Medical Biotechnology and Health Science Journal

Volume 2, Issue 1, July 2026

Molecular Activity of Bioactive Compounds from Mitragyna speciosa Using a Molecular Docking Approach

Tri Puji Lestari Sudarwati [1*] & Kavana Hafil Kusuma [2]

[1]Akademi Farmasi Surabaya, Jl. Ketintang Madya No.81, Ketintang, Kec. Gayungan, Surabaya, Jawa Timur 60232, Indonesia.

[2]Biologi, FMIPA, Brawijaya university, Jl. Veteran No.10-11, Ketawanggede, Kec. Lowokwaru, Kota Malang, Jawa Timur 65145, Indonesia

Article Info

Received : 30 June 2026
Received : 27 July 2026
Accepted : 28 July 2026
Published : 30 July 2026

Keywords:
Mitragyna speciosa, mitragynine, reseptor μ-opioid, MOR, molecular docking, PyRx.

***Corresponding author:**
tri.puji.ls@akfarsurabaya.ac.id

Abstract

Kratom (Mitragyna speciosa) is a medicinal plant that contains mitragynine, its principal alkaloid, which has been reported to interact with opioid receptors. This study aimed to evaluate the molecular interaction of mitragynine with the μ-opioid receptor (MOR) through a molecular docking approach. The three-dimensional structure of mitragynine was retrieved from the PubChem database, whereas the crystal structure of MOR was obtained from the Protein Data Bank (PDB). Molecular docking simulations were performed using PyRx, and the resulting ligand–receptor interactions were analyzed and visualized using PyMOL and BIOVIA Discovery Studio. The docking analysis yielded a binding affinity of −7.9 kcal/mol, indicating a favorable binding interaction between mitragynine and MOR. Further interaction analysis revealed that mitragynine established hydrogen bonds, hydrophobic interactions, and electrostatic interactions with several key amino acid residues located within the active binding site of MOR, including Tyr77, Gln126, Asp149, Trp295, His299, Trp320, His321, Ile324, Gly327, and Tyr328. These molecular interactions suggest that mitragynine exhibits a stable binding conformation within the MOR binding pocket, thereby supporting its potential pharmacological activity as an analgesic agent. The findings of this study provide molecular-level evidence supporting the interaction of mitragynine with MOR and contribute to the understanding of the mechanistic basis underlying the analgesic properties of Mitragyna speciosa.

INTRODUCTION

Kratom (*Mitragyna speciosa*) is a tropical plant native to Southeast Asia that has been traditionally used for pain relief, enhancement of physical endurance, and mood improvement. The pharmacological properties of M. speciosa are primarily attributed to its indole alkaloids, particularly mitragynine and 7-hydroxymitragynine, which have been reported to exhibit affinity for opioid receptors. Unlike classical opioids, such as morphine, which function as full agonists of the μ-opioid receptor (MOR), the major alkaloids of M. speciosa have been characterized as partial agonists exhibiting G protein-biased agonism. This distinctive pharmacological profile is believed to produce analgesic effects while potentially reducing the risk of adverse effects, particularly respiratory depression, commonly associated with conventional opioid analgesics. (Raini, 2017; Wahyono et al., 2019). These pharmacological characteristics make *M.speciosa* an attractive subject for investigation as a potential source of novel analgesic agents with an improved safety profile compared with conventional opioids.

MOR is the primary molecular target of opioid analgesics and plays a pivotal role in the modulation of pain. The interaction between a ligand and MOR is a critical determinant of a compound's pharmacological potency, receptor selectivity, and adverse effect profile (Lutz et al., 2014). Therefore, understanding the binding mechanism of the bioactive compounds of *M. speciosa* to MOR represents a crucial step toward elucidating their biological activity. Molecular-level analysis provides valuable insights into binding affinity,

ligand–receptor complex stability, and the key amino acid residues involved in ligand recognition and binding. Advances in computational approaches in structural biology have enabled the investigation of ligand–receptor interactions through *in silico* molecular docking. This approach is widely employed to predict the binding orientation, binding energy, and interaction patterns of ligands with target proteins, thereby providing preliminary insights into their potential biological activity.

In the context of *M. speciosa* research, molecular docking offers a valuable strategy for comparing the binding characteristics of mitragynine, 7-hydroxymitragynine, and other alkaloids with MOR relative to well-characterized opioid ligands. The findings of such analyses are expected to enhance the understanding of the structure–activity relationships of kratom alkaloids and provide a scientific basis for the rational development of novel analgesic agents with high therapeutic efficacy and an improved safety profile. Based on these considerations, investigating the molecular interactions of *M. speciosa* alkaloids with the MOR using a molecular docking approach is warranted to evaluate their binding affinity and interaction profiles with MOR, as well as to compare their binding characteristics with those of clinically established opioid ligands. Such an investigation is expected to provide valuable molecular insights into the receptor-binding behavior of kratom alkaloids and contribute to the identification of promising lead compounds for the development of safer and more effective opioid-based analgesics.

METHOD

1. Data Collection

This study employed an *in silico* approach to investigate the molecular interactions between mitragynine, the principal alkaloid of *M. speciosa*, and the MOR. Information on the secondary metabolites of *M. speciosa* was retrieved from the KNApSAcK Core Database by searching the metabolites reported for the species (https://www.knapsackfamily.com). Among the identified compounds, mitragynine was selected as the test ligand because it is the predominant alkaloid responsible for the pharmacological activity of kratom.

The three-dimensional (3D) structure of mitragynine was obtained from the PubChem database (CID: 3034396) in Structure Data File (SDF) format. The crystal structure of the target protein, the μ-opioid receptor (MOR), was retrieved from the Protein Data Bank (PDB) under accession code 8EF6 in PDB format. The retrieved ligand and protein structures were subsequently subjected to structural preparation prior to molecular docking simulations.

2. Molecular Docking Simulation

Molecular docking simulations were performed using PyRx software, which integrates AutoDock Vina as the docking engine. Prior to docking, the mitragynine ligand was subjected to energy minimization to obtain its most stable molecular conformation. The crystal structure of the μ-opioid receptor (MOR) was prepared by removing water molecules, the co-crystallized ligand, and other non-essential molecules. Subsequently, both the protein and ligand structures were converted into PDBQT format for docking analysis.

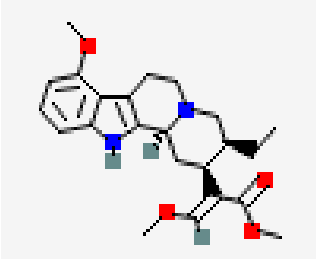
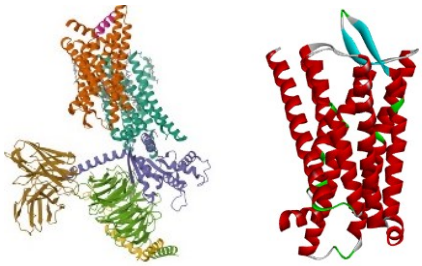
The binding site was defined based on the position of the co-crystallized ligand within the MOR crystal structure. A grid box encompassing the entire active binding pocket was generated to define the ligand search space during the docking simulation. Docking calculations were performed using AutoDock Vina, which generated multiple ligand binding poses along with their corresponding binding affinity values. The docking pose exhibiting the lowest binding energy was selected as the optimal binding model, as it represents the most thermodynamically favorable ligand–receptor complex.

The docking results were visualized using PyMOL to examine the three-dimensional orientation and binding mode of mitragynine within the active site of MOR. Detailed interaction analyses were subsequently performed using BIOVIA Discovery Studio Visualizer to identify the amino acid residues involved in ligand binding and to characterize the intermolecular interactions formed, including hydrogen bonds, hydrophobic interactions, π-alkyl interactions, π–π stacking, electrostatic interactions, and van der Waals forces. The docking results were analyzed descriptively based on the binding affinity values, the number and types of molecular interactions, and the amino acid residues involved in ligand binding. These analyses were conducted to evaluate the molecular binding characteristics of mitragynine toward MOR and to assess its potential as a candidate ligand for μ-opioid receptor-mediated analgesic activity.

RESULTS AND DISCUSSION

The molecular docking analysis demonstrated that mitragynine exhibited a binding affinity of −7.9 kcal/mol toward the μ-opioid receptor (MOR). The negative binding energy indicates that the formation of the ligand–receptor complex is thermodynamically favorable and occurs spontaneously. In general, a lower (more negative) binding affinity value reflects a stronger binding interaction between the ligand and its target protein. Therefore, the binding affinity of −7.9 kcal/mol suggests that mitragynine possesses a favorable binding capacity toward MOR and may exert its pharmacological effects through interaction with this receptor (Minami, 2004; Todd et al., 2020).

Table 1. Structure of Mitragynine and the μ-Opioid Receptor (MOR)

Struktur Mitragynine	Struktur MOR
 PubChem (ID. 3034396)	 PDB 8ef6

Interaction analysis performed using BIOVIA Discovery Studio revealed that mitragynine interacts with several amino acid residues located within the active binding pocket of MOR, including Tyr77, Gln126, Asn129, Asp149, Trp150, Met153, Trp295, Ile298, His299, Trp320, His321, Ile324, Gly327, and Tyr328. The ligand–receptor complex was stabilized by multiple intermolecular interactions, including van der Waals forces, conventional hydrogen bonds, carbon–hydrogen bonds, π–anion interactions, π–sigma interactions, alkyl interactions, and π–alkyl interactions. The diversity of these interaction types indicates that the stability of the ligand–receptor complex is not governed by a single interaction but rather arises from the combined contribution of multiple intermolecular forces acting synergistically to stabilize ligand binding within the receptor binding pocket.

The hydrogen bonds formed between mitragynine and the residues Trp320 and Gln126 are likely to play a critical role in maintaining the proper orientation of the ligand within the MOR binding pocket. Hydrogen bonding is one of the most important non-covalent interactions contributing to the specificity and stability of ligand binding to target proteins. In addition, the presence of a π–anion interaction with Asp149 suggests that electrostatic forces contribute to strengthening the binding affinity of mitragynine toward MOR.

Further analysis revealed that the hydrophobic residues Trp295, Ile298, Ile324, and Tyr328 establish π–alkyl and alkyl interactions with the aromatic ring system and carbon framework of mitragynine. Such hydrophobic interactions are recognized as key determinants of ligand stabilization within the binding pocket of opioid receptors, as the MOR binding site is predominantly composed of hydrophobic amino acid residues. Consequently, these interactions are expected to enhance the stability of the ligand–receptor complex and support the agonistic activity of mitragynine toward MOR.

Moreover, the involvement of aromatic residues, including Tyr77, Trp295, Trp320, and Tyr328, indicates that mitragynine binds to functionally important regions within the MOR binding pocket. These aromatic residues have been widely reported to play essential roles in opioid ligand recognition and in stabilizing ligand–receptor complexes through π-mediated interactions. The participation of these key residues suggests that mitragynine is capable of occupying the MOR binding pocket effectively and establishing an extensive interaction network that supports its biological activity (Goleman, 2019; Oktaviani.J, 2018; Yamamoto et al., 1999).

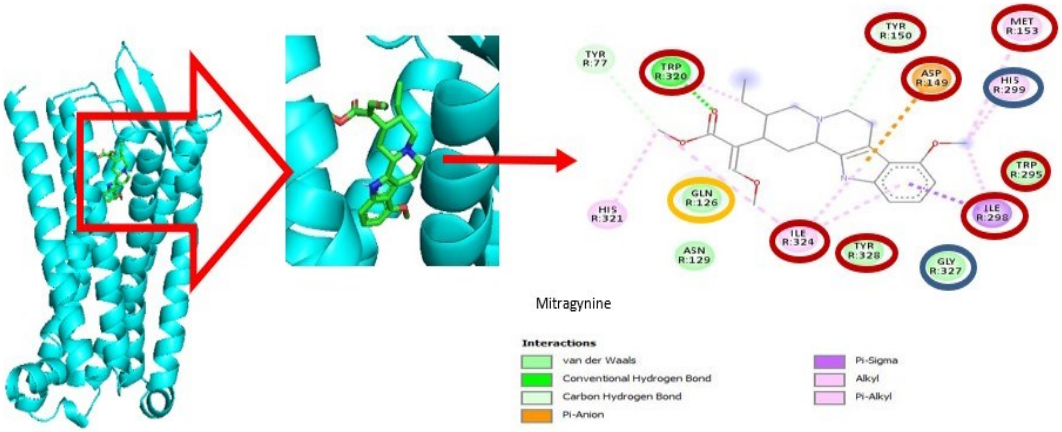


Figure 1. Molecular Docking Pose of Mitragynine in the Binding Pocket of the μ-Opioid Receptor (MOR)

The binding affinity value of −7.9 kcal/mol, together with the formation of hydrogen bonds, electrostatic interactions, and hydrophobic interactions, indicates that mitragynine exhibits a favorable binding affinity toward the μ-opioid receptor (MOR). These findings are consistent with previous pharmacological studies identifying mitragynine as the principal alkaloid of M. speciosa responsible for its analgesic effects through modulation of MOR.Nevertheless, molecular docking provides only a computational prediction of binding affinity and ligand–receptor interaction patterns at the molecular level. Therefore, further validation using molecular dynamics (MD) simulations, as well as in vitro and in vivo biological studies, is required to confirm the stability of the ligand–receptor complex and to verify the pharmacological activity of mitragynine.

CONCLUSION

The molecular docking analysis demonstrated that mitragynine exhibits favorable binding affinity toward the μ-opioid receptor (MOR), with a binding affinity value of −7.9 kcal/mol. Mitragynine interacts with several key amino acid residues within the MOR binding pocket through hydrogen bonds, hydrophobic interactions, and electrostatic interactions, all of which contribute to the stability of the ligand–receptor complex. These findings suggest that mitragynine has the potential to modulate MOR activity and support its role as a bioactive constituent of *M. speciosa* contributing to the plant's analgesic effects. Nevertheless, further investigations employing molecular dynamics simulations and experimental validation through *in vitro and *in vivo* studies are warranted to confirm its pharmacological activity and therapeutic potential.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to all individuals and institutions who supported the completion of this research. Their invaluable support and contributions were greatly appreciated and played a significant role in the successful completion of this study.

REFERENCES

Goleman. (2019). Reseptor. *Journal of Chemical Information and Modeling*, *53*(9), 1–30.

Lutz, P. E., Ayranci, G., Chu-Sin-Chung, P., Matifas, A., Koebel, P., Filliol, D., Befort, K., Ouagazzal, A. M., & Kieffer, B. L. (2014). Distinct Mu, Delta, and Kappa opioid receptor mechanisms underlie low sociability and depressive-like behaviors during heroin abstinence. *Neuropsychopharmacology*, *39*(11), 2694–2705. https://doi.org/10.1038/npp.2014.126

Minami, M. (2004). Molecular pharmacology of opioid receptors. *Folia Pharmacologica Japonica*, *123*(2), 95–104.

https://doi.org/10.1254/fpj.123.95

Oktaviani.J. (2018). 済無No Title No Title. *Sereal Untuk*, *51*(1), 51.

Raini, M. (2017). Kratom (Mitragyna speciosa Korth): Manfaat, Efek Samping dan Legalitas. *Media Penelitian Dan Pengembangan Kesehatan*. https://doi.org/10.22435/mpk.v27i3.6806.175-184

Todd, D. A., Kellogg, J. J., Wallace, E. D., Khin, M., Flores-Bocanegra, L., Tanna, R. S., McIntosh, S., Raja, H. A., Graf, T. N., Hemby, S. E., Paine, M. F., Oberlies, N. H., & Cech, N. B. (2020). Chemical composition and biological effects of kratom (Mitragyna speciosa): In vitro studies with implications for efficacy and drug interactions. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-76119-w

Wahyono, S., Widowati, L., Handayani, L., Sampurno, O. D., Haryanti, S., Fauzi, Ratnawati, G., & S, M. B. (2019). Kratom, Prospek Kesehatan dan Sosial Ekonomi. In *Journal of Chemical Information and Modeling* (Vol. 53, Issue 9).

Yamamoto, L. T., Horie, S., Takayama, H., Aimi, N., Sakai, S. I., Yano, S., Shan, J., Pang, P. K. T., Ponglux, D., & Watanabe, K. (1999). Opioid receptor agonistic characteristics of mitragynine pseudoindoxyl in comparison with mitragynine derived from Thai medicinal plant Mitragyna speciosa. *General Pharmacology*, *33*(1), 73–81. https://doi.org/10.1016/S0306-3623(98)00265-1