https://doi.org/10.48048/tis.2023.6395

Efficiency of Nitrification Inhibitor on Designing Nitrogen Fertilizer by Neem Compounds Based on Molecular Docking

Kharmila Rahmadani[1], Baso Manguntungi[2,*], Arwansyah[3], Oslan Jumadi[4], Muhammad Aziz Khizbullah[1], Alfin Hidayat[1], Nayeng Githa Aiyodya Ayunda[1], Muhammad Faiz[1], Leggina Rezzy Vanggy[1] and Ermin Septiawati[1]

[1]Department of Biotechnology, Faculty of Life Sciences and Technology, Sumbawa University of Technology, Sumbawa Besar, Indonesia
[2]Department of Biology Education, Universitas Sulawesi Barat, Majene, Indonesia
[3]Department of Chemistry Education, Faculty of Teacher Training and Education, Tadulako University, Palu, Indonesia
[4]Department of Biology, Universitas Negeri Makassar, Makassar, Indonesia

([*]Corresponding author's e-mail: manguntungibaso@unsulbar.ac.id)

Received: 5 September 2021, Revised: 28 October 2021, Accepted: 5 December 2021, Published: 24 November 2022

Abstract

In the agricultural trade, nitrogen fertilizer is one among the popular fertilizers within the world market. However, the high use of nitrogen fertilizers causes negative impacts within the type of environmental pollution, each in water, soil, and within the air. Nitrification inhibitor required to enhance the potency of nitrogen fertilizer use to realize additional economical production of food crops and minimize fertilizer related pollution of the environment. Neem (*Azadirachta indica*) act as an inhibitor nitrification that contained high tannin and the other secondary metabolite compounds. Neem plants encapsulated by urea as a nitrification inhibitor can increase nitrogen efficiency in the soil during fertilization. This research is a computational experiment research in solving problems using Molecular Docking Analysis. Molecular Docking method was performed using AutoDock Vina Tools 1.5.6 and Open Babel 2.4.1 Program. The use of molecular docking aims to predict ligand bonds and target proteins that focus on affinity and bond interactions. The high and low affinity of ligand-protein bonds is influenced by free bond energies, surface interactions, and intermolecular interactions. The results represent Neem (*A. Indica*) contains high tannin and the other secondary metabolite compounds (Nimbin, Nimbidin, Nimbic Acid, Nimbidinin, Nimbinin, Azadirachtin, Diepoxy Azadiradione, Dyhidrogedunin, Gallic Acid and Gedunin). Those metabolite compounds can inhibiting 4 enzymes such as methane monooxygenase, Hydroxylamine oxidoreductase, Nitric oxide reductase and Nitrite Reductase. From 10 metabolite compounds, Diepoxy Azadiradione and Gedunin may become the most stable complex that indicated become strength binding energy based on the lowest energy score of −10.3 kcal/mol.

Keywords: Nitrogen fertilizer, Neem, Nitrification inhibitor, Molecular docking, Binding energy

Introduction

In West Nusa Tenggara, agriculture is the leading sector of the existing seventeen sectors. Sumbawa Regency has a high contribution in the agricultural sector, amounting to 40.17 percent of the total 10 districts in NTB [1]. Agriculture in Sumbawa is well known as a corn production center (*Zea mays*) which supports the economic sector in Indonesia [2]. From 2008 to 2018, one of these mainstay sectors developed rapidly with an average growth rate of 30.7 percent per year [1]. However, agricultural production land in Sumbawa is not available so that much of the available agricultural land comes from converted forest land [3].

The increasing need for agricultural land is followed by the high use of urea fertilizer due to the lack of knowledge of the Sumbawa corn farmers regarding the negative impacts it has on the environment. Low fertilization efficiency is a crucial problem. This is indicated by the amount of nitrogen wasted in the process of ammonia volatilization, denitrification, surface runoff and nitrate leaching [4]. Fertilization inefficiency also causes an increase in the rate of greenhouse gas emissions through N_2O pollution [5]. The main factor in the inefficiency of nitrogen fertilization can be seen from the nitrification process of ammonium (NH_4^+) to nitrite (NO_2^-) to nitrate (NO^3). The conversion of these ions produces a secondary

product in the form of N_2O gases [5]. So that only 20 - 30 % of nitrogen is absorbed by maize plants while the rest is lost in the form of nitrates and nitrifying gas [6,7].

Based on these problems, plants that contain natural ingredients are needed to inhibit nitrification. Neem (*Azadirachta indica*) contains high tannin and the other secondary metabolite compounds [8]. Neem plants encapsulated by urea as a nitrification inhibitor can increase nitrogen efficiency in the soil during fertilization. Through the urea encapsulation mechanism with neem plants, the active ingredients of secondary metabolite will bind and inhibit the release of excessive urea in the soil and air. The inhibition of this nitrification process will reduce the percentage of N_2O gas in the air produced from the nitrification process itself or from the denitrification process and can increase the existence of the amount of ammonium contained in the soil. This innovation is a development of previous research using neem as an inhibitor of nitrification [9].

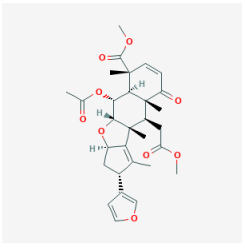
This research is a computational experiment research in solving problems using molecular docking analysis. Molecular docking plays an important role in ligand-protein docking. This method is effective for finding the active site of the enzyme to be inhibited. The interaction and affinity of metabolites in inhibiting target proteins can be an indication in predicting which compounds are most appropriate as natural nitrification inhibitors. This research is supported by basic bioinformatic data in order to increase the accuracy of the resulting data. The use of Molecular Docking-based on bioinformatic data to validate the types of secondary metabolites that can inhibit the enzyme action in the nitrification process. The neem encapsulated urea product based on nitrification inhibitors will be a superior product while maximizing the natural potential of Sumbawa Regency.

Materials and methods

Molecular docking simulation

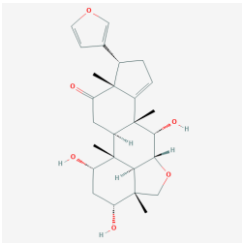
In this present investigation, molecular docking method is employed to obtain the binding pose of ligand into the catalytic site of receptor. AutoDock Vina package developed by Trott and co-workers [10] is applied to perform molecular docking on the ligand-receptor complex. All ligands are downloaded and saved to sdf extension. Open Babel 2.4.1 program packages are utilized to convert sdf files to pdbqt format. In receptor preparation, the tertiary structures of 4 enzymes that play a role in the nitrification process such as (a) Methane Monooxygenase, (b) Hydroxylamine Oxidoreductase, (c) Nitric Oxide Reductase, and (d) Nitrite Reductase are retrieved from RSCB database with PDB ID: 3RGB, 1FGJ, 3o0R, and 1H9Y, respectively (**Figure 2**). The polar hydrogen and Kollman's united atom charges are inserted to the receptor. Afterward, all enzymes are saved in pdbqt format. Molecular docking is performed by setting the grid box parameter. This parameter is required to decide for positional and rotational of the ligand into the site of the receptor. The grid box, including The grid box center (x, y, z), and spacing point for all systems are listed in **Table 1**. The exhaustiveness is set to 100. Other parameters are computed as the default of AutoDock Vina. The Broyden-Fletcher-Goldfarb Shanno (BFGS) algorithm is used for searching parameters. All parameters are created by using AutoDock Tools 1.5.6 developed by Morris and co-workers [11].

PubChem CID: 108058



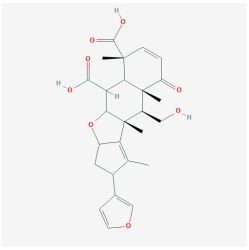
Structure of Nimbin

PubChem CID: 319426343



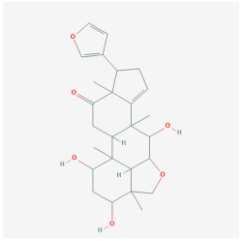
Structure of Nimbidinin

PubChem CID: 25446



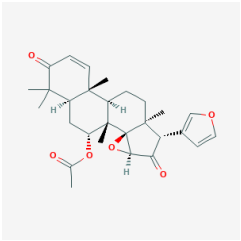
Structure of Nimbic Acid

PubChem CID: 169088



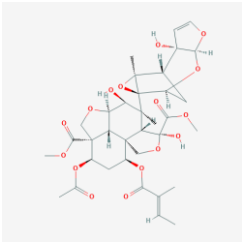
Structure of Nimbidin

PubChem CID: 49863985



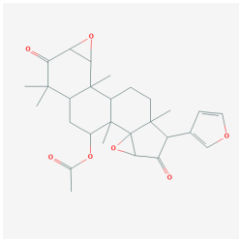
Structure of Nimbinin

PubChem CID: 5281303



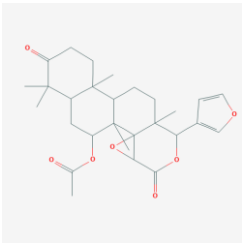
Structure of Azadirachtin

PubChem CID: 336301



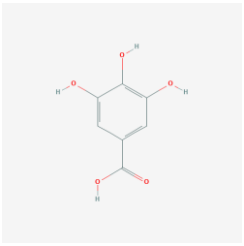
Structure of Diepoxy Azadiradione

PubChem CID: 542117



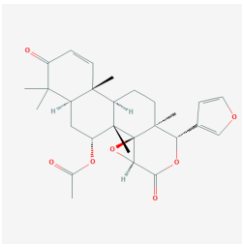
Structure of Dihydrogedunin

PubChem CID: 370



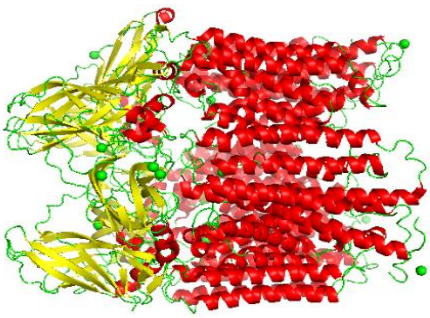
Structure of Gallic Acid

PubChem CID: 12004512

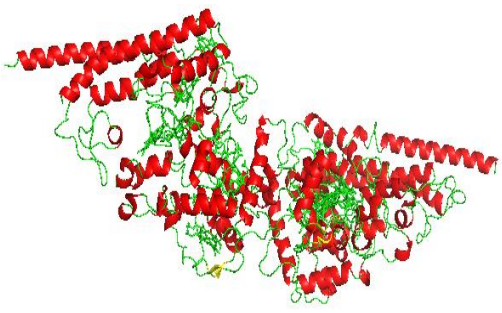


Structure of Gedunin

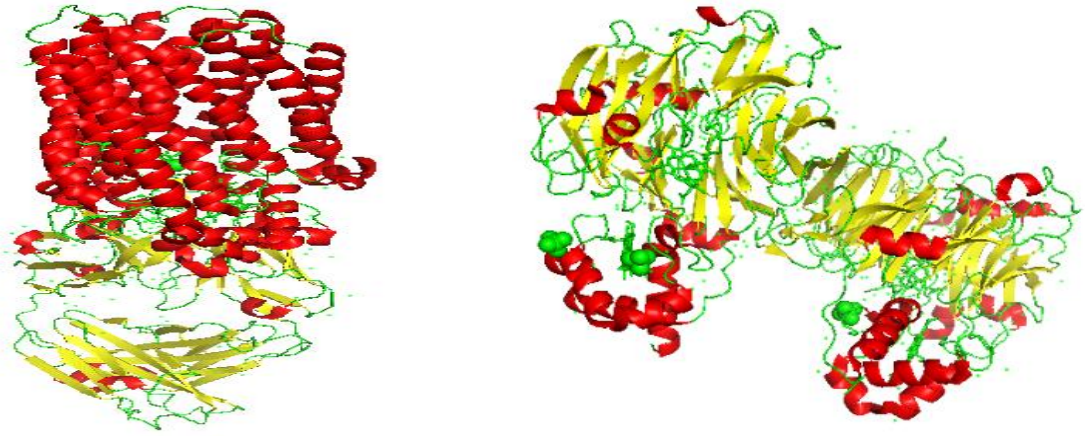
Figure 1 Ten chemicals structure of Neem compounds with highest energy binding score.
Source: https://pubchem.ncbi.nlm.nih.gov/.https://pubchem.ncbi.nlm.nih.gov/.



(a)



(b)



(c) (d)

Figure 2 The tertiary structure of enzymes; (a) Methane monooxygenase, (b) Hydroxylamine oxidoreductase, (c) Nitric oxide reductase and (d) Nitrite reductase. The structures of alpha-helix and beta-sheet are presented by red and green colors in cartoon models, respectively.

Table 1 The identity of box size for ligand/receptor complex.

No	Enzyme	Grid box size	The grid box center (x,y,z)	Spacing (Å)
1	Methane monooxygenase	50.464×129.509×47.846	106×86×74	1
2	Hydroxylamine oxidoreductase	29.363×22.795×26.701	72×40×126	1
3	Nitric oxide reductase	25.135×−1.105×21.846	64×126×86	1
4	Nitrite reductase	99.316×52.826×217.793	52×66×78	1

Results and discussion

Results

a) Inhibiting methane monooxygenase enzyme by *A. indica* metabolite

In order to analyze the stability of the complex, including the binding pocket of ligands into the moiety of receptors, molecular docking is performed by using AutoDock Vina 4 packages. In **Table 2**, we show the binding energy of 10 ligands in binding with Methane monooxygenase (receptor).

Table 2 The binding energy of ligands in complex with Methane monooxygenase enzyme obtained by molecular docking.

No	Compound	Binding energy (Kcal/mol)
1	Nimbin	−8.4
2	Nimbidin	−9.9
3	Nimbic acid	−9.1
4	Nimbidinin	−10.2
5	Nimbinin	−9.7
6	Azadirachtin	−9.3
7	Diepoxy azadiradione	−10.3
8	Dyhidrogedunin	−9.7
9	Gallic acid	−6.5
10	Gedunin	−10.3

From this table, all models have negative binding energies suggesting those ligands make a complex to the site of the receptor. Also, models 7 and 10 may become the most stable complex based on the lowest energy score of −10.3 kcal/mol. Further, the catalytic site of the receptor obtained by molecular docking can be predicted by evaluating the binding site of the ligand in complex with the receptor.

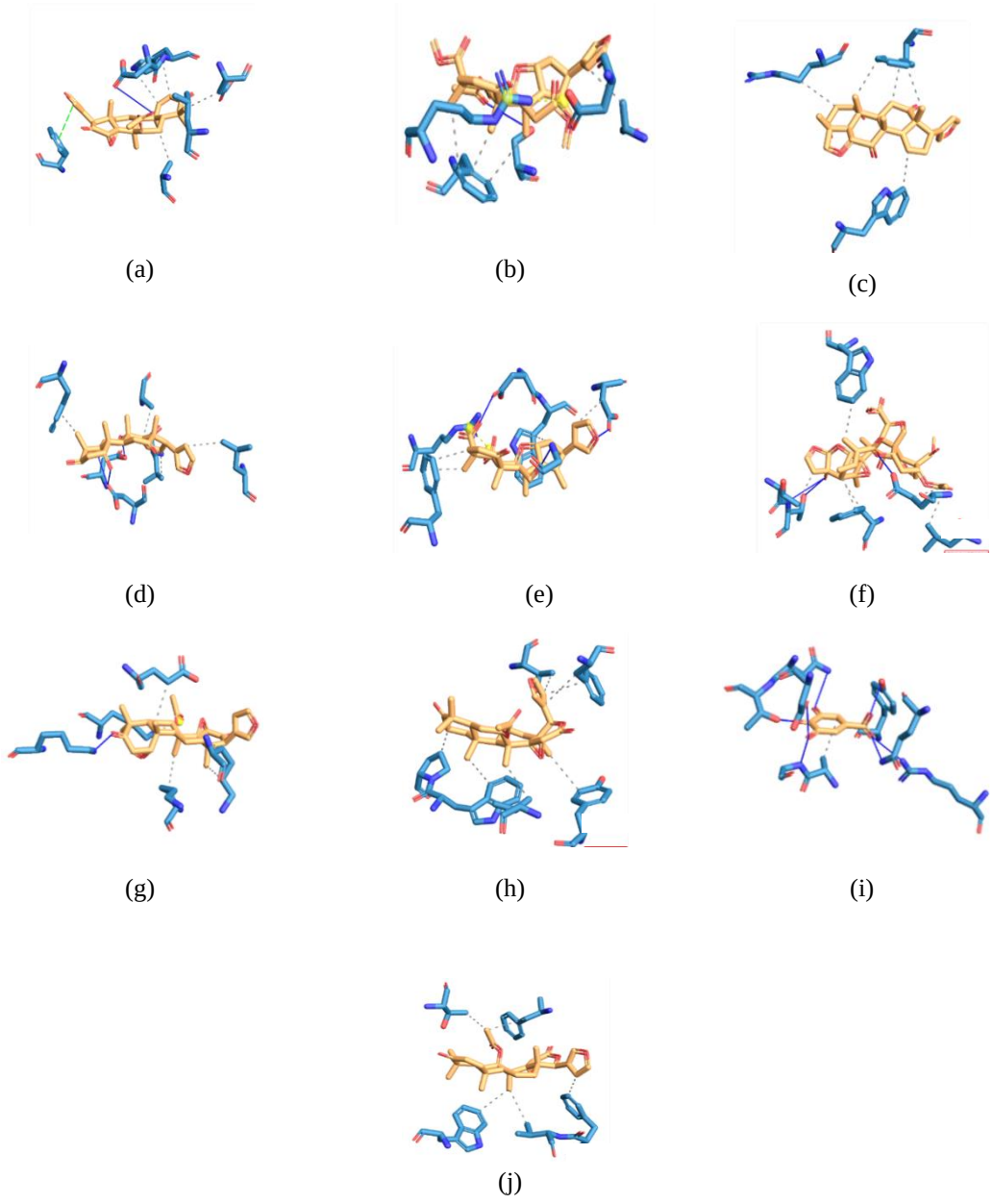


Figure 3 The binding pose of ligands to the site of Methane monooxygenase enzyme (a) Nimbin, (b) Nimbinin, (c) Nimbidin, (d) Nimbidinin, (e) Nimbic acid, (f) Azadirachtin, (g) Azadiradione, (h) Dihydrogedunin, (i) Gallic acid and (j) Gedunin.

Table 3 Hydrogen bonds between ligands and Methane monooxygenase enzyme obtained by molecular docking.

No.	Compounds	Residue	Distance H-A	Distance D-A
1	Nimbin	53F	3.06	3.39
		113E	3.65	4.02
2	Nimbinin	53F	3.60	4.04
3	Nimbidin		No hydrogen bond	
4	Nimbidinin	53F	2.59	3.33
		53F	3.11	4.5
		182F	2.60	3.36
5	Nimbic Acid	53J	2.79	3.12
		56J	2.46	3.35
		267I	3.09	3.80
6	Azadirachtin	215A	3.18	4.08
		215A	2.72	3.57
		215A	2.99	3.57
		447A	3.43	3.84
7	Azadiradione	77A	2.92	3.39
8	Dihydrogedunin		No hydrogen bond	
9	Gallic Acid	33A	2.20	2.92
		41A	1.98	2.81
		59A	2.21	2.99
		61A	2.52	3.26
		62A	2.09	2.84
		187A	3.11	3.73
		236A	1.84	2.81
10	Gedunin		No hydrogen bond	

The binding site, including the summary of the hydrogen bonds of ligands to the site of the receptor is provided in **Figure 3** and **Table 3**, respectively. From these results, the ligands, i.e. Nimbin, Nimbinin, and Nimbidinin, participate in hydrogen bonds with the similar amino acid of 53F. This finding suggests that the region of residue 53F may be assumed as the catalytic site of Methane Monooxygenase.

b) Inhibiting hydroxylamine oxidoreductase enzyme by *A. indica* metabolite

In **Table 4**, we show the binding energy of 10 ligands in binding with Hydroxylamine Oxidoreductase (receptor).

Table 4 The binding energy of ligands in complex with Hydroxylamine Oxidoreductase enzyme obtained by molecular docking.

No.	Compound	Binding energy (Kcal/mol)
1	Nimbin	−8.1
2	Nimbidin	−8.6
3	Nimbic acid	−8.5
4	Nimbidinin	−9.4
5	Nimbinin	−8.9
6	Azadirachtin	−8.5
7	Diepoxy azadiradione	−9.3
8	Dyhidrogedunin	−8.6
9	Gallic acid	−6.9
10	Gedunin	−9.0

From this table, all models have negative binding energies suggesting those ligands make a complex to the site of the receptor. Also, model 4 may become the most stable complex based on the lowest energy score of −9.4 kcal/mol. Further, the catalytic site of the receptor obtained by molecular docking can be predicted by evaluating the binding site of the ligand in complex with the receptor.

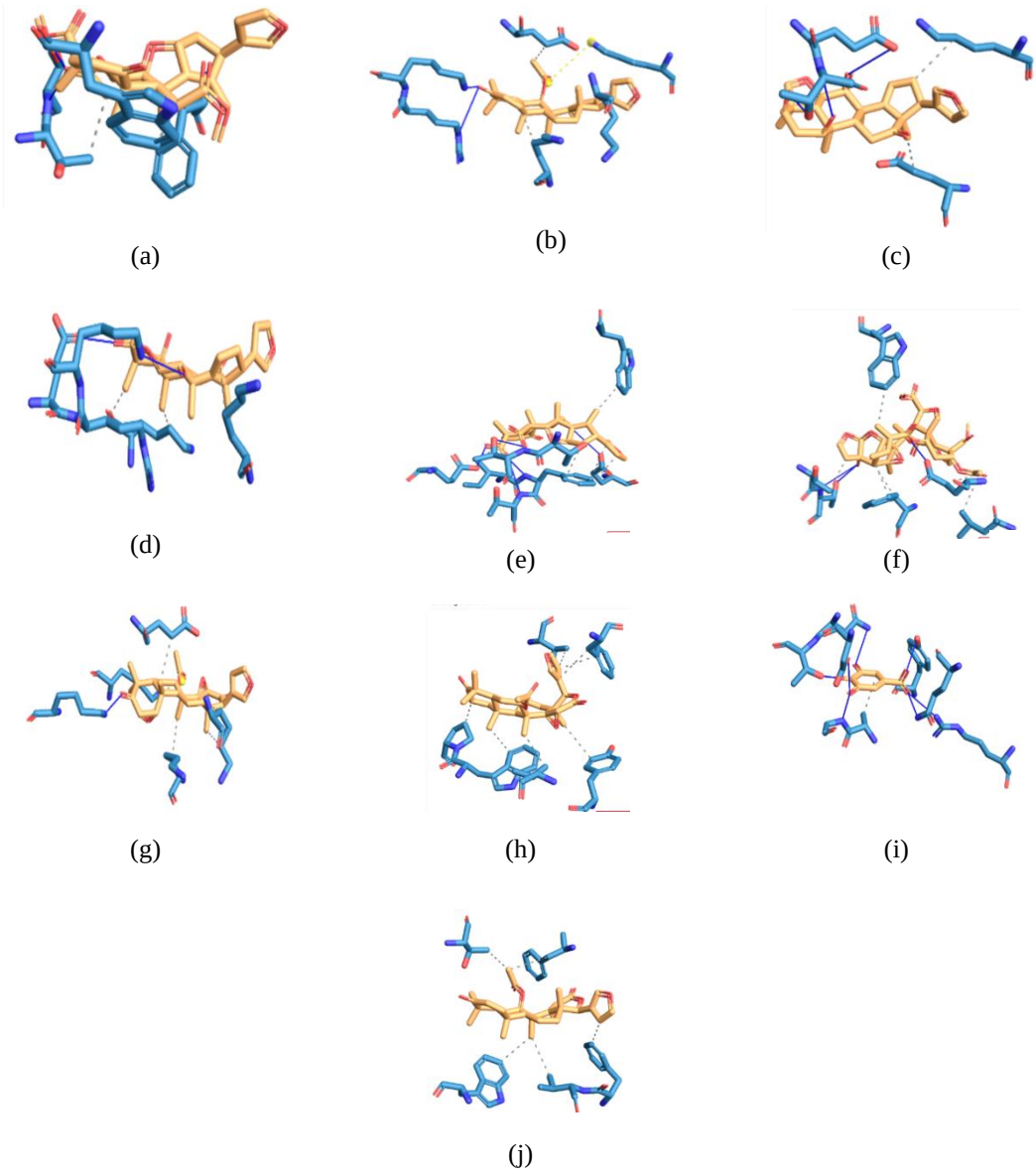


Figure 4 The binding pose of ligands to the site of hydroxylamine oxidoreductase enzyme (a) Nimbin, (b) Nimbinin, (c) Nimbidin, (d) Nimbidinin, (e) Nimbic acid, (f) Azadirachtin, (g) Azadiradione, (h) Dihydrogedunin, (i) Gallic acid and (j) Gedunin.

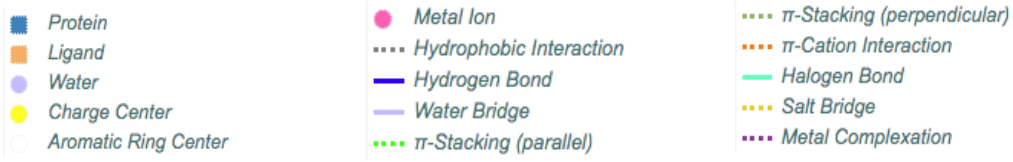


Table 5 Hydrogen bonds between ligands and hydroxylamine oxidoreductase enzyme obtained by molecular docking.

No.	Compounds	Residue	Distance H-A	Distance D-A
1	Nimbin	215B	2.07	3.00
		215B	2.11	3.09
2	Nimbinin	76A	3.66	4.02
		77A	3.09	3.68
3	Nimbidin	138B	3.54	3.99
		138B	2.80	3.33
		138B	2.46	3.40
4	Nimbidinin	75A	2.31	2.74
		77A	2.75	3.67
5	Nimbic Acid	215A	3.75	4.07
		417A	1.78	2.75
		424A	2.43	3.03
		425A	2.70	3.66
		447A	3.39	3.93
6	Azadirachtin	215A	3.18	4.08
		215A	2.72	3.57
		215A	2.99	3.57
		447A	3.43	3.84
7	Azadiradione	77A	2.92	3.39
8	Dihydrogedunin		No hydrogen bond	
9	Gallic Acid	33A	2.20	2.92
		41A	1.98	2.81
		59A	2.21	2.99
		61A	2.52	3.26
		62A	2.09	2.84
		187A	3.11	3.73
		236A	1.84	2.81
10	Gedunin		No hydrogen bond	

The binding site, including the summary of the hydrogen bonds of ligands to the site of the receptor is provided in **Figure 4** and **Table 5**, respectively. From these results, the ligands, i.e., Nimbinin, Nimbidinin, and Azadiradione, participate in hydrogen bonds with the similar amino acid of 77A. This finding suggests that the region of residue 77A may be assumed as the catalytic site of Hydroxylamine Oxidoreductase.

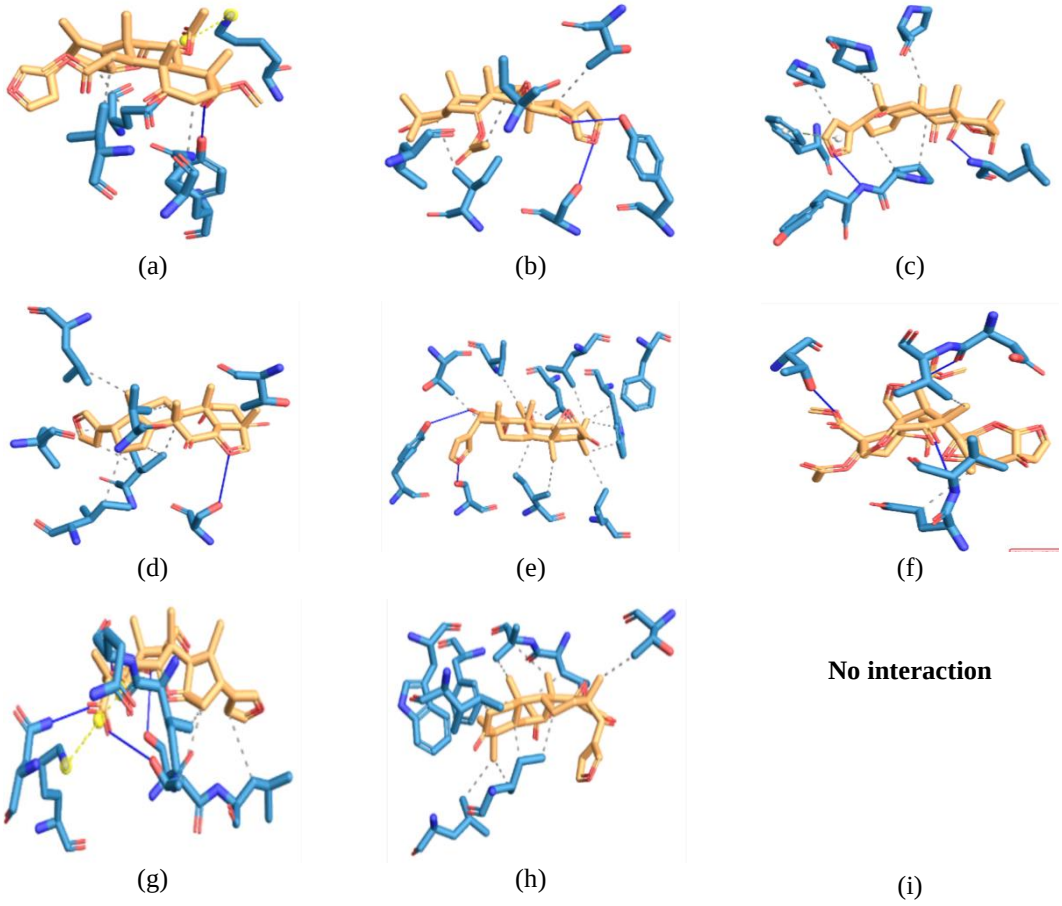
c) Inhibiting nitric oxide reductase enzyme by *A. indica* metabolite

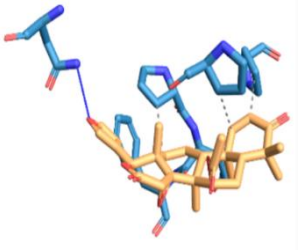
In **Table 6**, we show the binding energy of 10 ligands in binding with Nitric Oxide Reductase (receptor).

Table 6 The binding energy of ligands in complex with nitric oxide reductase enzyme obtained by molecular docking.

No.	Compound	Binding energy (Kcal/mol)
1	Nimbin	−7.5
2	Nimbidin	−8.4
3	Nimbic acid	−7.8
4	Nimbidinin	−8.3
5	Nimbinin	−7.8
6	Azadirachtin	−7.4
7	Diepoxy azadiradione	−8.7
8	Dyhidrogedunin	−8.1
9	Gallic acid	−6.3
10	Gedunin	−8.2

From this table, all models have negative binding energies suggesting those ligands make a complex to the site of the receptor. Also, model 7 may become the most stable complex based on the lowest energy score of −8.7 kcal/mol. Further, the catalytic site of the receptor obtained by molecular docking can be predicted by evaluating the binding site of the ligand in complex with the receptor.





(j)

Figure 5 The binding pose of ligands to the site of nitric oxide reductase enzyme (a) Nimbin, (b) Nimbinin, (c) Nimbidin, (d) Nimbidinin, (e) Nimbic acid, (f) Azadirachtin, (g) Azadiradione, (h) Dihydrogedunin, (i) Gallic acid and (j) Gedunin.

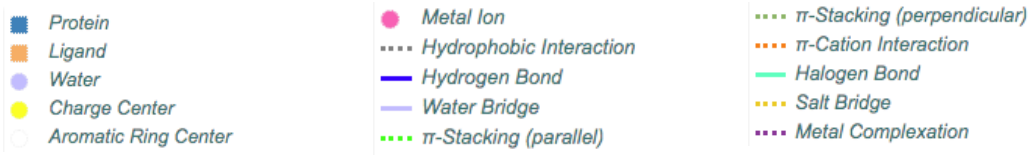


Table 7 Hydrogen bonds between ligands and nitric oxide reductase enzyme obtained by molecular docking.

No.	Compounds	Residue	Distance H-A	Distance D-A
1	Nimbin	87L	2.57	2.94
2	Nimbinin	14C	2.41	3.12
		18C	2.54	3.09
3	Nimbidin	114B	3.09	3.96
		117B	2.42	3.18
4	Nimbidinin	18C	3.26	4.06
5	Nimbic Acid	52L	2.31	3.10
		53L	3.17	3.90
		102C	2.67	3.08
		105C	2.63	2.98
6	Azadirachtin	2H	3.37	3.87
		43L	2.69	3.34
		112H	2.37	2.95
7	Azadiradione	14C	3.17	3.98
8	Dihydrogedunin		No hydrogen bond	
9	Gallic Acid		No interaction	
10	Gedunin	54B	2.30	3.25

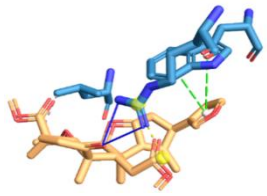
The binding site, including the summary of the hydrogen bonds of ligands to the site of the receptor, is provided in **Figure 5** and **Table 7**, respectively. From these results, the ligands, i.e., Nimbinin, Nimbidinin and Azadiradione, participate in hydrogen bonds with the similar amino acid of 18C. This finding suggests that the region of residue 18C may be assumed as the catalytic site of Nitric Oxide Reductase.

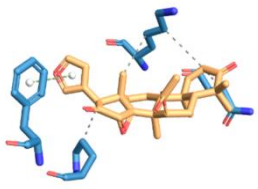
d) Inhibiting nitrite reductase enzyme by *A. indica* Metabolite
In **Table 8**, we show the binding energy of 10 ligands in binding with Nitrite Reductase (receptor).

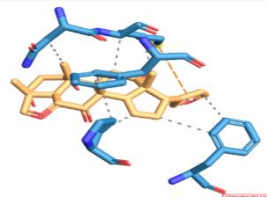
Table 8 The binding energy of ligands in complex with nitrite reductase enzyme obtained by molecular docking.

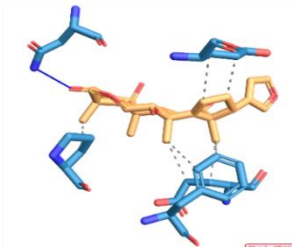
No.	Compound	Binding energy (Kcal/mol)
1	Nimbin	−7.6
2	Nimbidin	−8.3
3	Nimbic acid	−8.0
4	Nimbidinin	−8.3
5	Nimbinin	−8.0
6	Azadirachtin	−7.2
7	Diepoxy azadiradione	−7.6
8	Dyhidrogedunin	−7.9
9	Gallic acid	−7.3
10	Gedunin	−8.0

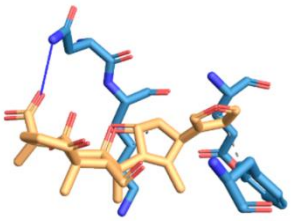
From this table, all models have negative binding energies suggesting those ligands make a complex to the site of the receptor. Also, model 2 and 4 may become the most stable complex based on the lowest energy score of −8.3 kcal/mol. Further, the catalytic site of the receptor obtained by molecular docking can be predicted by evaluating the binding site of the ligand in complex with the receptor.

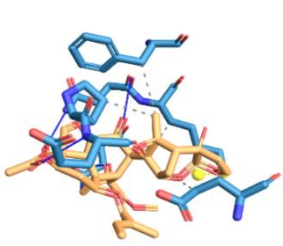

(a)

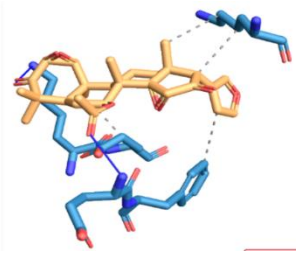

(b)

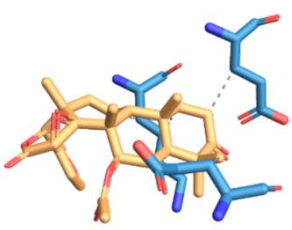

(c)

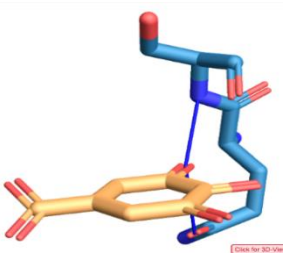

(d)

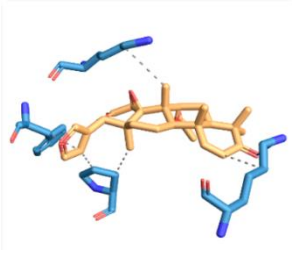

(e)


(f)


(g)


(h)


(i)



(j)

Figure 6 The binding pose of ligands to the site of nitrite reductase enzyme (a) Nimbin, (b) Nimbinin, (c) Nimbidin, (d) Nimbidinin, (e) Nimbic Acid, (f) Azadirachtin, (g) Azadiradione, (h) Dihydrogedunin, (i) Gallic acid and (j) Gedunin.

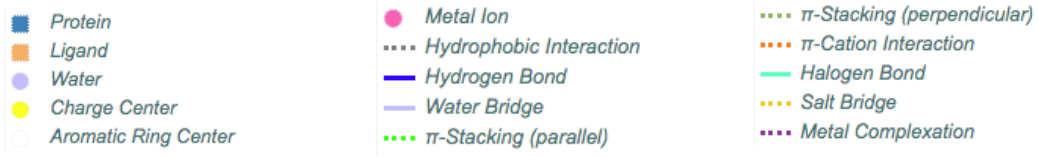


Table 9 Hydrogen bonds between ligands and Nitrite Reductase enzyme obtained by molecular docking.

No.	Compounds	Residue	Distance H-A	Distance D-A
1	Nimbin	174B	2.03	2.92
		174B	2.15	3.00
2	Nimbinin		No hydrogen bond	
3	Nimbidin		No hydrogen bond	
4	Nimbidinin	58A	2.84	3.80
5	Nimbic Acid	58A	2.04	2.96
6	Azadirachtin	55A	3.63	4.08
		58A	2.78	3.22
		58A	2.65	3.53
		136A	2.44	3.26
7	Azadiradione	136A	2.02	2.93
		140A	2.93	3.03
8	Dihydrogedunin		No hydrogen bond	
9	Gallic Acid	282B	2.06	2.97
		283B	2.66	3.15
10	Gedunin	140A	2.32	3.22

The binding site, including the summary of the hydrogen bonds of ligands to the site of the receptor, is provided in **Figure 6** and **Table 9**, respectively. From these results, the ligands, i.e., Nimbidinin, Nimbic Acid and Azadirachtin, participate in hydrogen bonds with the similar amino acid of 58A. This finding suggests that the region of residue 58A may be assumed as the catalytic site of Nitrite Reductase.

Discussion

In the agricultural trade, nitrogen fertilizer is one among the popular fertilizers within the world market. However, the high use of nitrogen fertilizers causes negative impacts within the type of environmental pollution, each in water, soil, and within the air. Nitrification inhibitor required to enhance the potency of nitrogen fertilizer use to realize additional economical production of food crops and minimize fertilizer related pollution of the environment. Except for ammonia volatilization, these losses area unit related to and follow the nitrification of ammonia to nitrate. The nitrification method converts the

comparatively immobile cationic ammonia to nitrate, that is incredibly mobile and usually not maintained by soil particles and subject to loss by natural action and denitrification. Nitrification is that the oxidisation of ammonia with oxygen into radical followed by the oxidisation of radical to nitrate, in alternative words nitrification is that the method by that ammonia is regenerate to radical (NO^2) and so nitrate (NO^3) [12]. This method happens naturally within the setting and is completed by a gaggle of Nitrosomonas and Nitrobacter. Nitrification is a crucial step within the organic process within the soil during which ammonia (NH_4^+) associate in nursing initial substrate for nitrification. However, there's continued got to improve the potency of N fertiliser use to realize additional economical production of food crops and minimize fertilizer related pollution of the setting.

The nitrification process involves the role of 4 enzymes, namely Methane Monooxygenese Enzyme, Hydroxylamine Oxidoreductase Enzyme, Nitric Oxide Reductase Enzyme and Nitrite Reductase. The 4 enzymes act as catalysts in the nitrification process. To inhibit these enzymes, bioactive compounds are needed to inhibit the nitrification process, namely neem. There are 10 metabolite candidates that have potential as inhibitors of the 4 enzymes including, Nimbin, Nimbidin, Nimbic Acid, Nimbidinin, Nimbinin, Azadirachtin, Diepoxy Azadiradione, Dyhidrogedunin, Gallic Acid, and Gedunin. In this study, *A. indica* acts as an inhibitor of nitrification by inhibiting the process or the first stage of nitrification, oxidation of ammonium (NH_4^+) into nitrite (NO^2) and inactivate the enzyme produced by nitrification bacteria that AMO (ammonia monooxygenase) [13]. Another study also showed that urea coated with *A. indica* at a dosage of 2.5 and 5 % could inhibit the nitrification process. The results showed that a reduced amount of nitrate and an increase in the amount of ammonium in the soil were signs that *A. indica* metabolites were able to inhibit the nitrification process. While the treatment of urea ammonium levels coated neem 5 % increase, this is caused by soil microorganisms has been active in decomposing organic material of neem 5 % and experienced ammonification in the soil that are available in large quantities [9]. The effectiveness of *A. indica* metabolites in inhibiting the nitrification process is needed to be known by using basic bioinformatic data based on molecular docking [14].

The use of molecular docking aims to predict ligand bonds and target proteins that focus on affinity and bond interactions [15]. The high and low affinity of ligand-protein bonds is influenced by free bond energies, surface interactions, and intermolecular interactions [16]. Free bond energy describes the stability and spontaneity of binding between the ligands and target proteins. The bonding is more spontaneous and stable if the free bond energy value is lower so that it is able to bind strongly to the target protein and has the potential to cause biological activity [17]. The value of free bonds also affects the level of bond affinity. The high value of ligand and receptor bond affinity is inversely proportional to the low value of free bond energy [18]. In addition, surface interactions play a role in recognizing ligands and target proteins by being influenced by the size of the ligand molecules. Another factor that affects the level of ligand-protein affinity is the intermolecular interaction, where the interaction is formed when there is resistance or attraction between the molecule and nearby particles, such as hydrogen bonds. Hydrogen bonding occurs when there is an intermolecular interaction that occurs between the element hydrogen and the electronegative ion [16].

Conclusions

Neem plants encapsulated by urea as a nitrification inhibitor can increase nitrogen efficiency in the soil and air during fertilization. The impact of a mimba's webbed fertilizer use is to see nitrification resistance from 10 metabolic compounds obtained from the extraction of the plant against 4 enzymes that are involved in the process of nitrification through the molecular docking method. Compounds of *A. indica* can inhibit 4 enzymes in nitrification process such as Nimbin, Nimbidin, Nimbic Acid, Nimbidinin, Nimbinin, Azadirachtin, Diepoxy Azadiradione, Dyhidrogedunin, Gallic Acid and Gedunin. Based on molecular docking analysis, those metabolite compounds on *A. indica* such as Diepoxy Azadiradione and Gedunin may become the most stable complex that indicated become strong binding energy based on the lowest energy score of −10.3 kcal/mol and has a strong ability to inhibiting nitrification from the other.

Acknowledgements

We acknowledge the support received from the Development Planning Agency at Sub-National Level for funding support and thanks to BPI (Beasiswa Pendidikan Indonesia) Lembaga Pengengola Dana Pendidikan (LPDP) for the support for the author in taking the Doctoral program at IPB University at this time.

References

[1] R Sayuti and M Taqiuddin. Performance analysis of the agricultural sector in poverty alleviation efforts in west nusa tenggara in the 2008-2018 period. *Agroteksos Agronomi Teknologi Dan Sosial Ekonomi Pertanian* 2020; **30**, 11-17.

[2] N Ardiani. The supply chain for maize in Indonesia's production centers. *J. Food* 2009; **18**, 73-85.

[3] NA Jariyah. Analysis of socio-economic aspects to support the management of das moyo, sumbawa regency, west nusa tenggara. *J. Forest. Res.* 2020; **4**, 95-114.

[4] J Pramono, D Prajitno and T Tohari. Utilization of natural materials as a nitrification inhibitor to increase the efficiency of lowland rice nitrogen fertilization. *Agrin* 2011; **15**, 92-102.

[5] O Jumadi. *The application of nitrification inhibitors to reduce emission gas of Nitrous Oxide and enhance of crop growth*. Makassar State University, Makassar, Indonesia, 2018.

[6] W Hartatik and H Wibowo. The effectiveness of several types of N fertilizer on palm oil nursery. *J. Ind. Crop. Res.* 2018; **24**, 29-38.

[7] NK Fageria. *The use of nutrients in crop plants*. CRC Press, New York, 2009, p. 40-74.

[8] LS Komariyah. 2010, Test the antioxidant activity of the methanol extract of leaves and seeds of prickly acacia (Acacia Nilotica (L.) Willd. Ex Delile). Master Thesis. University of Jember, Jember, Indonesia.

[9] AB Purnamasari, Y Hala and O Jumadi. The effect of Neem (Azadirachta indica) coated urea fertilizer against NH4+ and NO3− concentration in clay loam soil texture. *In*: Proceedings of the International Conference on Mathematic, Science, Technology, Education and their Applications, Makassar Indonesia. 2016, p. 416-22.

[10] O Trott and AJ Olson. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; **31**, 455-61.

[11] GM Morris, H Ruth, W Lindstrom, MF Sanner, RK Belew, DS Goodsell and AJ Olson. Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* 2009; **30**, 2785-91.

[12] AI Sawitri, P Purwanto and S Minardi. Study of the effectiveness of nitrification inhibition in the Rhizosphere of various Brachiaria plant species in alfisols. *J. Soil Sci. Agroclimatology* 2013; **10**, 153-64.

[13] O Jumadi, Y Hala, ABD Muis, A Ali, M Palennari, K Yagi and K Inubushi. Infuences of chemical fertilizers and a nitrification inhibitor on greenhouse gas fluxes in a corn (Zea mays L.) field in Indonesia. *Microb. Environ.* 2008; **23**, 29-34.

[14] NM Hassan, AA Alhossary, Y Mu and CK Kwoh. Protein-ligand blind docking using QuickVina-W with inter-process spatio-temporal integration. *Sci. Rep.* 2017; **7**, 1-13.

[15] RY Purnomo. Antimalarial potential of azadiractin, gedunin, and nimbolideto bind PfATP6 and inhibit lactate dehydrogenase: *In silico* study. *J. Bio Komplementer Med.* 2019; **6**, 199-204.

[16] B Davarcioglu. The general characteristic of weak intermolecular interactions in liquids and crystals. *Int. J. Res. Mod. Eng. Emerg. Tech.* 2011; **1**, 443-54.

[17] DS Damayanti, DH Utomo and C Kusuma. Revealing the potency of annona muricata leaves extract as FOXO1 inhibitor for diabetes mellitus treatment through computational study. *In Silico Pharmacol.* 2017; **5**, 3.

[18] RD Malmstrom and SJ Watowich. Using free energy of binding calculations to improve the accuracy of virtual screening predictions. *J. Chem. Inform. Model.* 2011; **51**, 1648-55.