

Interaction Prediction of Compounds Contained in Eleutherine Palmifolia with Serotonin, Norepinephrine, and BDNF Receptors by Computation

Sherly Limantara^{1*}, Masruroh Rahayu², Noorhamdani³, Moch. Istiadjid Eddy Santoso⁴

^{1*}Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Indonesia

²Lecturer at Doctoral Program of Medical Science, Faculty of Medicine, Universitas Brawijaya, Indonesia

³Professor of Microbiology, Faculty of Medicine, Universitas Brawijaya, Indonesia

⁴Professor of Neurosurgery, Faculty of Medicine, Universitas Brawijaya, Indonesia

Article History:

Submitted: 18.03.2020

Revised: 19.04.2020

Accepted: 25.05.2020

ABSTRACT

This study aims: to know the active compounds of Eleutherine palmifolia; and to predict the interaction between those compounds with serotonin, norepinephrine, and BDNF receptors by computation (in silico). Eleutherine palmifolia ethanolic extract was analyzed using LCMS at State Polytechnic of Malang. Proteins and ligands were prepared by Discovery Studio Client 3.5 and PyRx 0.8. Dockings were done using HEX 8.0, then interactions of proteins and ligands and energy bindings were analysed by Discovery Studio Client 3.5. Eleutherine palmifolia extract contains three dominant compounds, which are eleutherine, eleutherole, dan quercetin. These three compounds have a tendency to bind to 5HT1A, 5HT1B, 5HT1D, α 2, and trkB receptors. The smallest energy binding is between 5HT1B

and quercetin (-283.91 kJ / mol). The smallest average energy binding is at 5HT1B (-261.58 kJ / mol), while the largest is 5HT1A (-78.4 kJ / mol).

Keywords: Eleutherine palmifolia, serotonin, norepinephrine, BDNF.

Correspondence:

Sherly Limantara
Doctoral Program of Medical Science,
Faculty of Medicine, Universitas Brawijaya
Indonesia

E-mail: shelly_queen@yahoo.com

DOI: [10.31838/srp.2020.3.107](https://doi.org/10.31838/srp.2020.3.107)

©Advanced Scientific Research. All rights reserved

INTRODUCTION

Major depression disorder has the greatest lifetime prevalence compared to other psychiatric disorders, which is around 5-17% [1][2]. Given the high prevalence, comorbidity and losses due to depression, it is not surprising that Global Burden of Disease research by World Health Organization placed depression as the most burdensome disorder worldwide [2][3][4]. Serotonin and norepinephrine are the two neurotransmitters most implicated in the pathophysiology of depression [1].

Neuroplasticity aspect is one of the etiological factors of depression. Neuronal damages and structural changes in the hippocampus is one of the causes of depression [5]. Decreased brain-derived neurotrophic factor (BDNF) can contribute to neuronal damage and decreased hippocampal volume [5][6][7]. Serum BDNF levels decrease in depressed individuals [5][6][8][9]

St. John's wort (*Hypericum perforatum*) is one of the plants that has been used as herbal therapy in depression [10][11].

One of the main components of St. John's wort is quercetin, also found in onions [11]. *Eleutherine palmifolia* grow in Kalimantan forests and are used by local residents for generations as multifunctional medicinal plants [12][13][14][15]. Its bulbs contain naphthoquinones (elecanacine, eleutherine, eleutherole, eleutherinone), are known as antioxidants [13]. *Eleutherine palmifolia* also contains triterpenoids, quinones, alkaloids, steroids, tannins, saponins, glycosides, phenolics and flavonoids [12][13][15][16]. Flavonoids have neuroprotective and therapeutic effects on cell culture and animal model neurodegenerative diseases [11]. Research needs to be done about the interaction of compounds contained in *E. palmifolia*, especially quercetin, with serotonin, norepinephrine, and BDNF receptors.

Eleutherine palmifolia were obtained from Palangkaraya, Central Kalimantan, Indonesia. Plant identification was carried out at Plant Conservation Center of Purwodadi Botanical Garden – LIPI. Bulbs extraction is based on the protocol in Pharmacology Laboratory of Faculty of Medicine, Brawijaya University. *Eleutherine palmifolia* extract was dissolved in methanol, then analyzed using TSO Quantum Access Max Liquid Chromatography Mass Spectrometry (LCMS) from Thermo Fisher Scientific at the Chemical Engineering Laboratory, State Polytechnic of Malang, Indonesia.

The protein structure of receptors were obtained from PDB and SWISS MODEL. The 5HT1B structure (PDB ID: 5V54) is obtained from PDB. The structures of 5HT1A (Uniprot IDP19327), 5HT1D (Uniprot IDQ61224), norepinephrine α 2 receptor (Uniprot IDQ01338), and tropomyosin-related kinase B (trkB, Uniprot IDW8VY28) were obtained from the SWISS MODEL. Ligands were downloaded in 3D SDF format from PubChem. The compounds used were eleutherine (CID10166), eleutherole (CID120697), and quercetin (CID5280343). Protein preparation was carried out using Discovery Studio Client 3.5 to remove water molecules and ligands. Ligand preparation was done using PyRx 0.8 software by minimizing energy and changing the file format to .pdb [17].

Dockings were done using HEX 8.0, then interactions were analyzed by Discovery Studio Client 3.5. Data analyzed were energy binding, using HEX 8.0. Interaction data between ligands and receptors were analyzed using Discovery Studio Client 3.5 [18].

MATERIAL AND METHODS


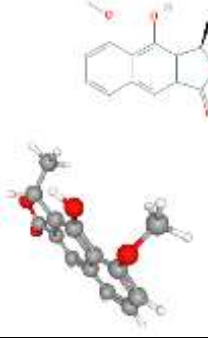

RESULTS



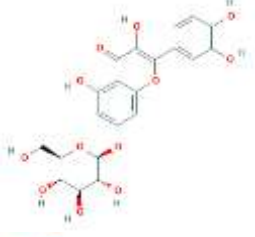




3.1 Examination of the active compounds of *E. palmifolia* extract

The analysis of the active compounds of *E. palmifolia* extract using LCMS find that there are 3 dominant compounds,

which are eleutherine, eleutherole, and quercetin. Chromatograms of these compounds are showed in Figure 1. Reference data of those compounds are obtained from PubChem [19] and listed in Table 1.

Table 1: Examination results of *E. palmifolia* extract using LCMS

Peak	Retention time (minutes)	Molecular weight (g/mol)	Compound name	Molecular formula	Structure 2D and 3D
1	6.14	272.29	<i>Eleutherine</i>	C ₁₆ H ₁₆ O ₄	
2	6.03	244.24	<i>Eleuthe-role</i>	C ₁₄ H ₁₂ O ₄	
3	6.01	302.3	<i>Quercetin</i>	C ₁₅ H ₁₀ O ₇	

4	5.66	626.5	Quercetin 3,4'-diglucoside	C ₂₇ H ₃₀ O ₁₇	 
5	5.01	464.4	Quercetin 7-O-beta-D-glucoside	C ₂₁ H ₂₀ O ₁₂	 
6	6.80	450.3	Quercetin 4'-galactoside	C ₂₀ H ₁₈ O ₁₂	 
7	6.32	610.5	Rutin (quercetin 3-rutinoside)	C ₂₇ H ₃₀ O ₁₆	

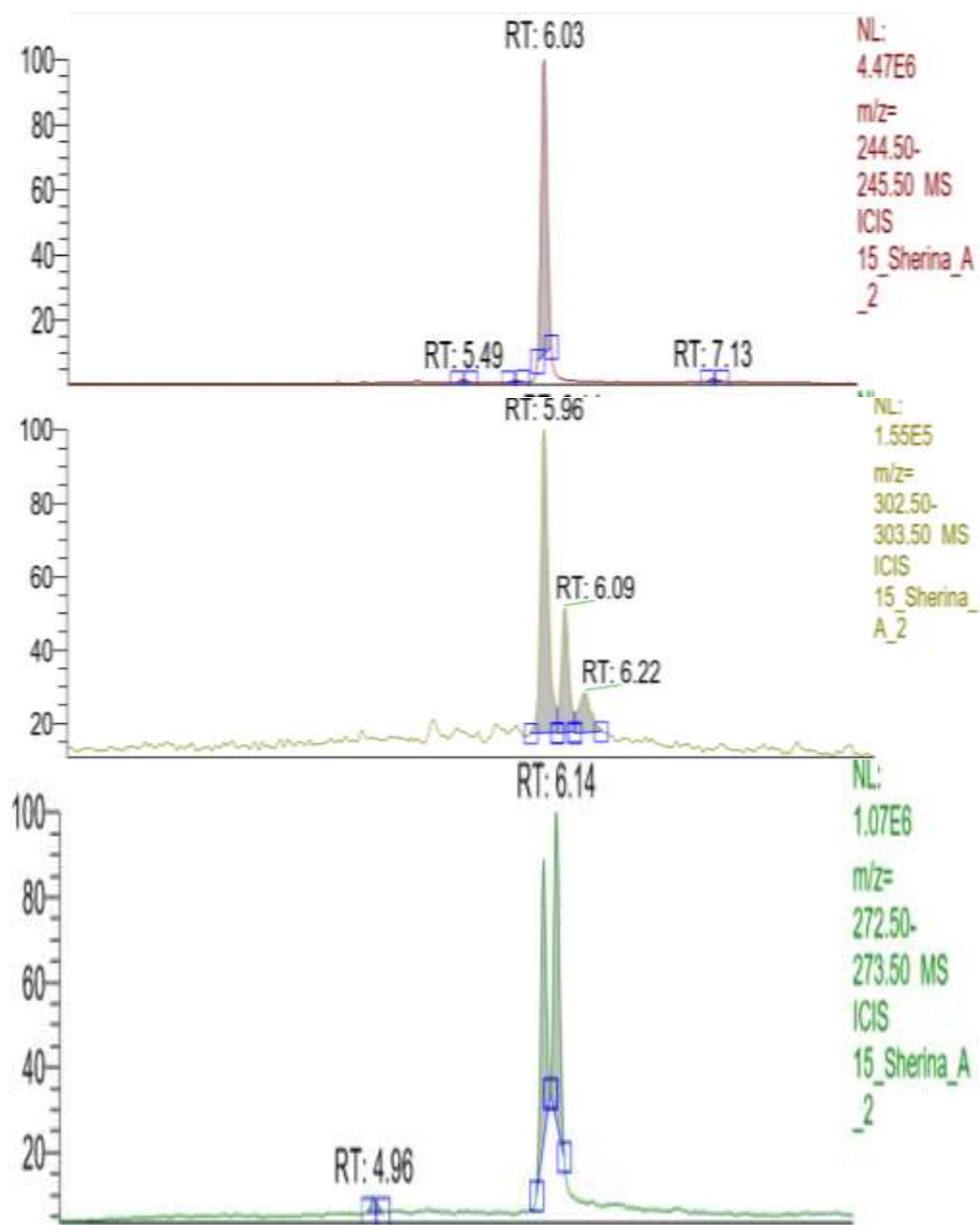


Fig 1: Chromatogram of LCMS. *Eleutherine* at RT 6.14, m/z 272.5-273.5 (top figure). *Eleutherole* at RT 6.03, m/z 244.5-245.5 (middle figure). *Quercetin* at RT 5.96, m/z 302.5-303.5 (bottom figure)

3.2 Prediction of interaction between active compounds of *E. palmifolia* extract and serotonin, norepinephrine, and BDNF receptors

Receptor model validation was using Ramachandran plot analysis. Figure 2 shows the validation of 5HT1A, 5HT1, α 2

receptors, and trkB before docking. The results show that the distribution of amino acid residues are most abundant in favored regions in the Ramachandran plot. These indicate that the receptors models have stereochemical qualities for structural analysis and validates the efficacy of protein-ligand bindings [18].

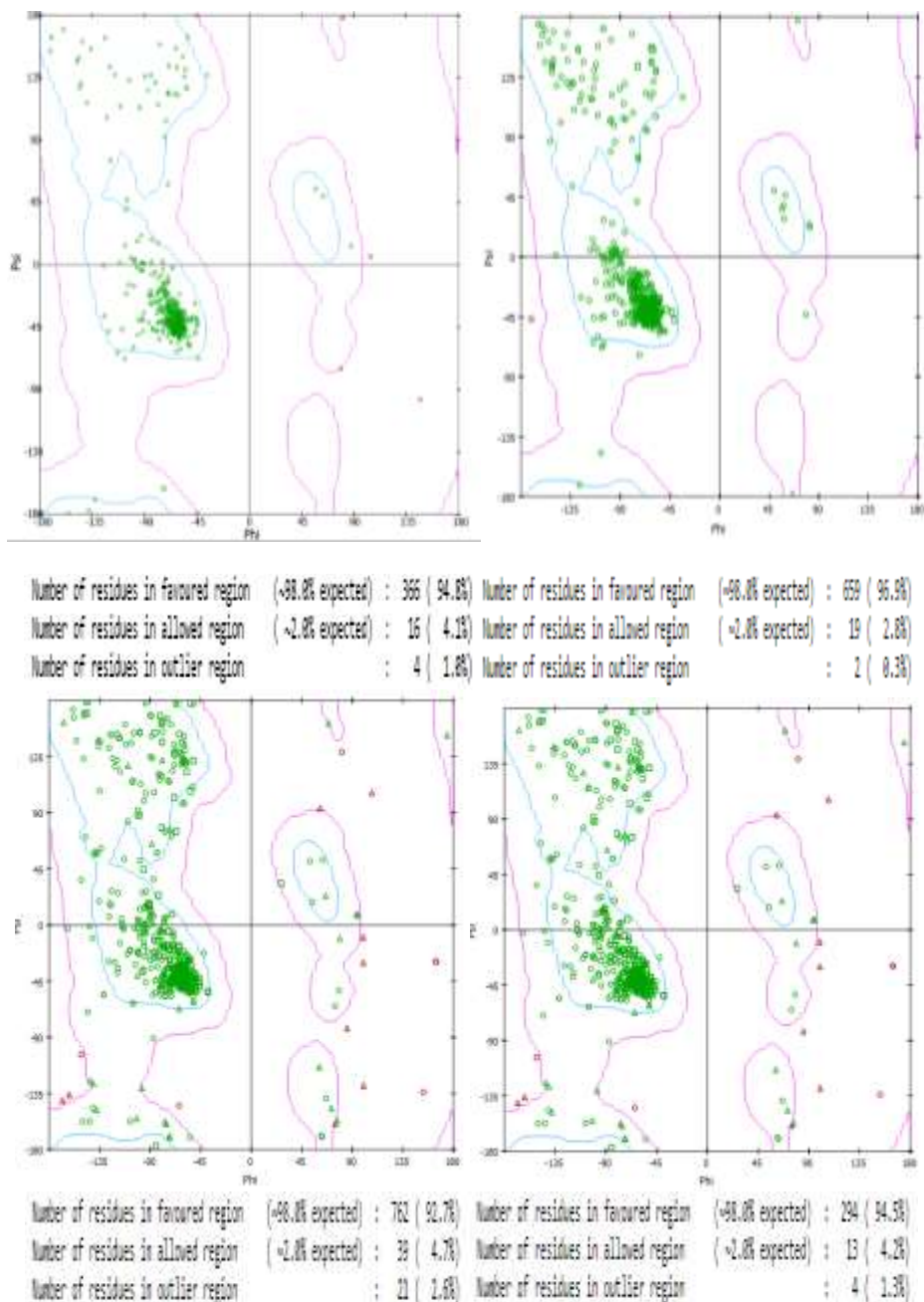


Fig 2: Ramachandran plot analysis. Residues in favored region : 5HT1A 94.8% (top left figure), 5HT1D 96.9% (top right figure), α 2 92.7% (bottom left figure), trkB 94.5% (bottom right figure)

Energy bindings between serotonin, norepinephrine, and BDNF receptors and eleutherine, eleutherol and quercetin by computation are listed in Table 2. The smallest energy

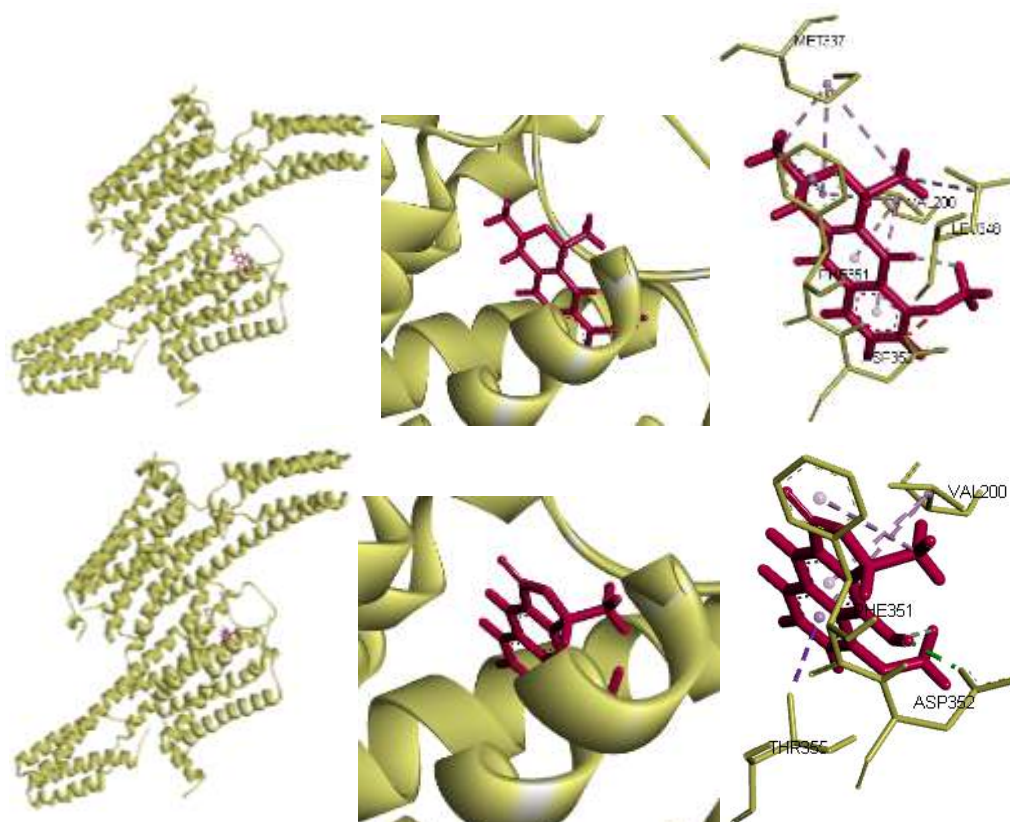
binding is 5HT1B-quercetin (-283.91 kJ/mol). Receptor that has the smallest average energy binding is 5HT1B (-261.58 kJ/mol), while the largest is 5HT1A (-78.4 kJ/mol).

Table 2: Energy bindings between serotonin, norepinephrine, and BDNF receptors and eleutherine, eleutherol and quercetin by computation.

Protein – ligand	Energy binding (kJ/mol)	Means of energy binding (kJ/mol)
5HT1A – eleutherine	-80.3	-78.40
5HT1A – eleutherol	-67.5	
5HT1A – quercetin	-87.4	
5HT1B – eleutherine	-264.06	-261.58
5HT1B – eleutherol	-236.78	
5HT1B – quercetin	-283.91	
5HT1D – eleutherine	-224.23	-222.42
5HT1D – eleutherol	-214.11	
5HT1D – quercetin	-228.92	
$\alpha 2$ – eleutherine	-215.02	-213.75
$\alpha 2$ – eleutherol	-201.74	
$\alpha 2$ – quercetin	-224.50	
trkB – quercetin	-247.21	-247.21

In silico analysis of protein-ligand interactions find that eleutherine, eleutherol and quercetin have tendencies to bind to serotonin receptors 5HT1A, 5HT1B, 5HT1D, norepinephrine $\alpha 2$ receptors, and BDNF trkB receptors.

Eleutherine, eleutherol and quercetin tend to bind more easily to 5HT1B, 5HT1D, $\alpha 2$, and trkB receptors than 5HT1A. Protein-ligand interactions between 5HT1B and these compounds are shown in Figure 3.



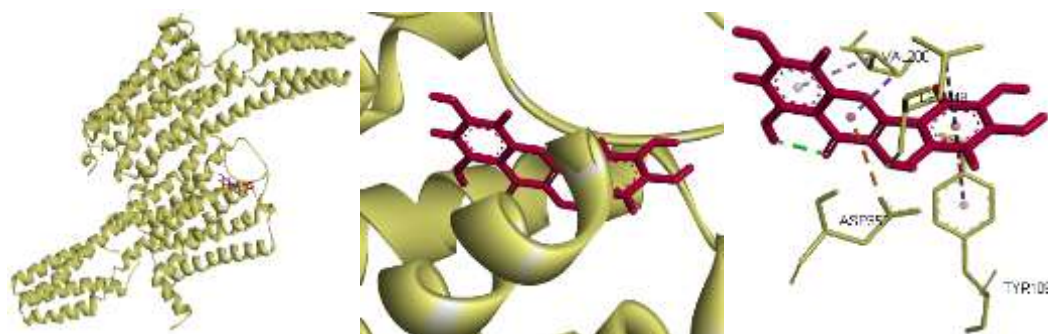


Fig 3: Protein-ligand interaction. 5HT1B-*eleutherine* (top figure), 5HT1B-*eleutherol* (middle figure), 5HT1B-*quercetin* (bottom figure)

DISCUSSION

Eleutherine palmifolia contains naphthoquinones (eleutherine, eleutherole) which are known as antioxidants [13]. *Eleutherine palmifolia* also contains flavonoids [12] [13][15][16]. Flavonoids are known as powerful antioxidants and can be turned into more active antioxidants [12][13]. Some studies have also found neuroprotective and therapeutic effects on cell cultures and animals model for neurodegenerative diseases [11].

Quercetin is one of the most studied polyphenolic flavonoids in onions. Low doses of quercetin reduce oxidative damage in neuron culture, but high doses of it damage neurons [11]. This research finds quercetin on the extract of *E. palmifolia* bulbs, both in a single form or bound to other compounds such as glucoside.

Protein-ligand interactions analysis in silico find that eleutherine, eleutherol and quercetin have tendencies to bind to serotonin receptors 5HT1A, 5HT1B, 5HT1D, norepinephrine $\alpha 2$ receptors, and BDNF receptors trkB. Activation of the trkB will induce dendrite formation, thereby increasing and maintaining the survival of serotonergic neurons [9].

Serotonin receptors 5HT1A, 5HT1B/D are postsynaptic receptors that regulate various neuronal pathways. The 5HT1A receptor inhibits pyramidal neurons in the cortex and regulates hormones, cognition, anxiety and depression. Norepinephrine $\alpha 2$ receptors are present in postsynaptics and presynaptics [20]. Binding with the postsynaptic receptor of monoamine neurotransmitter will trigger some processes in the cell membrane and intracellular [8]. Receptors in cell membranes interact with the intracellular environment via protein G. Protein G is associated with various intracellular enzymes, such as adenylate cyclase, phospholipase C, and phosphodiesterase. These enzymes regulate energy use and form 2nd messengers, such as cyclic nucleotides (e.g cyclic adenosine monophosphate, cyclic guanosine monophosphate), phosphatidylinositol (e.g inositol triphosphate, diacylglycerol) and calcium-calmodulin [1][8]. Phosphorylase enzymes, such as protein kinase A, protein kinase C, and calcium- and calmodulin-dependent protein kinase are the 3rd messengers that regulate genetic expression and phosphorylation of transcription factors (4th messenger) for the synthesis of proenkephaline, BDNF, neurotensin, or tyrosine hydroxylase [8].

This study find that the 3 main compounds of *E. palmifolia* have tendencies to bind to serotonin, norepinephrine, and BDNF receptors. This shows the potency of *E. palmifolia* to be herbal adjunctive therapy in depression. The smallest energy binding between the protein-ligand is 5HT1B-quercetin (-83.91 kJ/mol). Quercetin also has smaller energies binding than eleutherine and eleutherol at the 5HT1A, 5HT1D, $\alpha 2$, and trkB receptors. This indicates the early potential of quercetin as an antidepressant. Further investigation is needed to compare the interactions between serotonin and/or these compounds and serotonin autoreceptors.

REFERENCES

1. Sadock, B. J., Sadock, V. A., and Ruiz, P.: Kaplan & Sadock's Synopsis of Psychiatry: Behavioral Sciences/Clinical Psychiatry. pp. 347-386 (2015)
2. Hamilton, J. P., Furman, D. J., and Gotlib, I. H.: Neural Foundations of Major Depression: Classical Approaches and New Frontiers in Lopez-Munoz F, and Alamo C (eds), Neurobiology of Depression. CRC Press, Boca Raton. pp. 57-74 (2012)
3. Li, H., Luo, X., Ke, X., Dai, Q., Zheng, W., Zhang, C., Cassidy, R. M., Soares, J. C., Zhang, X. Y., and Ning, Y.: Major depressive disorder and suicide risk among adult outpatients at several general hospitals in a Chinese Han population. (2017)
4. Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., Vos, T., and Whiteford, H. A.: Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. PLoS Med; 10(11)(2013)
5. Sakata, K.: Brain-Derived Neurotrophic Factor and Major Depression in Lopez-Munoz F, and Alamo C (eds), Neurobiology of Depression. CRC Press, Boca Raton. pp. 391-418 (2013)
6. Higgins, E. S., and George, M. S.: The Neuroscience of Clinical Psychiatry: The Pathophysiology of Behavior and Mental Illness. Lippincott Williams & Wilkins, Philadelphia. pp. 47-57 (2013)
7. Kusumawardhani, A. A. A. A.: Panduan Gangguan Depresi Mayor PP PDSKJI. Jakarta. pp. 5-24 (2013)
8. Alamo, C., and Lopez-Munoz, F.: Neurobiological Basis for Development of New Antidepressant Agents in Lopez-Munoz F, and Alamo C (eds), Neurobiology

- of Depression. CRC Press, Boca Raton. pp. 455-481 (2012)
9. Erickson, K. I., Miller, D. L., and Roecklein, K. A.: The Aging Hippocampus: Interactions between Exercise, Depression, and BDNF. *Neuroscientist*; 18(1): 82-97 (2012)
 10. Ceremuga, T. E., et al.: Evaluation of the anxiolytic and antidepressant effects of asiatic acid, a compound from Gotu kola or *Centella asiatica*, in the male Sprague Dawley rat. *AANA J*; 83(2): 91-98 (2015)
 11. Lee, J., Jo, D., Park, D., Chung, H. Y., and Mattson, M. P.: Adaptive Cellular Stress Pathways as Therapeutic Targets of Dietary Phytochemicals: Focus on the Nervous System. *Pharmacol Rev*; 66: 815-868 (2014)
 12. Nurcahyawati, D. G., Plumeriastuti, H., and Maslachah, L.: Protection of Dayak Onion Tuber Extract (*Eleutherine palmifolia*) against Kidney Histopathological Appearance of Albino Male Rat Strain Wistar which was Induced by Alloxan. The Veterinary Medicine International Conference, KnE Life Sciences, pp. 702-711 (2017)
 13. Kuntorini, E. M., Dewi, M., and Misrina.: Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine palmifolia*) on different plant age. *Biodiversitas*; 17: 229-233 (2016)
 14. Febrinda, A. E., Yuliana, N. D., Ridwan, E., Wresdiyati, T., and Astawan, M.: Hyperglycemic control and diabetes complication preventive activities of Bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extracts in alloxan-diabetic rats. *International Food Research Journal*; 21(4): 1405-1411 (2014)
 15. Liestyani, E., Fikri, E. N., and Fitriyanti, D.: Kemampuan Serbuk Bawang Dayak Menekan Serangan *Meloidogyne* spp. pada Tomat. *Agroscentia*; 20(2): 53-55 (2013)
 16. Yuswi, N. C. R.: Ekstraksi Antioksidan Bawang Dayak (*Eleutherine palmifolia*) dengan Metode Ultrasonic Bath (Kajian Jenis Pelarut dan Lama Ekstraksi). *Jurnal Pangan dan Agroindustri*; 5(1): 71-79 (2017)
 17. Krisnamurti, G. C., and Fatchiyah, F.: Interaction of acetaminophen and caffeine towards cyclooxygenase-2 (COX-2) in inhibition of prostaglandin (PGH₂) synthesis. *J. Phys.: Conf. Ser.* 1146; (2019)
 18. Raharjo, S. J., Mahdi, C., Nurdiana, N., Kikuchi, T., and Fatchiyah, F.: Binding Energy Calculation of Patchouli Alcohol Isomer Cyclooxygenase Complexes Suggested as COX-1/COX-2 Selective Inhibitor. *Advances in Bioinformatics* (2014)
 19. www.pubchem.ncbi.nlm.nih.gov
 20. Stahl, S. M.: *Stahl's Essential Psychopharmacology: Neuroscientific Basis and Practical Application*. Cambridge University Press, Cambridge. pp. 253-283 (2013).