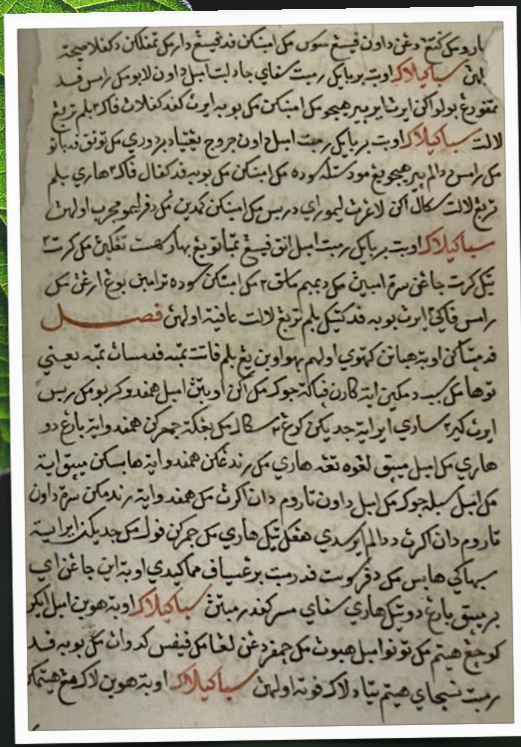
International Journal of Allied Health Sciences (IJAHS)

Special issue of the

4TH INTERNATIONAL CONFERENCE ON MALAY MEDICAL MANUSCRIPTS 2023



MSS 1292 PNM p.28

5-6 September 2023
via Online Platform

SUSTAINABLE DEVELOPMENT GOALS

HIKAM
HEALTH INFORMATION AND KNOWLEDGE FROM MALAY MEDICAL MANUSCRIPTS
AN IIUM FLAGSHIP

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
Garden of Knowledge and Virtue

CALL FOR PAPERS

4TH INTERNATIONAL CONFERENCE ON MALAY MEDICAL MANUSCRIPTS 2023

"TOWARDS MAINSTREAMING MALAY MEDICAL MANUSCRIPTS"

5 - 6 SEPTEMBER 2023

HYBRID (ZOOM/IIUM KUANTAN)

REGISTER HERE:
http://bit.ly/icommm2023

ICOMMM 5-6 SEPT 2023

PLANTS WITH ANTINOCICEPTIVE ACTIVITY FROM MSS 3048 FOR SHINGLES TREATMENT: A MOLECULAR DOCKING STUDY OF THE ACTIVE COMPOUNDS WITH P2X4 RECEPTOR

Farah Nazihah Zulkifli[1] and **Izzuddin Ahmad Nadzirin**[1*]

[1]Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jln Sultan Ahmad Shah Bandar Indera Mahkota 25200 Kuantan, Pahang, Malaysia.

*Corresponding author's email: izzuddin_a@iium.edu.my

ABSTRACT

Malay medical manuscripts (MMM) discuss diseases and remedies according to Malay traditional medicine. One of them, MSS 3048, compiles a variety of diseases including shingles; a viral disease that manifests itself with neuropathic pain. One of the mechanisms that cause neuropathic pain is through the activation of P2X4, an ionotropic receptor. Hence, one of the strategies for treating neuropathic pain is by inhibiting the receptor. However, current antinociceptive drug can cause serious side effects like respiratory depression. Thus, the goal is to identify potential compounds that can block P2X4 based on the information from MSS 3048 and *in silico* study. Firstly, the content of MSS 3048 was analysed to find the plants used for shingles. Then, the active compounds possessed by the plants were studied and the ones that had been shown to have antinociceptive effect based on literature studies were selected. Then the 3D structures of the plant's active ingredients were docked on the human P2X4 homology model using Autodock Vina software. The software produced results that showed the binding affinity of the active ingredients towards P2X4. The inhibitors' affinity was compared to known ligands which are 5-(3-Bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one(5-BDBD),adenosine triphosphate (ATP), cytidine triphosphate (CTP) and α,β-methylene ATP (α,β-meATP) as a reference. The result shows that the top six compounds with high affinity towards P2X4 are dioscin, ampelopsin F, corilagin, ellagic acid pentoside, punicalin and proanthocyanidins. Then the top six compounds were analysed by looking at the chemical interaction. The compound that showed the highest potential was dioscin, one of the compounds from nipah palm, with binding affinity of -9.87 kcal/mol. This compound may have the potential to block P2X4 receptor and could be studied in *in vitro* and *in vivo* study before being used as treatment for shingles.

Keywords: Malay medical manuscript, shingles, P2X4 receptor, *in silico* study, molecular docking

INTRODUCTION

Shingles, or known as *kayap* in Malay, is caused by Varicella-zoster virus (VZV). People with previous infections to chickenpox are more prone to develop shingles as it is caused by the same virus. Peate (2010) stated that this virus infects the nerves and manifests itself with a painful rash on the side supplied by the infected nerves. Wilson and Wilson (2021) explained that the symptoms include pain before and during the rash, fever, malaise, and headache, which can last up to four weeks. This type of pain is called neuropathic pain as it arises from nerve damage. One of the treatments is to manage the pain with the use of painkillers. Ibuprofen and acetaminophen have fewer side effects but are not strong enough to relieve the pain. On the other hand, Tylenol and codeine are among the stronger painkillers but with more serious side effects. Other than painkillers, antiviral medicine is one of the treatments for shingles. Center for Disease Control (CDC) (2023) stated that the administration of antiviral is to shorten the length and severity of the pain, not eliminating the virus fully. Hence, herbal medicine becomes an option when modern medicine is ineffective in the treatment of disease or due to concern about the adverse effects.

The treatment for shingles had been discussed in many Malay medical manuscripts including MSS 3048. The manuscript is divided into two sections, part A, Pelbagai Petua and Part B, Kitab Tib. *Katalog Manuskrip Melayu Koleksi Perpustakaan Negara Malaysia Tambahan Keempat* (2006) added that among the contents covered in part B includes traditional remedies for seizures, coughs, stomach aches, eye

diseases, shingles, urinary stones, and amenorrhea. This manuscript also describes a few plants, herbs, and food that are used to treat the previously mentioned diseases. Besides that, MSS 3048 also contains non-medical information such as amulets and advice for the community's daily lives at the time.

One of the receptors involved in pain signalling is P2X4 in which its activation, induced by binding of adenosine triphosphate (ATP), results in the propagation of nociception. Natural ingredients in plants are being investigated to develop new antinociceptive drugs with potential therapeutic effects in pain management. Makoto et al. (2012) stated that the P2X4 receptor is involved in the pathogenesis of neuropathic pain when expressed on microglia. The activated glia elicits responses such as the release of cytokines and neurotrophic factor, resulting in the hyperexcitability of dorsal horn neurons and neuropathic pain. Thus, blocking the P2X4 receptor will reverse the injury and is said to be a potential therapeutic target for neuropathic pain (Tsuda et al., 2013). It serves as the basis for this research to find compounds that can bind to P2X4 receptors in which the compounds are tested for antinociceptive activity.

Since the conventional drug development process is time-consuming, *in silico* method such as computer-aided drug design (CADD) are introduced. In CADD, drug targets are identified, chemical compounds are screened and optimized for drug candidates, and toxicity is assessed. All these are done using computational methods. These strategies can reduce the number of chemical compounds that need to be tested experimentally while increasing the success rate by eliminating ineffective and harmful chemical compounds from consideration (Shaker et al., 2021). Hence, *in silico* study help in reducing time and cost in identifying active ingredients that have high affinity to P2X4 receptor.

MATERIALS & METHODS

Materials

All the molecular docking and modelling jobs were performed using a laptop with processor of Intel® core ™i5-10210U CPU@1.60GHz 2.11GHz with Windows 11 operating system. The molecular docking was conducted using Autodock Vina version 1.1.2, The Scripps Research Institute, United States of America. (Trott & Olson, 2010).

Identification of P2X4 and Ligands

P2X4 was chosen as the receptor of interest in this study. Since the crystal structure of P2X4 in Protein Data Bank (PDB) is only available for zebra fish, the homology model of human P2X4 was obtained from Zayuri (2020).

Ligands are the active ingredients of the plants and were obtained from the transliteration of MSS 3048 by Ahmad Radzaudin (2021). A total of 19 formulations were listed for shingles. From the formulations, a total of 22 plants were listed. The active ingredients of each plant were identified through literature search. The inclusion criteria for the literature were articles from year 2000 until present, written in Malay or English, and in full paper. The database used was Google Scholar, using the keywords such as "scientific name of plants" "antinociceptive" "analgesic" "phytochemical analysis" together with the use of Boolean operators, AND and OR.

A total of 112 compounds were found altogether through literature search. These compounds were filtered based on Lipinksi's Rule using an online tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp), which is useful for identifying molecules that are drug-like and those that are not.

Receptor and Ligands Preparation

The 3D structure of the P2X4 receptor was obtained from Zayuri (2020) in *.pdb format. The structure was modified to be used for molecular docking by removing water molecules, adding hydrogen atoms, and merging polar and non-polar hydrogens, using a software called AutoDockTools, The Scripps Research Institute, United States of America. (Sanner et al., 1999). The structure then was converted to *.pdbqt format.

The structures for active ingredients were obtained from the PubChem Open Chemistry database. The 3D structure of the compounds was downloaded in *.sdf format. Certain compounds only have 2D structure available in the database, so Avogadro software (open-source tool) was used to generate the 3D structure. The 3D structure was optimized in Avogadro using Generalized Amber Force Field (GAFF), a type of force field. Then, Open Babel software (SourceForge, United States of America) was used to convert *.sdf to *.mol2 format and hydrogen had been added to polar atoms only. The conversion of *.mol2 to *.pdbqt format was done in AutoDockTools. This process started by detecting the root of the torsion tree. Then, the root was chosen, and torsion was added to that root.

Docking Parameter and Control Docking

AutoDock Vina software (The Scripps Research Institute, United States of America) was used for docking. The parameter for docking needs were set to search for a binding site. The amino acid residues were Lys67, Lys69, Thr186, Lys190, Ile218, Asn293, Arg295, and Lys313 (Ahmad, 2018). These residues acted as binding sites for the ligands in the target structure. The receptor grid box size and coordinates are the important parameters in AutoDock Vina. The size of the grid box was set to 32.0 Å by 36.0 Å by 28.0 Å with 1.0 Å spacing, centered at coordinates x=69.393, y=-24.195, z=-8.315 and the exhaustiveness was set to twelve.

Control docking was done using AutoDock Vina to optimize the correct parameters and to make sure it is reliable for the receptor. It was done on a known agonist which are, adenosine triphosphate (ATP), cytidine triphosphate (CTP) and α, β-methylene ATP (α, β-meATP) and the antagonist which is 5-(3-Bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (5-BDBD). This step was repeated three times, and the average binding energy for each agonist was recorded and compared to the known P2X4 agonist rank of EC_{50} (Ahmad, 2018). After the parameter was verified, the prepared ligands were docked with the P2X4 receptor in triplicate, and the average binding affinity was then reported. Control docking was performed, and the outcome shows the reliability of the binding site based on the ranking of affinities.

Docking and Analysis

For Autodock Vina, several files were required after downloading and preparing the ligands that bind to the P2X4 receptor. The files required are receptor files with residue information, ligand files, and configuration files with docking parameter information. The docking was carried out using the Windows command prompt (cmd). The docking process was done in triplicate to get the average for better confirmation results. Vina produced 9 conformations for each docking in a text document (.txt) file and .pdbqt file format.

The Ligplot+ software (European Bioinformatics Institute, United Kingdom) was used to analyse hydrogen bonding and hydrophobic interaction between ligands and P2X4. The PDB file for the complex of P2X4 and the docked compound was created. Types of interactions between ligands and P2X4 were examined and important residues that made up the binding were recorded.

RESULTS

Malay medical manuscript MSS 3048 was referred to find the plants associated with shingles treatment. A total of 22 plants were listed from 19 formulations related to shingles. Then, its active compounds were searched through literature using the inclusion criteria. The articles include review and experimentation articles. A total of 112 compounds with antinociceptive activity were found. The full result of the plants' scientific names and its active compounds is listed in Appendix A.

Control Docking

The results of binding between P2X4 with its antagonist and agonists are shown in Table 1. The results were consistent with the known EC_{50} level that ATP is higher than CTP and $\alpha\beta$-meATP (Ahmad, 2018).

Table 1 Average binding affinity of P2X4 antagonist and agonists

Compounds	Average binding affinity (kcal/mol) (σ=0.46)
5-BDBD	-8.50
ATP	-7.57
CTP	-7.50
$\alpha\beta$-meATP	-7.30

Molecular Docking

Table 2 shows the binding affinity of the top six hit compounds produced by Vina. Binding affinity is the strength of interaction between a protein and a ligand. The highest binding affinity towards P2X4 was produced by dioscin then followed by ampelopsin F, corilagin, ellagic acid pentoside, punicalin, and proanthocyanidins. The result for binding affinity of all 112 compounds is listed in Appendix B.

Table 2 Binding affinity of top six hit compounds produced by Vina.

Compounds	Average binding affinity (kcal/mol) (σ=0.21)
Dioscin	-9.87
Ampelopsin F	-9.47
Corilagin	-9.43
Ellagic acid pentoside	-9.43
Punicalin	-9.27
Proanthocyanidins	-9.20

Analysis of Protein-Ligand Complexes

The analysis of protein-ligands complexes was presented in Figure 1. Figure 1(a) shows the interaction between P2X4 and dioscin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. Two hydrogen bonds were formed between oxygen atom of Asn287 with 14th and 15th oxygen of dioscin with length 2.95 Å and 2.91Å respectively. Hydrophobic interactions occurred between the 15th oxygen of dioscin and residue Thr211. Another hydrophobic interaction was formed between 43rd carbon of dioscin and residues Pro290, His172 and Glu249.

Figure 1(b) shows the interaction between P2X4 and ampelopsin F. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the fifth oxygen of ampelopsin F and nitrogen of Tyr299 with distance of 2.82Å. The second hydrogen bond is between the third oxygen of ampelopsin F and oxygen of Ala297(A) with length of 2.69Å. Another hydrogen bond occurred between the fourth oxygen of ampelopsin F and oxygen of Ala297(B) with distance of 2.98Å. Hydrophobic interaction occurred between the first oxygen of ampelopsin F and residue Ala9(A), second oxygen of ampelopsin F and residues Ala93(C) and Pro92. Another hydrophobic interaction formed between the third, fourth and fifth oxygen of ampelopsin F and residues Lys298(A), Phe296(B) and Lys298(C) respectively. Hydrophobic interaction also occurred between the 21st and 22nd carbon of ampelopsin F and residue Ala297. Another hydrophobic interaction was between the 27th carbon of ampelopsin F and residues Ala87 and Phe296(C).

Figure 1(c) shows the interaction between P2X4 and corilagin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the eighth oxygen of corilagin with the nitrogen and oxygen of Ala297 and oxygen of Ala87(B) with distance of 2.94Å, 3.13Å and 3.10Å respectively. The second hydrogen bond is between the 12th oxygen of P2X4 with the

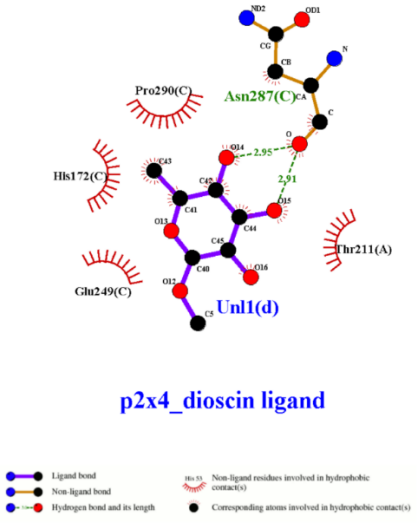
nitrogen of Ala93 with distance of 2.81Å. Another hydrogen bond is between the 16th oxygen of corilagin with oxygen atom of Val90(C) and Ala87(C) with length 2.76Å and 2.72Å respectively. The 18th oxygen of corilagin also formed hydrogen bond with oxygen atom of Ala87(C) with distance 2.93Å. The next hydrogen bond is between the eleventh oxygen of corilagin with oxygen atom of Val90(A) with length 3.2Å. Another hydrogen bond is between the ninth oxygen of corilagin with oxygen of Ala87(B) and Val90(B) with length 2.7Å and 3.31Å respectively. The tenth oxygen of corilagin also formed hydrogen bond with the oxygen of Val90(B) with distance 3.16Å. The 18th oxygen of corilagin formed hydrophobic interaction with residues Ile91(C), Asp88(C) and Tyr299(B). The 13th oxygen of corilagin formed hydrophobic interaction with residues Lys298(B) and Ala297(B). The fourth, eighth, tenth, and 15th oxygen of corilagin formed hydrophobic interaction with residues Phe296(C), Tyr299(A), Ile91(B) and Lys298(C) respectively. The next hydrophobic interaction is between eleventh oxygen of corilagin with residues Pro92(A) and Ile91(A). Another hydrophobic interaction is between 13th carbon of corilagin with residues Phe296(A), Pro92(B) and Ala93(B).

Figure 1(d) shows the interaction between P2X4 and ellagic acid pentoside. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 11th oxygen of ellagic acid pentoside with nitrogen of Ala297 (B) and Ala87(C) with distance 3.12Å and 3.06Å respectively. The next hydrogen bond is between the 7th oxygen of ellagic acid pentoside with nitrogen and oxygen of Ala297(A) and oxygen of Ala87(B) with distance of 2.93Å, 2.94Å and 3.26Å respectively. The 12th oxygen of ellagic acid pentoside also formed hydrogen bond with hydroxide of Tyr299(B) and oxygen of Ala87(C) with length 3.22Å and 2.82Å. The hydrogen bond is formed between the eight oxygen of ellagic acid pentoside and oxygen of Ala87(B) with distance of 2.95Å. Another hydrogen bond is between the ninth oxygen atom of ellagic acid pentoside with oxygen of Val90(B) with distance 2.73Å. The hydrophobic interaction occurred between the ninth oxygen of ellagic acid pentoside with residues Ile91(B) and Pro92(B). The fourth oxygen of ellagic acid pentoside also shows hydrophobic interaction with residues Phe296(C) and Ile91(C). Another hydrophobic interaction is with the third and fifth oxygen and 17th carbon of ellagic acid pentoside with residues Phe296(B), Ala93(B) and Tyr299(A) respectively.

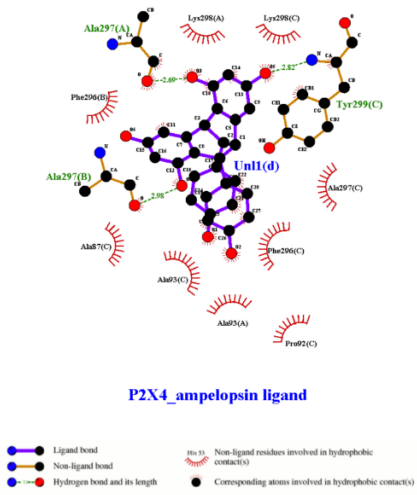
Figure 1(e) shows the interaction between P2X4 and punicalin. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 16th oxygen of punicalin with nitrogen of Asn293 (C) with length 3.03Å. The second hydrogen bond is between the fourth oxygen of punicalin with nitrogen of Lys67 (A) with distance of 3.28Å. Another hydrogen bond is between the 21st oxygen of punicalin with the second nitrogen of His172 (C) with distance of 3.12Å. The 12th oxygen of punicalin shows hydrogen bond with oxygen of His140(C) with distance 2.94Å. The hydrogen bond between the 14th oxygen of punicalin and oxygen of Thr186(A) has 3.03Å. The 19th oxygen of punicalin also shows hydrogen bond with the oxygen of Thr171(C) with length 3.12Å. Another hydrogen bond is between the 21st oxygen of punicalin with the nitrogen of His172(C) with length 3.13Å. Hydrophobic interaction occurred between the 19th oxygen of punicalin with residue Glu249(C). Hydrophobic interaction also occurred between the 30th carbon of punicalin with residue Lys313(C). The 13th oxygen of punicalin shows hydrophobic interaction with residues Leu188(A) and Ile229(A). Other hydrophobic interaction is between the eight oxygen of punicalin with residue Lys69(A), the 14th oxygen of punicalin with residue Thr139(C) and the 15th oxygen of punicalin with residue Lys215(A).

Figure 1(f) shows the interaction between P2X4 and proanthocyanidins. Autodock Vina predicted that the ligands consist of two types of interaction which are hydrogen bonds shown in green dotted lines and hydrophobic bonds shown in red eyelashes. The hydrogen bond is formed between the 13th oxygen of proanthocyanidins with nitrogen of Ala297 (B) with distance 2.80Å. The fourth oxygen of proanthocyanidins formed hydrogen bond with the oxygen of Ala87(A) and Val90(A) with length 2.73Å and 3.13Å respectively. The fifth oxygen of proanthocyanidins formed hydrogen bond with the hydroxide of Tyr299(C) with distance 3.07Å. Another hydrogen bond is formed between the seventh

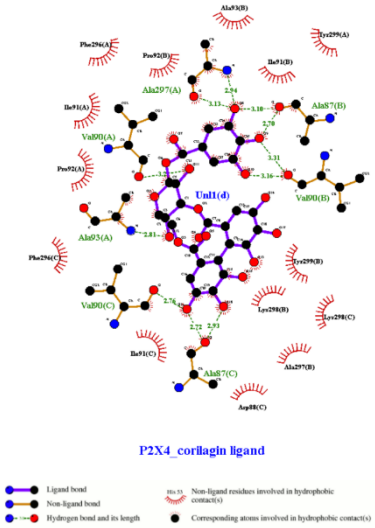
oxygen of proanthocyanidins with the oxygen of Ala87(B) with distance 2.77Å. Hydrophobic interaction occurred between the fifth oxygen of proanthocyanidins with residue Asp88(A). The eight oxygen of proanthocyanidins show hydrophobic interaction with residues Pro92(B) and Ile91(B). The 12th oxygen of proanthocyanidins also shows hydrophobic interaction with residues Phe296(C) and Ile91(C). Another hydrophobic interaction is between the eighth carbon of proanthocyanidins with residues Tyr299(B) and Ala87(C). The 17th carbon of proanthocyanidins shows hydrophobic interaction with residues Val90(B) and Ala93(B). Other hydrophobic interaction is with the ninth carbon of proanthocyanidins with residue Phe296(B), 14th carbon of proanthocyanidins with residue Phe296(A), 20th carbon of proanthocyanidins with residue Ile91(A) and the 30th carbon of proanthocyanidins with residue Ala297(C).



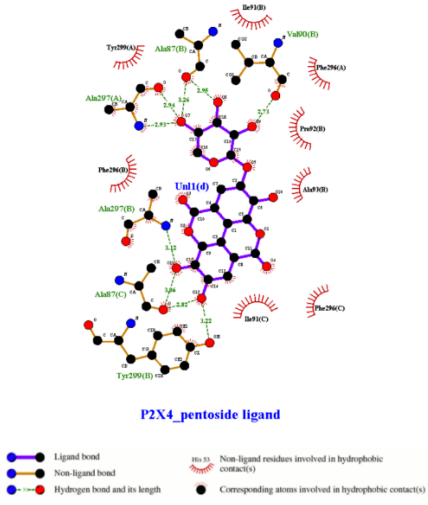
(a) Dioscin-P2X4 complex



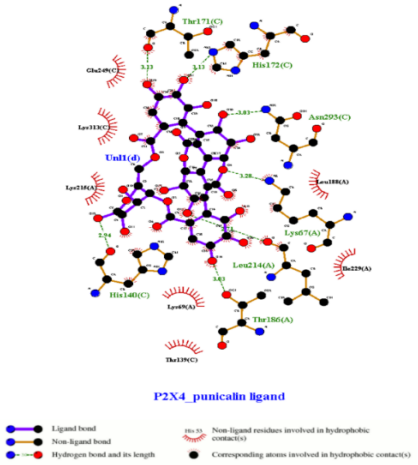
(b) Ampelopsin F-P2X4 complex



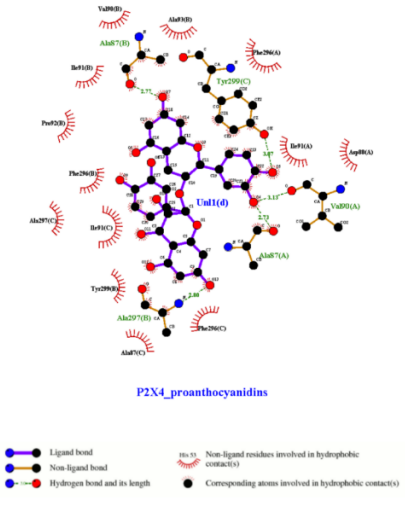
(c) Corilagin-P2X4 complex



(d) Ellagic acid pentoside-P2X4 complex



(e) Punicalin-P2X4 complex



(f) Proanthocyanidins-P2X4 complex

Figure 1 Analysis of protein-ligands complexes

DISCUSSION

The extraction of information from Malay medical manuscript MSS 3048 is a continuation of previous study by Ahmad Radzaudin (2021). MSS 3048 contains traditional remedies for diseases including shingles. This study focuses on shingles treatment since the use of the existing drugs may cause unwanted side effects and may not be fully effective. Since there is a long list of remedies listed, a virtual screening needs to be done to identify which ingredients possess the desired pharmacological action. *In silico* method is used to aid the drug development since it can save time and cost compared to the conventional drug design. This includes molecular docking, that can predict the binding of ligand to the target's binding site, and then is scored based on the strength of binding affinity (Batool et al., 2019).

Control docking was done beforehand to make sure the parameter is correct and reliable for the subsequent docking tests. This was done by docking 5-BDBD, ATP, CTP and α, β-meATP. The result shows that the binding site is correctly validated as it follows the characteristics of P2X4 receptor as described in earlier study (Ahmad, 2018). A total of 112 compounds were docked with P2X4 using the binding parameters set. The compound with the highest binding affinity is dioscin at -9.87 kcal/mol.

Literature search of *Nypa fruticans* or nipah palm reveal that it has 10 active ingredients. Studies from Reza et al (2011) stated that the extract of the plants inhibits prostaglandin synthesis and downregulates the expression of transient receptor potential vanilloid 1 (TRPV1). The most important active ingredient is dioscin with binding affinity at -9.87 kcal/mol indicating high interaction towards P2X4 receptor. Dioscin is a steroidal saponin that possesses anti-inflammatory properties by inhibiting toll-like receptor 2 (TLR2) signalling pathway in mice and rats (Zhao et al., 2018). Furthermore, dioscin also reduce inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions (Marahatha et al., 2021). Dioscin potential as anti-inflammatory may contribute to antinociceptive effects.

Ligplot+ shows the binding of the compounds to P2X4 is based on hydrogen bond and hydrophobic interactions. Dioscin has two hydrogen bonds and four hydrophobic interactions. This compound is a potential lead compound as it has strong interaction with P2X4, shown by the binding affinity. From the screening through Lipinski's rule, this compound has low molecular weight at 312 Dalton, low lipophilicity (log P) at -0.053 and low hydrogen-bonding capacity which helps in absorption and permeability (Benet et al., 2016).

CONCLUSION

The objective of this study, which is to identify the plants used for shingles treatment in MSS 3048, was achieved. Overall, 19 formulations related to shingles were listed and a total of 22 plants were identified from the manuscript such as *Nypa fruticans*, *Punica granatum* and *Capsicum*. Next, the active compounds of the plants that showed antinociceptive properties were also identified through literature review with a total of 112 compounds. The last objective, which is to identify the active ingredients that have high affinity to the P2X4 receptor was achieved. All 112 active compounds were docked using Autodock Vina and the compounds with high affinity towards P2X4 were identified. The top six compounds that show high affinity to P2X4 are dioscin, ampelopsin F, corilagin, ellagic acid pentoside, punicalin and proanthocyanidins. The compound that showed the highest potential based on its binding affinities is dioscin, with binding affinity of -9.87 kcal/mol. Further study in *in vivo* and *in vitro* method is recommended for validation as there is few studies on dioscin as antinociception.

ACKNOWLEDGEMENT

I would like to express my gratitude to my supervisor, Asst. Prof Dr Izzudin Ahmad Nadzirin for his guidance and assistance during the study period.

REFERENCES

1. Ahmad Radzaudin (2021). Transliteration and Extraction of Medicinal Information in an Accordion-Folded Malay Medical Manuscript MSS 3048, and Medical Insight on Shingles Treatment.

2. Ahmad, I. Bin. (2018). Identification and structure activity relationship of small molecule Antagonists of the human P2X 4 receptor. Philosophy to the University of East Anglia

3. Benet, L. Z., Hosey-Cojocari, C., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. *Advanced Drug Delivery Reviews*, *101*, 89–98. https://doi.org/10.1016/j.addr.2016.05.007

4. Centers for Disease Control and Prevention. (2023, May 10). *Prevention and treatment (herpes zoster)*. Centers for Disease Control and Prevention. https://www.cdc.gov/shingles/about/treatment.html

5. *Katalog Manuskrip Melayu Koleksi Perpustakaan Negara Malaysia: Tambahan Keempat*. (2006). Perpustakaan Negara Malaysia.
6. Tsuda, M., Hidetoshi, T. S., & Inoue, K. (2012). P2X4R and P2X7R in neuropathic pain. *Wiley Interdisciplinary Reviews*, *1*(4), 513–521. https://doi.org/10.1002/wmts.47

7. Marahatha, R., Gyawali, K., Sharma, K., Gyawali, N., Tandan, P., Adhikari, A., Timilsina, G., Bhattarai, S., Lamichhane, G., Acharya, A., Pathak, I., Devkota, H. P., & Parajuli, N. (2021). Pharmacologic activities of phytosteroids in inflammatory diseases: Mechanism of action and therapeutic potentials. *Phytotherapy Research*, *35*(9), 5103–5124. https://doi.org/10.1002/ptr.7138

8. Peate, I. (2010). Preventing shingles: Symptoms, treatment and management. British Journal of Healthcare Assistants, 4(3), 120–123. https://doi.org/10.12968/bjha.2010.4.3.47061

9. Reza, H., Haq, W. M., Das, A. K., Rahman, S., Jahan, R., & Rahmatullah, M. (2011). Antihyperglycemic and antinociceptive activity of methanol leaf and stem extract of Nypa fruticans Wurmb. *Pakistan journal of pharmaceutical sciences*, *24*(4), 485–488.
10. Shaker, B., Ahmad, S., Lee, J., Jung, C., & Na, D. (2021). In silico methods and tools for Drug Discovery. *Computers in Biology and Medicine*, *137*, 104851. https://doi.org/10.1016/j.compbiomed.2021.104851

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

12. Tsuda, M., Masuda, T., Tozaki-Saitoh, H., & Inoue, K. (2013). P2X4 receptors and neuropathic pain. *Frontiers in Cellular Neuroscience*, *7*. https://doi.org/10.3389/fncel.2013.00191

13. Wilson, M., Wilson, P.J.K. (2021). Shingles. In: Close Encounters of the Microbial Kind. Springer, Cham. https://doi.org/10.1007/978-3-030-56978-5_8

14. Zayuri (2020). Identification of P2X4 Inhibitor using *In-Silico* Method for Treatment of Multiple Sclerosis

15. Zhao, X., Yin, L., Fang, L., Xu, L., Sun, P., Xu, M., Liu, K., & Peng, J. (2018). Protective effects of dioscin against systemic inflammatory response syndromevia adjusting TLR2/MyD88/NF-κb signal pathway. *International Immunopharmacology*, *65*, 458–469. https://doi.org/10.1016/j.intimp.2018.10.036

APPENDIX A

Scientific name of plants and its active compounds obtained from literature.

No.	Scientific names	Active compounds	Mechanism of Action	References
1.	*Allium cepa*	Allicin	The compounds prevent the formation of pro-inflammatory messengers and inhibit COX-2 enzyme and LOX (lipoxygenase)	Tigu et al., 2021, Ranjan et al., 2014
		Alliin		
		β-sitosterol		
		Cycloartenol		
		Quercetin		
2.	*Areca catechu*	Arecaidine	*A. catechu* possesses significant anti-nociceptive effects. Its effect is closely related to suppression of COX-2 expression	Ghelardini et al., 2001, Ansari et al., 2021
		Arecaine		
		Arecoline		
		Choline		
		Gallic acid		
		Guvacine		
		Guvacoline		
3.	*Baeckea frutescens*	1,8-Cineole	Possess anti-inflammatory activity against lipopolysaccharide (LPS)-induced nitric oxide production in macrophage	Saad et al., 2021, Chen et al., 2008, Hou et al., 2017
		Baeckeol		
		Caryophyllene		
		Ethyl gallate		
		Oleanolic acid		
		P-cymene		
4.	*Capsicum*	Capsaicin	Reduced oedema generated by the carrageenan in rat paw	Da Silva Antonio et al., 2018, Hernandez-Ortega et al., 2012
		Capsiate		
		Dihydrocapsaicin		
		Nonivamide		

5.	*Ceriops tagal*	2-methyl-4-(3′ -phenylpropyl)	Inhibits the release of inflammatory mediators	Biswas et al., 2023
		5-nitro-2-aminobenzophenone thiosemicarbazone		
		α-guaiene		
		β-sitosterol		
		Betulinic acid		
		Lupeol		
		Tagalsin P		
6.	*Champeira*	β-carotene	Inhibit nuclear factor kappa light chain enhancer of activated B cells (NF-κB)	Ragasa et al., 2015
		Lutein		
		Phytol		
		Squalene		
7.	*Croton tiglium*	2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4- diazine	Inhibit the acetic acid-induced abdominal writhing in mice	Liu et al., 2012), (Wu et al., 2007, Dey et al., 2018
8.	*Curcuma longa*	Curcumin	Curcumin selectively inhibits lipoxygenase, phospholipase A2 and COX-2, but not COX-1	Eke-Okoro et al., 2018 Kalirajan et al., 2020
		Cyclocurcumin		
9.	*Cyclea laxiflora*	Dicentrine	Inhibit nociceptive response of transient receptor potential ankyrin 1 (TRPA1)	Lemmens & Horsten, 2022, Montrucchio et al., 2013
10.	*Dipterocarpus*	Ampelopsin F	Inhibit the production of inflammatory cytokines	Wan Mohd Zain et al., 2021, Toan Phan et al., 2015, Aslam et al., 2015
		Davidiol A		
		E-viniferin		
		Laevifonol		
		Myricetin		
		Myricitrin		
		Scopoletin		

		Stenophyllol B		
		Tiliroside		
11.	*Eurphobia hirta*	Afzelin	Reduce inflammation in rat's ear	Al-Snafi, 2017, Vallisuta & Olimat, 2012, Kumar et al., 2010
		β-sitosterol		
		Campesterol		
		Cycloartenol		
		Euphorbin E		
		Stigmasterol		
12.	*Hedyotis phillipensis*	Asperuloside	Studies have shown this compound possesses anti-inflammatory activity. A study in treating rheumatoid arthritis done by Li et al. has found that the compound exhibited an inhibitory effect on the release of TNF	Mahbob et al., 2014
13.	*Mangifera foetida*	Daidzein	Inhibit acetic-acid writhing test in mice	Khoo et al., 2016, Fitmawati et al., 2019), Chang et al., 2020
		Genistein		
		Mangiferin		
14.	*Myristica fragrans*	Myristicin	Inhibit the release of interleukin-1β and interleukin-8 by resident peritoneal cells or to suppression of prostaglandins and bradykinin	Hayfaa et al., 2013, Santos et al., 2013
		Nordihydroguaiaretic acid		
		Trimyristin		
15.	*Nypa fruticans*	β-sitostenone	The extract inhibits prostaglandin synthesis and downregulate the expression of transient receptor potential vanilloid 1 (TRPV1)	Kang & Hyun, 2020,Reza et al., 2011
		β-sitosterol		
		Chlorogenic acid		
		Daucosterol		
		Dioscin		
		Diosgenin		
		Kaempferol		
		Protocatechuic acid		
		Stigmasta-4, 22- dien-3-one		

		Stigmasterol		
16.	*Oryza sativa*	Catechin	Rice bran oil reduces the inflammation by interfering the arachidonate metabolites	Tyagi et al., 2022, Ghasemzadeh et al., 2018, Ashraf et al., 2019
		Cinnnamic acid		
		GABA		
		Tocotrienol		
		Myricetin		
		Oryzanol		
		P-coumaric acid		
		Quercetin		
17.	*Piper betel*	4- allyl phenyl acetate	inhibit the increase of the intracellular Ca2+ through transient receptor potential ankyrin 1 (TRPA1)	Alam et al., 2013, Sakinah et al., 2020, Chan & Wong, 2014
		4-allylphenol		
		β-sitosterol		
		Cepharadione		
		Chavibetol		
		Eugenol		
		Hydroxychavicol		
		Piperine		
18.	*Pisonia grandis*	α-spinasterol	Inhibit the release of inflammatory mediators	(Radha et al., 2008) (Shubashini et al., 2011)
		Allantoin		
		β-sitosterol		
		β-sitosterolglucoside		
		Dulcitol		
		Pinitol		
19.	*Pterocarpus*	Calocedrin	Inhibit production of inflammatory mediators like histamine, cyclooxygenase, and arachidonic acid metabolites	Bulle et al., 2016, Navada & Vittal, 2014, Kumar, 2011
		Pterocarpol		
		Pterolinus L		
		Santalin A		

		Santalin B		
		Savinin		
20.	*Punica granatum*	Anthocyanins	Ellagic acid's antinociceptive effect could be due to COX inhibition. Anthocyanin inhibits prostaglandin synthesis and suppresses COX2 transcription.	Saad et al., 2014 Guerrero-Solano et al., 2020, Yu et al., 2021
		Corilagin		
		Ellagic acid hexoside		
		Ellagic acid pentoside		
		Gallic acid		
		Proanthocyanidins		
		Punicalagin		
		Punicalin		
		Punicic acid		
21.	*Quercus infectoria*	Ellagic acid	Increase reaction time of rats towards stimulus	Fan et al., 2014, Elham et al., 2021
		Gallic acid		
		Syringic acid		
22.	*Schima wallichii*	Coumarin	Inhibit the release of inflammatory mediators	Banjara et al., 2022, Dewanjee et al., 2009
		Terpenoid		

APPENDIX B

Binding affinity arranged from highest to lowest.

Number	Compounds	Binding affinity (kcal/mol)			
		Run 1	Run 2	Run 3	Average
1	Dioscin	-9.7	-9.9	-10.9	-9.87
2	Ampelopsin F	-9.7	-9.3	-9.4	-9.47
3	Corilagin	-8.7	-11.0	-8.6	-9.43
4	Ellagic acid pentoside	-9.1	-9.6	-9.6	-9.43
5	Punicalin	-9.3	-9.3	-9.2	-9.27
6	Proanthocyanidins	-10.3	-9.4	-7.9	-9.20
7	Tiliroside	-8.9	-9.3	-9.3	-9.17
8	Cepharadione	-9.0	-9.0	-9.0	-9.00
9	Ellagic acid hexoside	-9.0	-9.0	-8.8	-8.93
10	Stenophyllol B	-9.2	-8.3	-9.0	-8.83
11	Punicalagin	-8.7	-8.9	-8.9	-8.83
12	Oryzanol	-8.8	-8.7	-8.6	-8.70
13	Cycloartenol	-8.7	-8.7	-8.7	-8.70
14	Cycloartenol	-8.7	-8.7	-8.7	-8.70
15	β-sitosterol	-8.9	-8.3	-8.5	-8.57
16	β-sitosterol	-8.9	-8.3	-8.5	-8.57
17	β-sitosterol	-8.9	-8.3	-8.5	-8.57
18	Stigmasterol	-8.6	-8.5	-8.6	-8.57
19	β-sitosterol	-8.9	-8.3	-8.5	-8.57
20	Stigamasterol	-8.6	-8.5	-8.6	-8.57
21	β-sitosterol	-8.9	-8.3	-8.5	-8.57
22	β-sitosterol	-8.9	-8.3	-8.5	-8.57
23	Calocedrin	-8.6	-8.2	-8.7	-8.50
24	Diosgenin	-8.5	-8.5	-8.4	-8.47
25	Savinin	-8.5	-8.5	-8.4	-8.47
26	Betulinic acid	-8.4	-8.4	-8.4	-8.40
27	E-viniferin	-8.2	-8.5	-8.5	-8.40
28	Laevifonol	-8.4	-8.3	-8.5	-8.40
29	Campesterol	-8.4	-8.4	-8.4	-8.40
30	Stigmasta-4, 22- dien-3-one	-7.7	-8.9	-8.6	-8.40
31	Lupeol	-8.4	-8.3	-8.4	-8.37
32	Santalin A	-8.4	-8.4	-8.3	-8.37
33	Santalin B	-8.4	-8.4	-8.3	-8.37
34	α-spinasterol	-8.3	-8.1	-8.6	-8.30
35	Davidiol A	-8.1	-8.4	-8.4	-8.30
36	Myricitrin	-8.4	-8.1	-7.9	-8.13

37	Cyclocurcumin	-8.5	-8.0	-7.8	-8.10
38	β-sitostenone	-7.8	-8.6	-7.9	-8.10
39	Tocotrienol	-8.0	-8.3	-8.0	-8.10
40	Dicentrine	-8.4	-7.9	-7.9	-8.07
41	Afzelin	-8.0	-8.1	-8.1	-8.07
42	Oleanolic acid	-8.0	-7.9	-8.0	-7.97
43	Tagalsin P	-7.9	-7.9	-7.9	-7.90
44	Asperuloside	-8.2	-7.6	-7.7	-7.83
45	β-sitosterolglucoside	-8.3	-7.3	-7.7	-7.77
46	Caryophyllene	-7.7	-7.7	-7.7	-7.70
47	Ellagic acid	-7.9	-7.9	-7.3	-7.70
48	Daucosterol	-7.7	-7.7	-7.6	-7.67
49	Euphorbin e	-7.4	-8.2	-7.2	-7.60
50	Mangiferin	-7.7	-7.6	-7.5	-7.60
51	Daidzein	-7.6	-7.6	-7.6	-7.60
52	Anthocyanins	-7.6	-7.6	-7.6	-7.60
53	Chlorogenic acid	-7.6	-7.5	-7.6	-7.57
54	Terpenoid	-7.7	-7.2	-7.8	-7.57
55	Nordihydroguaiaretic acid	-7.3	-7.8	-7.4	-7.50
56	Curcumin	-7.4	-7.5	-7.5	-7.47
57	Kaempferol	-7.4	-7.8	-7.5	-7.47
58	Quercetin	-7.1	-7.6	-7.6	-7.43
59	Genistein	-7.5	-7.4	-7.4	-7.43
60	Quercetin	-7.1	-7.6	-7.6	-7.43
61	Myricetin	-7.6	-7.0	-7.6	-7.40
62	Myricetin	-7.6	-7.0	-7.6	-7.40
63	Catechin	-7.3	-7.5	-7.3	-7.37
64	β-carotene	-7.3	-7.6	-7.2	-7.34
65	α-guaiene	-7.3	-7.3	-7.3	-7.30
66	Piperine	-7.1	-7.2	-7.1	-7.13
67	5-nitro-2-aminobenzophenone thiosemicarbazone	-7.0	-7.1	-7.0	-7.03
68	Pterocarpol	-7.1	-6.9	-7.1	-7.03
69	Pterolinus L	-6.9	-7.0	-6.9	-6.93
70	Squalene	-6.6	-7.1	-6.9	-6.87
71	Capsiate	-6.8	-6.7	-6.4	-6.63
72	2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4- diazine	-6.8	-6.5	-6.5	-6.60
73	Scopoletin	-6.6	-6.6	-6.6	-6.60
74	Capsaicin	-6.4	-6.5	-6.6	-6.50
75	Dihydrocapsaicin	-6.5	-6.5	-6.5	-6.50
76	Allantoin	-6.5	-6.5	-6.5	-6.50
78	Nonivamide	-6.7	-6.4	-6.3	-6.47

79	2-methyl-4-(3′ -phenylpropyl)	-6.4	-6.4	-6.4	-6.40
80	Gallic acid	-6.3	-6.3	-6.3	-6.30
81	Ethyl gallate	-6.3	-6.3	-6.3	-6.30
82	Hydroxychavicol	-6.3	-6.3	-6.3	-6.30
83	Gallic acid	-6.3	-6.3	-6.3	-6.30
84	Gallic acid	-6.3	-6.3	-6.3	-6.30
85	Baeckeol	-6.2	-6.2	-6.3	-6.23
86	Phytol	-6.4	-6.1	-6.1	-6.20
87	Chavibetol	-6.2	-6.2	-6.2	-6.20
88	Trimyristin	-6.6	-5.4	-6.5	-6.17
89	Cinnnamic acid	-6.1	-6.1	-6.1	-6.10
90	4- allyl phenyl acetate	-6.1	-6.1	-6.1	-6.10
91	Eugenol	-6.1	-6.1	-6.1	-6.10
92	Pinitol	-6.1	-6.1	-6.1	-6.10
93	Myristicin	-6.0	-6.0	-6.0	-6.00
94	Protocatechuic acid	-6.0	-6.0	-6.0	-6.00
95	P-coumaric acid	-6.0	-6.0	-6.0	-6.00
96	Coumarin	-5.9	-5.9	-5.9	-5.90
97	Punicic acid	-6.0	-5.7	-6.0	-5.90
98	P-cymene	-5.8	-5.8	-5.8	-5.80
99	4-allylphenol	-5.8	-5.8	-5.8	-5.80
100	Syringic acid	-5.8	-5.8	-5.8	-5.80
101	1,8-Cineole	-5.7	-5.7	-5.7	-5.70
102	Dulcitol	-5.5	-5.6	-5.4	-5.50
103	Arecaine	-5.4	-5.5	-5.5	-5.47
104	Guvacine	-5.3	-5.3	-5.3	-5.30
105	Arecaidine	-5.2	-5.2	-5.2	-5.20
106	Arecoline	-5.1	-5.1	-5.1	-5.10
107	Guvacoline	-5.1	-5.1	-5.1	-5.10
108	Alliin	-4.9	-5.1	-5.1	-5.03
109	Lutein	-0.9	-6.0	-6.4	-4.43
110	Allicin	-4.3	-4.3	-4.3	-4.30
111	GABA	-4.1	-4.1	-4.1	-4.10
112	Choline	-3.6	-3.6	-3.6	-3.60