: nornicotine. Nicotine presents a methyl group in amide.

Molecular docking studies are a good option for analyzing the interactions of compounds of interest on a protein, such as nicotine, nornicotine, and the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Molecular docking is used to predict the interaction between two molecules by creating a model of union between them. These models consider molecular mechanisms and experimental parameters (charges, torsion, and geometric angles). Usually, the ligand-protein interaction is studied (Lopes *et al*., 2015; Prieto-Martínez *et al*., 2018). Therefore, the objective of this work is to perform a molecular docking study to analyze the inhibitory activity of nicotine and nornicotine on the enzyme 5-enolpyruvylshikimate-3-phosphate synthase.

MATERIALS AND METHODS

PROTEIN PREPARATION

The three-dimensional crystal structure of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase was downloaded in .pdb format from the Protein Data Bank (PDB ID: 1RF6) (Park *et al*., 2004). The protein was prepared using Chimera 1.16 (Pettersen et al., 2004) to remove domains B, C, and D, likewise ligands and water molecules were removed. Finally, it was prepared as a macromolecule and the file was obtained in .pdbqt format using PyRx (Dallakyan and Olson, 2015).

LIGAND PREPARATION

The three-dimensional structure of the compounds nicotine (CID: 89594), nornicotine (CID: 412), and glyphosate (CID: 3496) were downloaded from PubChem in .sdf format. PyRx was used to minimize the energy of the compounds and obtain the files in .pdbqt format (Dallakyan and Olson, 2015).

DOCKING

Docking was carried out centered on the residue using AutoDock Vina and AMDock (Trott and Olson, 2010; Valdes-Tresanco *et al*., 2020). The docking box was located at the protein's binding site, which is constituted by the residues Lys 20, Ser 21, Arg 25, Thr 93, Ser 166, Ala 167, Gln 168, Ile 311, Asp 312, Lys 339.

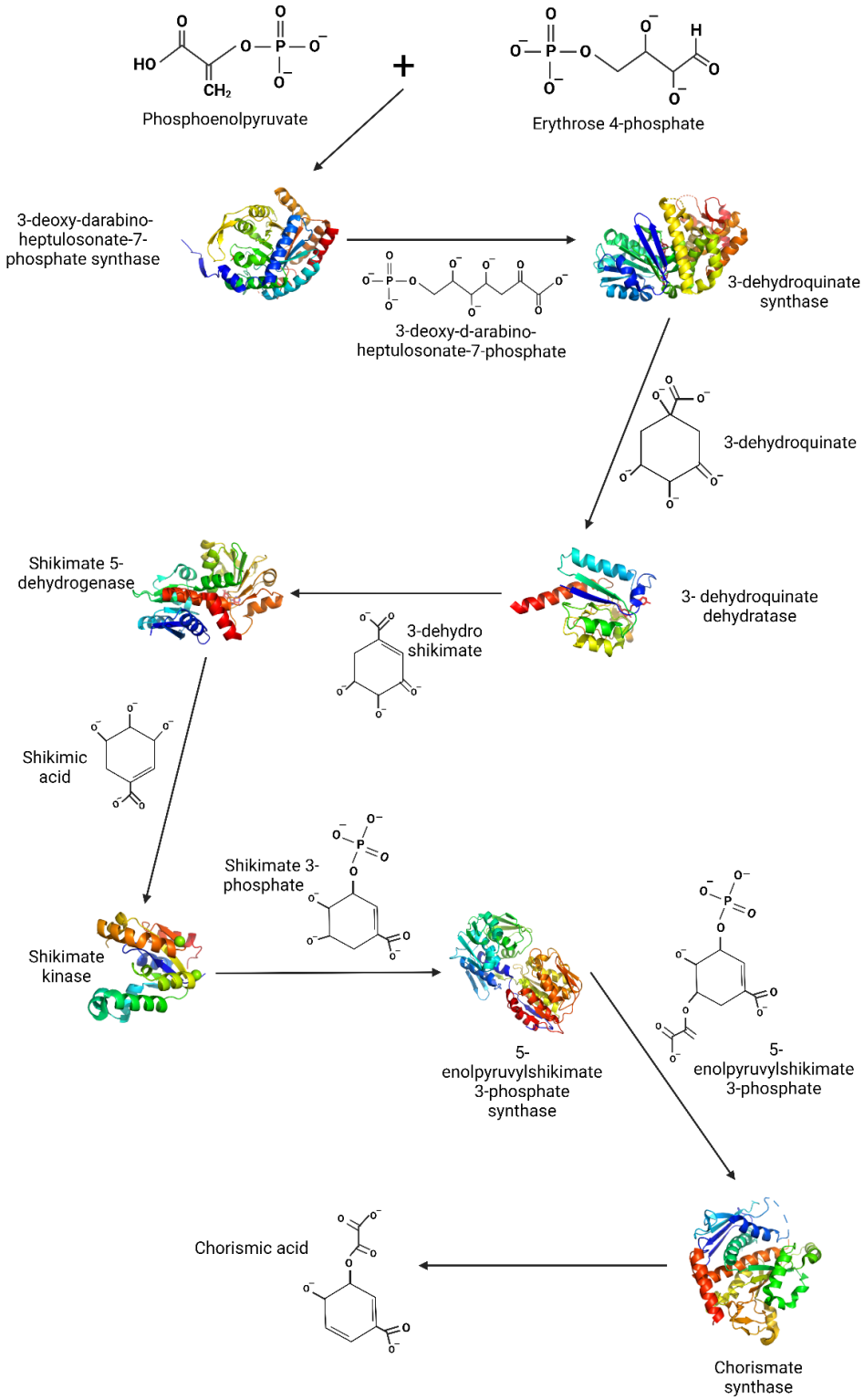

Figure 2. Shikimate pathway with representation of the enzymes and products involved (Modified from Oliveira *et al.*, 2020).

RESULTS

The binding model of glyphosate with the enzyme 5-enolpyruvylshikimate-3-phosphate synthase showed greater stability than the models of nicotine and nornicotine. Nicotine presented a more stable model than nornicotine according to the values of free binding energy (ΔG). Both nicotine and nornicotine showed the same efficiency, both lower than that presented by glyphosate. (Table 1).

Ligand	ΔG(kcal/mol)	ΔG(kcal/mol) Reported	Ligand Efficiency
Nicotine	- 4.8	NA	- 0.40
Nornicotine	- 4.4	NA	- 0.40
Glyphosate	- 6.2	- 5.9[a], - 6.0[a], - 6.3[b], - 6.3[c]	- 0.62

NA: No available, a: Filiz y Cock, 2016, b: Almihyawi *et al*., 2022, c: Cuadros-Siguas *et al*., 2023.

Table 1. Free binding energy and ligand efficiency presented by nicotine, nornicotine, and glyphosate at the binding site of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase.

Glyphosate showed the highest affinity towards the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, both nicotine and nornicotine showed low affinity, likewise, glyphosate showed higher inhibitory capacity (Table 2).

Ligand	K_i (μM)	IC_{50} (μM)
Nicotine	300	600
Nornicotine	600	1200
Glyphosate	28.53	57.06

Table 2. Inhibition constant and IC_{50} presented in the binding models of each ligand with the enzyme 5-enolpyruvylshikimate-3-phosphate synthase.

Nornicotine showed greater interaction with residues of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase by interacting with 18 residues, followed by glyphosate which interacted with 17 residues, finally, nicotine interacted with 12 residues. Glyphosate had ten hydrogen-bond interactions with the residues Lys 20, Asp 47, Gly 92, Thr 93, Arg 120, Glu 340, and Arg 343, nornicotine only had one hydrogen-bond interaction with residue Asp 312, while nicotine did not have interactions of this type (Figure 4).

DISCUSSION

The binding model obtained from the docking showed glyphosate interacting with residues Lys 20, Asn 90, Gly 92, Thr 93, Arg 120, Gln 168, Asp 312, Glu 340, Arg 343, Arg 385, which have been reported to participate in glyphosate binding to the enzyme (Park *et al*., 2003). The ΔG presented by glyphosate was the highest of the three ligands and is similar to that reported in the literature (Table 1) (Filiz and Koc, 2016), thus it presented the most stable binding model, as well as the greatest number of hydrogen-bond interactions. These interactions are related to the stability of the model; more hydrogen-bond interactions represent greater stability in the binding model and a reduction in energy (Glowacki *et al*., 2013). Glyphosate also showed the highest affinity and inhibitory activity among the three ligands according to the K_i and IC_{50} values obtained. IC_{50} values ranging between 1 and 40 μM are considered efficient (Fam *et al*., 2023). There are several studies reporting the effect of glyphosate on the growth of plant and microbial organisms, this effect is due to these organisms having the shikimate pathway where glyphosate exhibits its inhibitory effect (Ruiz, 2000; Priestman *et al*., 2005; Bórtoli *et al*., 2012). However, there are several reports of adverse effects from glyphosate exposure, making this compound a health risk. Among the adverse effects reported are increased

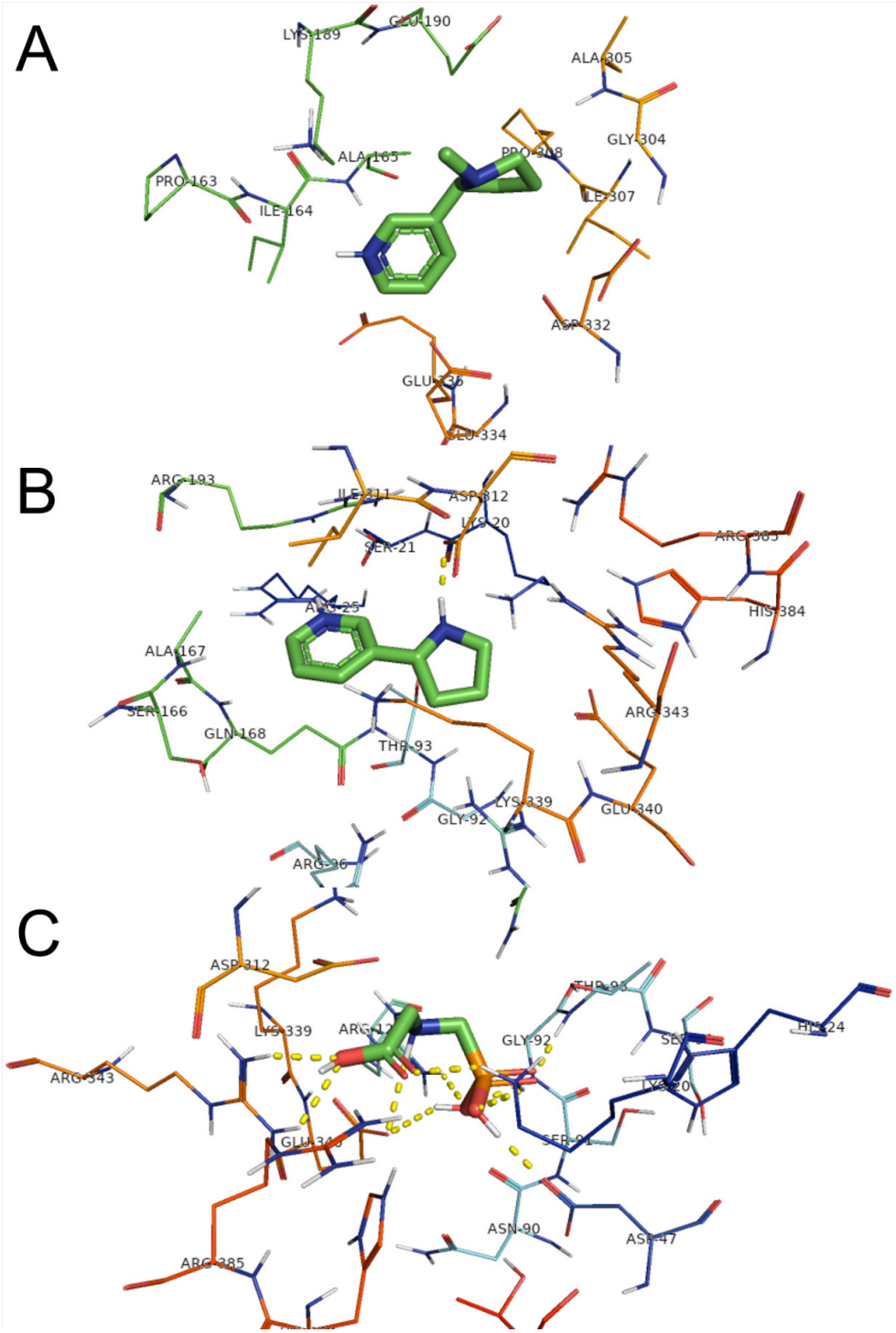

Figure 4. Residues of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase interacting with the ligands. A: Nicotine interacting with residues Pro 163, Ile 164, Ala 165, Lys 189, Glu 190, Gly 304, Ala 305, Ile 307, Pro 308, Asp 332, Glu 334, and Glu 335; B: Nornicotine interacting with residues Lys 20, Ser 21, Arg 25, Gly 92, Thr 93, Arg 96, Arg 120, Ser 166, Ala 167, Gln 168, Arg 193, Ile 311, Asp 312, Lys 339, Glu 340, Arg 343, His 384, and Arg 385; C: Glyphosate interacting with residues Lys 20, His 24, Asp 47, Asn 90, Ser 91, Gly 92, Thr 93, Ser 94, Arg 120, Gln 168, Asp 312, Lys 339, Glu 340, Arg 343, His 384, Arg 385, and Thr 412. Obtained using PyMOL (DeLano, 2002).

lipoperoxidation, DNA fragmentation, cellular alteration, and infertility (Barbosa *et al*., 2017; Cardona, 2019).

Nicotine showed greater stability than nornicotine in the binding models, but both ligands presented the same efficiency. Nicotine showed greater affinity toward the enzyme 5-enolpyruvylshikimate-3-phosphate synthase compared to nornicotine according to the K_i and IC_{50} values obtained (Fam *et al*., 2023). However, nornicotine interacted with residues Lys 20, Ser 21, Arg 25, Thr 93, Ser 166, Ala 167, Gln 168, Ile 311, Asp 312, and Lys 339, which are part of the protein's binding site. With residue Asp 312, it showed a hydrogen-bond interaction. Nicotine did not interact with any residue at the binding site and did not show any hydrogen-bond interactions, therefore, the presence of the methyl group in nicotine prevents interaction with the residues at the enzyme's binding site but increases its binding stability and affinity. Nornicotine has no reports of herbicidal activity, whereas nicotine has the ability to inhibit photosynthetic pigments (Chiellini *et al*., 2022), so greater inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase by nicotine was expected.

CONCLUSIONS

Nicotine and nornicotine showed low affinity with the enzyme 5-enolpyruvylshikimate-3-phosphate synthase compared to glyphosate. The methyl group present in nicotine and absent in nornicotine affects the stability and affinity of the models, as well as the interactions with the residues at the binding site.

ACKNOWLEDGMENT

LANCAD-UNAM-DGTIC-413 for the access to supercomputer Miztli.

REFERENCES

Alcántar, A. F. C. (2005). **Identificación y cuantificación de metabolitos secundarios en plantas de tabaco (*Nicotiana tabacum*) transformadas con sistemina y prosistemina de jitomate en respuesta a daño mecánico, herbivoría con Manduca sexta e infestación con mosquita blanca (*Bemisia tabaci*).** Tesis de maestría. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. IPN. México. 131 p.

Almihyawi, R. A. H., Naman, Z. T., Al-Hasani, H. M. H., Muhseen, Z. T., Zhang, S. & Chen, G. (2022). **Integrated computer-aided drug design and biophysical simulation approaches to determine natural anti-bacterial compounds for *Acinetobacter baumannii***. *Scientific Reports*, 12, 6590.

Barbosa, M. C., Aiassa, D. & Mañas, F. (2017). **Evaluación de daño al ADN en leucocitos de sangre periférica humana expuestos al herbicida glifosato**. *Revista Internacional de Contaminación Ambiental*, 33(3), 403-410.

Bórtoli, P. V., Verdenelli, R. A., Conforto, C., Vargas, G. S. & Meriles, J. M. (2012). **Efectos del herbicida glifosato sobre la estructura y el funcionamiento de comunidades microbianas de dos suelos de plantaciones de olivo**. *Ecología Austral*, 22, 33-42.

Botte, M., Mabon, F., LeMouillour, M. & Robins, R. J. (1997). **Biosynthesis of nornicotine in root cultures of *Nicotiana alata* does not involve oxidation at C-5' of nicotine**. *Phytochemistry*, 46, 117-122.

Burger, M & Fernández, S. (2004). **Exposición al herbicida glifosato: aspectos clínicos toxicológicos**. *Revista Médica del Uruguay*, 20, 202-207.

Cardona, M. W. D. (2019). **El glifosato afecta negativamente a los espermatozoides humanos: evidencia *in vitro***. *Revista Urología Colombiana*, 29(2), 96-98.

Chiellini, C., Mariotti, L., Huarancca-Reyes, T., de Arruda, E. J., Fonseca, G. G., & Guglielminetti, L. (2022). **Remediation Capacity of Different Microalgae in Effluents Derived from the Cigarette Butt Cleaning Process**. *Plants*, 11(13), 1770.

COFEPRIS (Comisión Federal para la Protección contra Riesgos Sanitarios). (2009). **Catálogo de Plaguicidas**. Available in: http://www.cofepris.gob.mx/wb/cfp/catalogo_de_plaguicidas. Consulted: October 20, 2020.

Cruz, S. J. G. (2013). **Glifosato: uso, efecto y regulación en calidad del agua**. Tesis de licenciatura. Facultad de Estudios Superiores Iztacala. UNAM. México. 123 p.

Cuadros-Siguas, C. F., Herrera-Calderon, O., Batiha, G. E.-S., Almohmadi, N. H., Aljarba, N. H., Apesteguia-Infantes, J. A., Loyola-Gonzales, E. Tataje-Napuri, F. E., Kong-Chirinos, J. F., Almeida-Galindo, J. S., Chávez, H. & Pari-Olarte, J. B. (2023). **Volatile Components, Antioxidant and Phytotoxic Activity of the Essential Oil of *Piper acutifolium* Ruiz & Pav. from Peru**. *Molecules*, 228(8), 3348.

Dallakyan, S. & Olson, A. J. (2015). **Small-molecule library screening by docking with PyRx**. *Methods in Molecular Biology*, 1263, 243–250.

DeLano, W. L. (2002). **The PyMOL Molecular Graphics System**.

Devine, M. D., Duke, S. O. & Fedtke, C. (1993). **Physiology of herbicide action**. Englewood Cliffs, Nueva Jersey, The United States Prentice Hall.

Fam, M. S., Sedky, C. A., Turky, N. O., Breitinger, H. G., & Breitinger, U. (2023). **Channel activity of SARS-CoV-2 viroporin ORF3a inhibited by adamantanes and phenolic plant metabolites**. *Scientific reports*, 13(1), 5328.

Filiz, E. & Koc, I. (2016). **Genome-wide identification and comparative analysis of EPSPS (aroA) genes in different plant species**. *Journal of Plant Biochemistry and Biotechnology*, 25, 21–29.

Forbes. (2020). **Semarnat pone fecha para el fin del glifosato presente en herbicidas (y en el maíz)**. Available in: https://www.forbes.com.mx/semarnat-pone-fecha-para-el-fin-del-glifosato-presente-en-herbicidas-y-en-el-maiz/. Consulted: October 23, 2020.

Glowacki, E. D., Irimia-Valdu, M., Bauer, S. & Sariciftci, N. S. (2013). **Hydrogen-bonds in molecular solids - from biological systems to organic electronics**. *Journal of Materials Chemistry*, 31, 3742-3753.

Lopes, P. E., Guvench, O. & MacKerell, A. D. (2015). **Current status of protein force fields for molecular dynamics simulations**. *Methods in molecular biology*, 1215, 47–71.

Nuñez, G, T. (1963). **Estudios sobre el tabaco: Los alcaloides.-Farmacología y Toxicología**. *Ars Pharmaceutica*, 4(2), 133-148.

Oliveira, M. D., Araújo, J. O., Galúcio, J. M. P., Santana, K. & Lima, A. H. (2020). **Targeting shikimate pathway: In silico analysis of phosphoenolpyruvate derivatives as inhibitors of EPSP synthase and DAHP synthase**. *Journal of Molecular Graphics and Modelling*, 101, 1-12.

Park, H., Hilsenbeck, J. L., Kim, H. J., Shuttleworth, W. A., Park, Y. H., Evans, J. N., & Kang, C. (2003). **Structural Studies of *Streptococcus pneumoniae* EPSP Synthase in S3P-GLP Bound State**.

Park, H., Hilsenbeck, J. L., Kim, H. J., Shuttleworth, W. A., Park, Y. H., Evans, J. N., & Kang, C. (2004). **Structural studies of *Streptococcus pneumoniae* EPSP synthase in unliganded state, tetrahedral intermediate-bound state and S3P-GLP-bound state**. *Molecular microbiology*, 51(4), 963–971.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). **UCSF Chimera--a visualization system for exploratory research and analysis.** Journal of computational chemistry, 25(13), 1605–1612.

Priestman, M. A., Healy, M. L., Becker, A., Alberg, D. G., Bartlett, P. A., Lushington, G. H., & Schönbrunn, E. (2005). **Interaction of phosphonate analogues of the tetrahedral reaction intermediate with 5-enolpyruvylshikimate-3-phosphate synthase in atomic detail**. *Biochemistry*, 44(9), 3241–3248.

Prieto-Martínez, F. D., Arciniega, M. & Medina-Franco, J. L. (2018). **Molecular docking: current advances and challenges**. *Revista Especializada en Ciencias Químico-Biológicas*, 21, 65-87.

Ruiz, F. E. (2000). **Control de Lirio acuático (*Eichhornia crassipes*) con el herbicida glifosato en combinación con ácidos húmicos y fúlvicos**. Tesis de licenciatura. Facultad de Estudios Superiores Cuautitlán. UNAM. México. 94p.

Salazar, L. N. J. & Aldana, M. M. L. (2011). H**erbicida Glifosato: usos, toxicidad y regulación**. *Revista de Ciencias Biológicas y de la Salud de la Universidad de Sonora*, 13(2), 23-28.

Trott, O. & Olson, A. J. (2010). **AutoDock Vina: improving the speed and accuracy of docking with a ner scoring function, efficient optimization and multithreading**. *Journal of Computational Chemistry*, 31, 455-461.

Valdes-Tresanco, M. S., Valdes-Tresanco, M. E., Valiente, P. A. & Moreno, E. (2020). **AMDock: a versatile graphical tool for assisting molecular docking with Autodock Vina and Autodock4**. *Biology Direct*, 15(12), 1-12.

Williams, G. M., Kroes, R. & Munro, I. C. (2000). **Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans**. *Regulatory toxicology and pharmacology*: *RTP*, 31(2), 117–165.