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Inhibition of main protease enzyme (M^{pro}) by active compounds in cabya (*Piper retrofractum* Vahl.) for covid-19 treatment via *in silico* experiment

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Abstract. Covid-19 is caused by aetiological agent for SARS-CoV-2. The disease has caused pandemic responsible for deaths and economic loss worldwide. Therefore, novel drugs of covid-19 primarily using herbs are urgently needed. Cabya (Piper retrofractum Vahl.) is a popular spice and also traditionally applied for herbal medicines. This study conducted an *in silico* experiment to screen methanolic active compounds in cabya and test their inhibitory activities against main protease enzyme (M^{pro}) as receptor of SARS-CoV-2. The *in silico* approach complied with molecular docking protocols enabling to evaluate performance of the compounds to inhibit M^{pro}. Two common drugs were used as control, i.e. chloroquine and ivermectin. As the results, molecular docking showed a promising inhibition by active compounds in cabya; in this regard, beta-sitosterol demonstrated the strongest inhibition against Mpro with binding affinity -7,5 kcal/mol, which is better than chloroquine (-4.8 kcal/mol) and close to ivermectin (-8,5 kcal/mol). The interaction resulted from two hydrogen bonds with amino acids ARG A131 and ASP A289 at distance of 15Å and 2,49 Å, respectively. The inhibition site of beta-sitosterol was similar to that of ivermectin. This research revealed the potential use of cabya for covid-19 treatment through restriction of molecular binding between virus and receptor.

Keywords: Cabya, *Piper retrofractum* Vahl., covid-19, main protein, docking, *in silico*

1. Introduction

The development of spice-based products has demonstrated great interest worldwide, and in this case, emergence of covid-19 pandemic provokes the attempts in search of effective treatments, such as plant-based medicine. Spices can be a very potential source in development of functional food and medicine due to their bioactive compounds exerting anti-oxidants [1][2] and antibacterial or anti-viral activities [3]. Practically, various spices are often formulated into products in the form of extract, including oleoresin [4][5]. In this regard, oleoresin can be extracted from cabya (*Piper retrofractum* Vahl.) [6].

To date, various industries have used oleoresin in their products, including flavor enhancer, medicines, and cosmetics, and it is only extracted from some sources such as black pepper [7], white pepper [6], cinnamon [5, 8], and ginger [9]. In attempt of finding potential oleoresin source, cabya (*Piper retrofractum* Vahl.) is considerable commodity, which is cultivated in various regions in Indonesia, such as Wonogiri, Jember, Lamongan, and Madura. In Madura, cabya—locally known as *cabbhi jemo*—is popular as an additive ingredient in coffee beverages. Meanwhile, it also serves as

one of the top ingredients for traditional medicine industries. The demand for industrial uses relates to the health effects of cabya, besides it is safe for food and medicine purposes [10].

To acquire a satisfied functionality of plant extract, understanding the optimum extraction procedures and oleoresin characterization is essential. Extraction of by ultrasonics has been widely used for herbs and foods [11, 12]. Principally, ultrasonic extraction—also known as ultrasound-assisted extraction (UAE)—produces shock waves that intensively generates pressure and shear force. The intensive pressure and force damages structure of plant cells, causing the swelling and pore size enlargement. The damaged cell walls increase the extractability of bioactive compounds in solvent without high-temperature processes [13].

Performance of UAE in oleoresin isolation was reported to be more effective compared with maceration. Budiastra, Mardjan, & Azis (2020) reported the advantages of UAE in nutmeg oleoresin extraction, including higher yield (12-16%) and shorter time (≤1.25 h) than maceration method (10% yield, 7 h). In addition, the extraction of oleoresin from *Capsicum annuum* by using UAE is faster than conventional method, even though no difference in yield [4].

Oleoresin isolated from cabya can be alternative to covid-19 drugs; and therefore, it requires sufficient scientific evidence and crucial stages. For the first stage, in silico experiment is needed in order to facilitate the simulation of the activity of a chemical compound against certain diseases, including covid-19. This technique predicts the affinity between an active compound (ligand) and a target protein (receptor) to form a stable complex [15]. *In silico* procedure constitutes a method of approaching a real condition or state into a computer simulation using a specific program. This work aimed to simulate inhibition of main protease enzyme (M^{pro}) by active compounds in cabya for covid-19 treatment via *in silico* experiment.

2. Materials and Method

2.1 Materials

The three-dimensional structure of the compounds present in cabya was collected from Pubchem database via https://pubchem.ncbi.nlm.nih.gov/. Meanwhile, target protein structure, i.e. main protease (M^{pro}), is downloaded in protein data bank website (http://www.rcsb.org/pdb/home/home.do).

2.2. In Silico Procedures

2.2.1. Target protein. The target protein determination employed SEA Target Prediction (http://sea16.docking.org/) and Swiss Target Prediction (http://www.swisstargetprediction.ch/) webservers by including the Cannonical SMILE of each compound. The target protein prediction approach conformed to the similarity of the compound composition connected with the standard or known compound capable of interacting with certain proteins.

2.2.2 Molecular docking. Docking was carried using Autodock Vina in protein program PyRx 9.5 targeting MPro. The protein is used as a target protein because it is generally applied in the treatment of covid-19. Furthermore, PyMol 2.3.1 program is used to visualize docking results, while the LigPlot 2.1 program is used to observe interactions of amino acids.

2.2.3. Construction of molecular structure and code. Ligands used for docking analysis included active compounds in cabya. The SMILES code of the tested compounds is converted into a 3D structure in Protein Data Bank (PDB) format using BIOVIA Discovery Studio 4.5 . This structure was used for ligand docking. The receptor structure was accessed from PDB for M^{Pro}. The protein or receptor was then interpreted using BIOVIA Discovery Studio.

3. Results and Discussion

The active compounds in cabya refers to database in http://www.knapsackfamily.com. The search successfully provides chemical compounds, including glucose, piperine, piperlongumine, beta sitosterol, guineensine, fructose, filfiline, methyl piperate, and sylvatine. Subsequently, we followed Lipinski Rule of Five to screen the identified compounds of cabya, enabling to check which compounds best fit the criteria. The criteria include as follows: molecular weight <500 Da, high lipophilicity <10, hydrogen bond donors <5, hydrogen bond acceptors <10, and molar refractivity 40-130. Test results using Lipinski's rule of five are shown in Table 1.

Table 1. Lipinski Rule of Five for compounds in cabya

Compounds	Lipinski Rule of Five				
	Molecular mass (<500 Da)	High lipophilicity (<10)	Hydrogen bond donors (<5)	Hydrogen bond acceptors (<10)	Molar refractivity (40-130)
Glucose	180.0634	0.272440	5	6	37.607491
Fructose	180.0634	0.272440	5	6	37.607491
Piperine	285.1365	2.997199	0	4	81.169983
Piperlongumine	317.1263	2.040700	0	6	85.608978
Beta sitosterol	414.3862	8.024803	1	1	128.216736
Guineensine	383.2460	4.979700	1	3	115.317177
Filfiline	389.3658	6.895873	1	1	136.769211
Methyl piperate	232.0736	1.989620	0	4	54.497993
Sylvatine	383.2460	5.653700	1	4	115.428665

The results show that there are 3 compounds, i.e glucose, fructose, and filfiline, not meeting the criteria due to low molar refractivity (less than 40). Drug interactions can occur when the substance is able to penetrate the cell membrane and reaches the target [16]. Chilistone and Hardman (2018) argued that penetration of a substance relied on its molecular weight, in which the high molecules are difficult to pass through cell membrane since it interferes diffusion process. In addition, a very high value of hydrogen donor and hydrogen receptor leads to the intense formation of hydrogen bonds, which delays the contact between drug and target [17]. Moreover, drug discovery setting also considers LogP value, and in this case, the high value (>5) is not preferable, which means high hydrophobicity. A drug with a high hydrophobicity is retained in the lipid bilayer and the drug is widely distributed in the body. This condition causes the low selectivity on target receptor [18].

The molecular docking was conducted after the Lipinski Rule of Five test. In this regard, Main protease (M^{Pro}) serves as receptor, while the selected compounds, i.e piperine, piperlongumine, beta sitosterol, guineensine, methyl piperate, and sylvatine, act as ligands. For comparison, ivermectin and chloroquine are used as drug control. Molecular docking is used to predict binding affinity of one or more compounds [19]. Affinity binding values between ligands and receptors are displayed in Table 2.

Table 2. Binding Affinity ligand with M^{Pro} receptor

Ligan	Binding Affinity
Piperine	-6.8
Piperlongumine	-6.4
Beta sitosterol	-7.4
Guineensine	-5.4
Methyl piperate	-5.2
sylvatine	-5.9
Ivermectin (drug)	-8.5
Chloroquinon (drug)	-4.8

Table 2 shows that all compounds of cabya possess a higher affinity binding value than chloroquine, but lower than ivermectin as a drug control. Affinity binding for ivermectin and chloroquine is -8.5 and -4.8, respectively. Compared with other compounds, beta sitosterol shows the lowest affinity, but it is close to ivermectin, i.e. -7.4. This suggests that the compound can be a potential drug for covid-19 treatment. Lower affinity is preferable, which represents the adequate stability of binding between ligand and receptor [20].

The affinity relies on formation of bonds. Hydrogen bonds play an important role in the affinity formed from the docking. Hydrogen bonds contribute to a higher energy than hydrophobic bonds, considering the difference in their binding energy, i.e. 7 kcal/mol and 1 kcal/mol [21]. Based on visualizations using Discovery Studio and Ligplus, hydrogen bonds of beta sitosterol and ivermectin are 2 and 7, respectively, with different amino acids. Figure visualizes the complex of ligand and receptor.

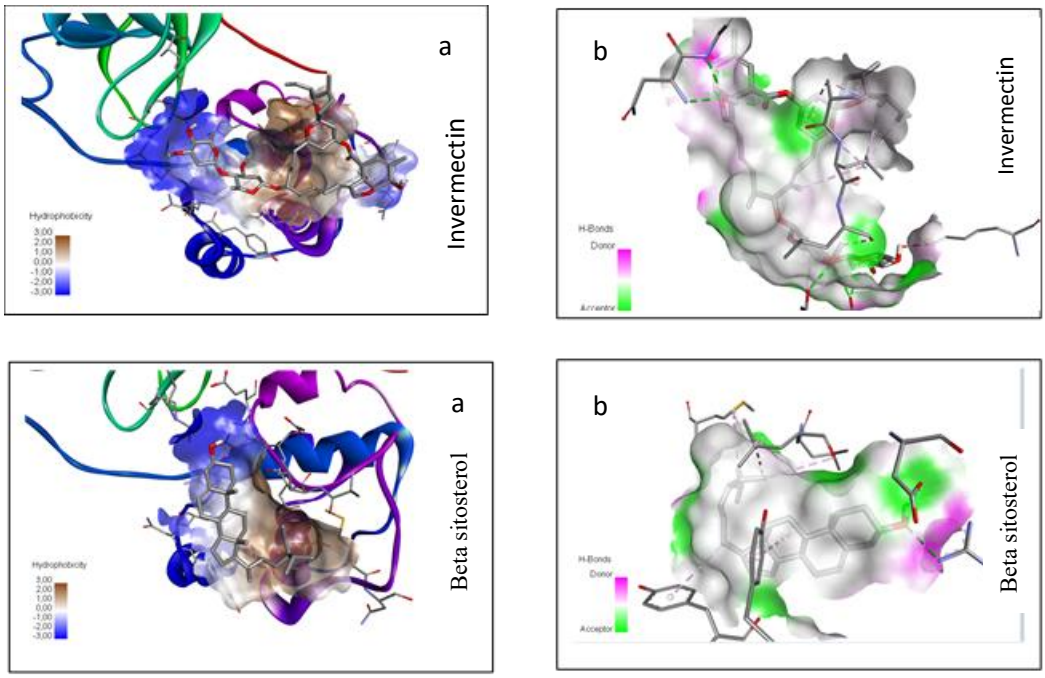

Figure 1. Visualization of the interaction of ligands and receptors with bond distance (Ivermectin and M^{pro} and Beta sitosterol and M^{pro}) (a) hydrophob bond (b) Hydrogen bond

Binding affinity is also affected by distance, besides type of bond. Ivermectin has 4 hydrogen bonds with amino acid residues THR, TYR, GLY and ASN, showing bond distances of 2.94, 2.81, 3.13 and 292 Å, respectively. In case of beta sitosterol, there are 2 hydrogen bonds with ARG and ASP residues. The distance is recorded at 3.15 and 2.49 Å, respectively (Figure 2).

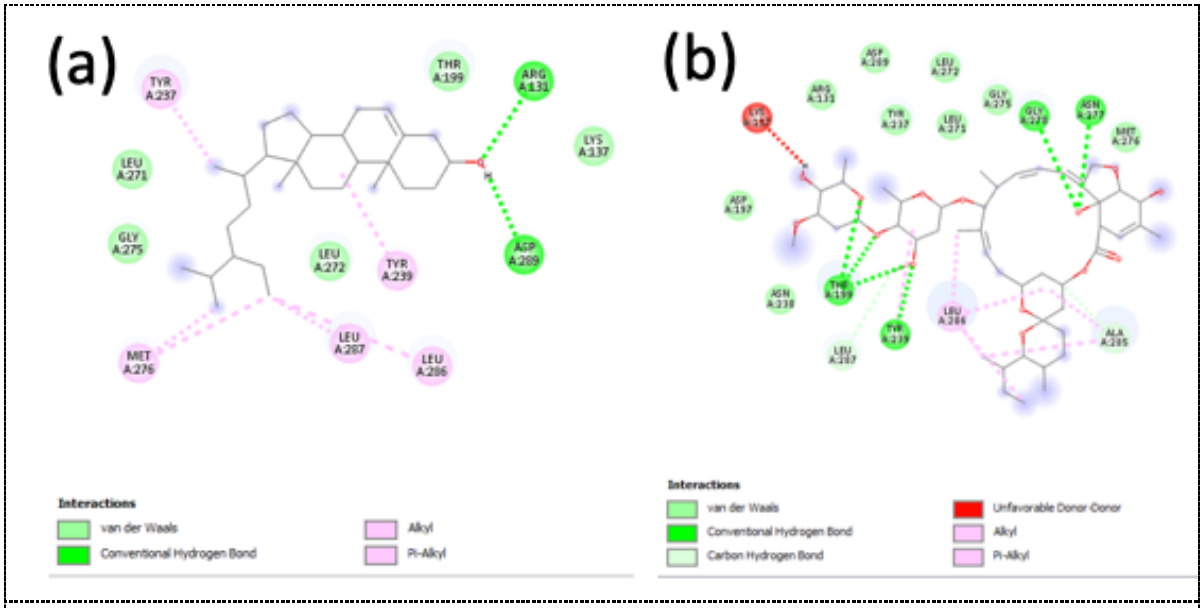

Figure 2. Visualization of the hydrogen position and amino acid residues in beta sitosterol (a) and ivermectin (b).

4. Conclusion

Molecular docking results show that the active compounds in cabya demonstrate their bioactivity as anti covid-19, especially through strong bonds between beta sitosterol and M^{Pro} with affinity binding of -7.5 kcal/mol, which is better than chloroquine (-4.8 kcal/mol) and close to ivermectin (-8.5 kcal/mol). Furthermore, beta sitosterol has two hydrogen bonds with ARG A131 and ASP A289 at bond distances of 3.15 Å and 2.49 Å, respectively. The binding side is similar to ivermectin.

References

[1] M. Takahashi, N. Hirose, S. Ohno, M. Arakaki, and K. Wada, "Flavor characteristics and antioxidant capacities of hihatsumodoki (Piper retrofractum Vahl) fresh fruit at three edible maturity stages," J. Food Sci. Technol., vol. 55, no. 4, pp. 1295–1305, 2018, doi: 10.1007/s13197-018-3040-2.

[2] Y. Lu and B. Cui, "Extraction and purification of capsaicin from capsicum oleoresin using a combination of tunable aqueous polymer-phase impregnated resin (TAPPIR) extraction and chromatography technology," Molecules, vol. 24, no. 21, 2019, doi: 10.3390/molecules24213956.

[3] W. Panphut, T. Budsabun, and P. Sangsuriya, "In Vitro Antimicrobial Activity of Piper retrofractum Fruit Extracts against Microbial Pathogens Causing Infections in Human and Animals," Int. J. Microbiol., vol. 2020, 2020, doi: 10.1155/2020/5638961.

[4] M. P. Fernández-Ronco, I. Gracia, A. de Lucas, and J. F. Rodríguez, "Extraction of Capsicum annuum Oleoresin by Maceration and Ultrasound-Assisted Extraction: Influence of Parameters and Process Modeling," J. Food Process Eng., vol. 36, no. 3, pp. 343–352, Jun. 2013, doi: https://doi.org/10.1111/j.1745-4530.2012.00702.x.

[5] L. U. Khasanah, B. K. Anandhito, Q. Uyun, R. Utami, and G. J. Manuhara, "Optimization of Two Stages Extraction Process dan Characterization of Cinnamon Leaf Oleoresin (Cinnamomum Burmanii)," Indones. J. Essent. Oil, vol. 2, no. 1, pp. 20–28, 2017, doi: 10.21776/ub.ijeo.2017.002.01.03.

[6] O. A. Olalere, N. H. Abdurahman, O. R. Alara, and O. A. Habeeb, "Parametric optimization of microwave reflux extraction of spice oleoresin from white pepper (Piper nigrum)," J. Anal. Sci. Technol., vol. 8, no. 1, pp. 0–7, 2017, doi: 10.1186/s40543-017-0118-9.

[7] Sulhatun, Jalaluddin, and Tisara, "Pemanfaatan Lada Hitam Sebagai Bahan Baku Pembuatan Oleoresin dengan Metode Ekstraksi," J. Teknol. Kim. Unimal, vol. 2, no. 2, pp. 16–30, 2013.
[8] L. U. Khasanah and B. K. Anandito, "Oleoresin Production Made From Raw Sewage Distillation of Cinnamon (Cinnamomum burmannii.)," J. Teknol. Has. Pertan., vol. VII, no. 1, pp. 1–11, 2014.
[9] Sri Hartuti and M. D. Supardan, "Optimasi Ekstraksi Gelombang Ultrasonik untuk Produksi Oleoresin Jahe (Zingiber officinale Roscoe) Menggunakan Response Surface Methodology (RSM)," agriTECH, vol. 33, no. 4, pp. 415–423, 2014, doi: 10.22146/agritech.9537.
[10] W. Haryudin and O. Rostiana, "Karakteristik morfologi tanaman cabe jawa (Piper retrofractum Vahl.) di beberapa sentra produksi," Bul. Penelit. Tanam. Rempah dan Obat, vol. 20, no. 1, pp. 1–10, 2009.
[11] M. Vinatoru, "An overview of the ultrasonically assisted extraction of bioactive principles from herbs," Ultrason. Sonochem., vol. 8, no. 3, pp. 303–313, 2001, doi: 10.1016/S1350-4177(01)00071-2.
[12] T. S. Awad, H. A. Moharram, O. E. Shaltout, D. Asker, and M. M. Youssef, "Applications of ultrasound in analysis, processing and quality control of food: A review," Food Res. Int., vol. 48, no. 2, pp. 410–427, 2012, doi: 10.1016/j.foodres.2012.05.004.
[13] M. D. Supardan, A. Fuadi, P. N. Alam, and N. Arpi, "Solvent extraction of ginger oleoresin using ultrasound," MAKARA Sci. Ser., vol. 15, no. 2, pp. 163–167, 2012, doi: 10.7454/mss.v15i2.1066.
[14] I. W. Budiastra, S. S. Mardjan, and A. A. Azis, "Pengaruh amplitudo ultrasonik dan waktu ekstraksi terhadap rendemen dan mutu oleoresin pala," JTEP J. Keteknikan Pertan., vol. 8, no. 2, pp. 45–52, 2020.
[15] R. Hakim, Y. R. Bintari, and D. S. Damayanti, "Studi Insilico Potensi Minyak Astiri dan Ekstrak Etanol Daun Annona muricata Sebagai Calon Herbal Terstandart Untuk Analgesik dan Antiinflamasi," J. Kesehat. Islam Islam. Heal. J., vol. 7, no. 01, pp. 39–44, 2018, doi: 10.33474/jki.v7i01.977.
[16] S. Tian, J. Wang, Y. Li, D. Li, L. Xu, and T. Hou, "The application of in silico drug-likeness predictions in pharmaceutical research," Adv. Drug Deliv. Rev., vol. 86, pp. 2–10, 2015, doi: 10.1016/j.addr.2015.01.009.
[17] G. Patrick, Instant Notes in Medical Chemistry, 1st Editio. London: Taylor & Francis, 2001.
[18] S. Ekins, Y. Nikolsky, and T. Nikolskaya, "Techniques: Application of systems biology to absorption, distribution, metabolism, excretion and toxicity," Trends Pharmacol. Sci., vol. 26, no. 4, pp. 202–209, 2005, doi: 10.1016/j.tips.2005.02.006.
[19] M. Gaba, A. Shaheed, B. Ajit, S. Jujhar, and S. Memorial, "An overview on Molecular Docking International Journal of Drug Development & Research ISSN 0975-9344 Available online http://www.ijddr.com Review Paper," Int. J. Drug Dev. Res., vol. 2, no. 2, pp. 219–231, 2015.
[20] A. Arwansyah, L. Ambarsari, and T. I. Sumaryada, "Simulasi Docking Senyawa Kurkumin dan Analognya Sebagai Inhibitor Reseptor Androgen pada Kanker Prostat," Curr. Biochem., vol. 1, no. 1, pp. 11–19, 2014, doi: 10.29244/cb.1.1.11-19.
[21] M. A. H. and A. Rathinavelu, Basic Pharmacology Understanding Drug Actions and Reactions, 1st Editio. Boca Raton, 2017.