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Detection of Secondary Metabolites in Cucumber (*Cucumis Sativus*) Leaves and Its Potential as Candidates for Acne Drug Using Histochemical Analysis and In Silico Study

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ABSTRACT

Acne is a facial skin problem that is generally experienced by 75-80% of adolescents as a result of excessive production of oil glands and sweat, inflammatory mediators. The acne-causing bacteria, Propionibacterium acnes, involves KAS III and JNK 1 proteins, so it is expected to be an effective anti-inflammatory and antibacterial strategy. Cucumber leaves are known to have biological activity as a candidate for acne medication. This study aims to detect secondary metabolites in cucumber leaves through histochemical, and in silico analysis. Histochemical analysis was carried out by preparing fresh leaves through lower leaf incisions by dropping secondary metabolite detection reagents and then observing the color changes microscopically. The in silico test aims to determine the physicochemical properties, pharmacokinetic properties, and interactions of the active compounds with KAS III and JNK1 as acne drug targets through molecular docking. Histochemical analysis showed that cucumber leaves positively contained secondary metabolites, namely terpenoids, flavonoids, alkaloids, tannins, and phenols. Meanwhile, it was known that isovitexin and cucurbitacin B & C had fairly good physicochemical properties, but only isovitexin and cucurbitacin C had the best pharmacokinetic properties. Based on the results of molecular docking, there are the same amino acid bonds between isovitexin and the control at the JNK1 receptor, namely the amino acids Ile:32, Val:40, and Leu:168 and the binding affinity value is low so it is predicted to be the most effective in inhibiting JNK1. Therefore, cucumber leaves open up a new potential as a candidate for natural acne preparations.

Keywords: Acne, Cucumber,* Secondary Metabolites*, Histochemical, In Silico

1. INTRODUCTION

Acne is a facial skin problem that can generally be caused by excessive oil gland production and the release of inflammatory mediators [1], one of the bacteria that is on the skin and can generally cause acne is *Propionibacterium acnes* [2], *acnes* is a gram-positive, anaerobic bacterium with oxygen tolerance. Bacterial survival is also supported by fatty acid production, so proteins involved in fatty acid synthesis are also attractive targets for antibacterial agents. The role of *P. acnes* bacteria in acne formation is to produce lipase which breaks down triglycerides into free fatty acids and β-ketoacyl acyl carrier protein (ACP) synthase III (KAS III) is a protein that plays a role in fatty acid synthesis. *P. acnes* bacteria multiply and exacerbate inflammation by inducing monocytes to produce proinflammatory cytokines. *P. acnes* activates c-Jun N-Terminal Kinase 1 (JNK1) through Toll-like receptor (TLR) 2-mediated inflammatory signaling, causing inflammation. Therefore suppression of fatty acid synthesis through inhibition of KAS III activity and suppression of inflammation through inhibition of JNK1 protein is expected to be an effective anti-inflammatory and antibacterial strategy [3], the immune response to inflammation induced by *P. acnes* plays an important role in the pathogenesis of acne. Therefore, it is important to develop antibacterial and anti-inflammatory agents so that they can be used as acne drug candidates. Many types of medications are given

to treat this condition, including topical agents, oral antibiotics, and oral hormone therapy. However, due to increasing resistance to antibiotics and existing side effects, public interest in natural products for the treatment of acne has increased significantly [4].

One of the plants that has been proven to be efficacious as an acne medicine is cucumber (*Cucumis sativus*). Apart from being consumed by the public, cucumbers are also widely used to cure diseases, one of which is acne [5], previous studies have explained a lot about cucumber as an acne medicine, but there is still little research that discusses the benefits of the leaves. Phytochemical screening of cucumber leaf chloroform extract contains alkaloids, glycosides, steroids, flavonoids, saponins, and tannins [6], based on the results of the study, there were several phenolic compounds identified in cucumber leaves including cucurbit-asides B, cucurbit-asides C, and ferredoxin and also flavone glycosides such as isovitexin and saponarin which can act as anti-inflammatory, anti-allergic, antimicrobial and antiviral. [7], [8], therefore, further research is needed regarding the potential of these compounds as acne drug candidates.

Research in the context of finding new drugs continues to be carried out, including the exploration of secondary metabolites in plants and their benefits. One method that can be used to identify secondary metabolites in plants is through histochemical analysis. Secondary metabolites in a plant have many derivatives of active compounds. The existence of active compounds can provide benefits and are often researched to be developed into medicinal agents for various diseases [9], research is often carried out to analyze the potential of active compounds as drug candidates are to utilizing molecular docking methods [10], [11], molecular docking is generally evaluated based on the Root Mean Squared Deviation (RMSD) value which compares the position of the ligand from the docking results with that of natural co-crystalline ligands in proteins [12], [13], the role of in silico studies in the discovery of new drugs has been vital and interesting in recent years, where the results can be used as predictions before further testing or as confirmation of the results of in potential as an anti-acne agent through an in vitro and in vivo tests carried out experimentally in the laboratory. Based on this background, it is important to test the content contained in cucumber leaves, namely the histochemical method. as well as its silico approach because research on cucumber leaves is still limited

2. METHOD

Material (Analysis Histokimia*)*

The materials used in the histochemical analysis were Cucumber leaves, 70% alcohol, Wagner reagent, 10% AlCl3, 10% FeCl3, 5% CuSo4, and 5% NaCo3. The tools used in the histochemical analysis are beaker glass, petri dish, watch glass, tongs, object glass, cover glass, pipette, tweezers, measuring cup, label, water bath, test tube, razor blade, and microscope (Olympus CX331 Microscope).

Analysis In Silico

The materials used in the cucurbit-asides B, cucurbit-asides C, ferredoxin, isovitexin, and saponarin. The silico analysis is. protein structure of KAS III (6A9N) and JNK1 (3V3V) and 3D structure of active compounds including tools used for in silico analysis are computer equipment and some supporting software, namely PubChem, Protein Data Bank, Discovery Studio Visualizer 2019 Client, Chimera 1.14, and Pyrx.

Methods (Histochemical Analysis)

Cucumber plants were obtained from the Splendid market. The sample used in this study was the leaves of Cucumber (*Cucumis sativus*). Cucumber leaves obtained are washed in running water so that the dirt can be removed. After washing, it is then sliced to prepare it for the test preparations. The results of the incisions obtained were tested histochemically under a microscope to determine the content of secondary metabolites found in Cucumber leaves. Various kinds of suitable reagents detect the histochemical analysis:

(1). Analysis of Terpenoid, compounds The detection of terpenoid compounds was carried out by immersing the sample incisions in 5% $CuSO_4$ solution for 24 hours then dripping with glycerin and observing under a microscope. Positive results are indicated by a change in color to brownish yellow [14].

(2). Analysis of Flavonoid, compounds Flavonoid compounds were detected by giving $AlCl_3$ solution in 85% ethanol. A change in color to yellow or blue indicates the presence of flavonoids [15].

(3). Analysis of Alkaloid, compounds Compound detection was carried out by immersing the sample in Wagner's reagent for 48 hours and then observing it using a microscope. The presence of alkaloid compounds is indicated by a brownish-red color [14].

(4). Analysis of Tannin, compounds Compound analysis was carried out by dripping a 10% $FeCl_3$ solution into the sample incision. The change in color to dark brown indicates a positive result for the presence of tannin compounds [15].

(5). Analysis of Fenol, compounds The sample incisions were immersed in 10% $FeCl_3$ solution which had been given $NaCO_3$ solution, and allowed to stand at room temperature for 15 minutes. Phenol content is indicated by a change in color to dark green or black [14].

In Silico Study

(1). Protein preparation of KAS III and JNK. The KAS III (6A9N) and JNK 1 (3V3V) proteins were downloaded via the Protein Data Bank with the website (http://www.rcsb.org/pdb). The first step in protein preparation is to remove water molecules (H2O) from the target protein. Then the original ligand on the target protein was removed by the Chimera 1.14 program. Separate native ligands are used for the molecular docking method validation process (re-docking).

(2). Molecular docking method validation, the validation of the molecular docking method was carried out by interacting with the original ligand with a previously prepared target protein (removing the original ligand) using the PyRx program to determine the Binding affinity values of the original ligand so that it can be compared with the results of the tested compounds.

(3). Preparation of Active Compounds, the test compound preparations were downloaded from PubChem software (http://PubChem.ncbi.nlm.nih.gov) and prepared using Chimera 1.14 software by changing the sdf format to pdb format so that it can adapt to the software when the process is running.

(4). Molecular Docking of Active, compounds with KAS III and JNK1 the prepared test compound interacted with the target protein using the PyRx program. Molecular docking results will show the compound with the lowest conformation and binding energy to bind to the target protein.

3. RESULT AND DISCUSSION

Detection of Cucumber Leaf Secondary Metabolites using Histochemical Analysis

Histochemical analysis was analyzed on cross-sectional preparations of Cucumber leaves or epidermal cells using a microscope at 100x magnification with the observed secondary metabolites being Terpenoids, Flavonoids, Alkaloids, Tannins, and Phenols. The results of the detection of secondary metabolites can be seen in the following figure:

Figure 1. Preparation of cucumber leaves (control), dripped with distilled water and without detection reagent, cucumber leaf preparations did not change color and remained the original color, namely green.

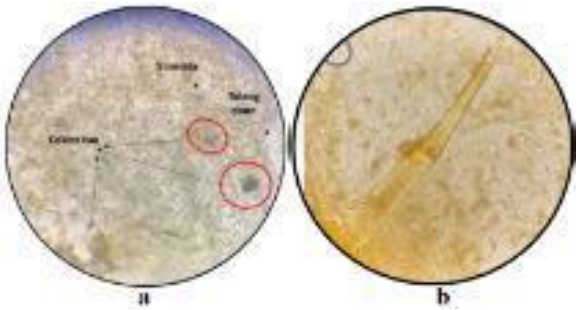

Figure 2. Histochemical detection of tannins on cross-section of cucumber leaves, 10% FeCl3 detection solution, a change in color to dark brown indicates positive tannins, (a) 1000x magnification, color change of cucumber leaf preparations (red circle), (b) cucumber leaf trichomes [16].

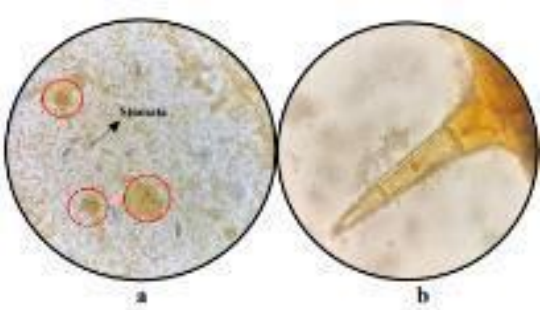

Figure 3. Detection of Alkaloid Compounds Histochemical cross-section of cucumber leaves, detection solution of Wagner's reagent for 48 hours, there is a change in color to brownish red indicating positive Alkaloids, (a) 1000x magnification, color change of cucumber leaf preparations (red circle), (b) cucumber leaf trichomes [17].

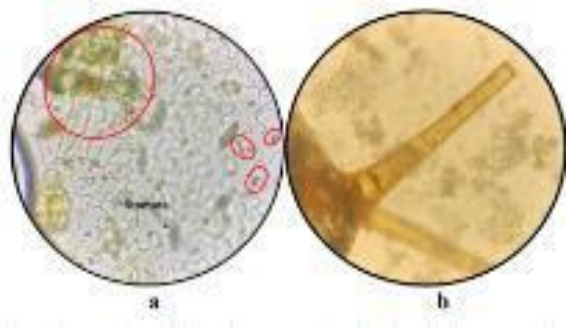

Figure 4. Detection of Phenol Compounds Histochemically cross-section of cucumber leaves, 10% FeCl3 + NaCO3 detection solution, there was a change in color to dark green indicating positive Phenol, (a) 1000x magnification, color change of cucumber leaf preparations (red circle), (b) cucumber leaf trichomes [18].

Figure 5. Detection of Flavonoid Compounds Histochemically cross-section of cucumber leaves, AlCl3 detection solution in 85% ethanol, there is a change in color to yellow indicating positive Flavonoids, (a) 1000x magnification, color change of cucumber leaf preparations (red circle), (b) cucumber leaf trichomes [19].

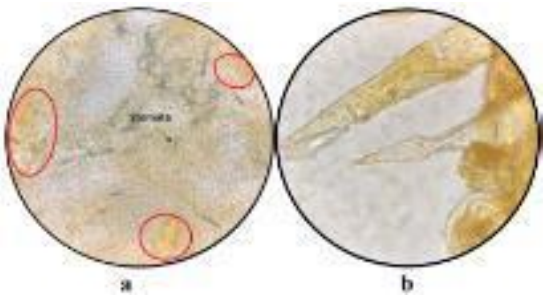

Figure 6. Histochemical detection of Terpenoids in cross-section of cucumber leaves, 5% CuSO4 detection solution for 24 + Glycerin, there was a change in color to brownish yellow indicating positive Terpenoids, (a) 1000x magnification, color change of cucumber leaf preparations (red circle), (b) cucumber leaf trichomes [17].

Each preparation that positively contained these compounds experienced a color change after being given the reagent and detection solution. Based on the results of all the preparations tested compared to the control (Figure 1), cucumber leaves positively contained tannin secondary metabolites with a color change from green to dark brown (Figure 2), Alkaloids to brownish red (Figure 3), Phenol to dark green (Figure 4), Flavonoids turned yellow (Figure 5) and Terpenoids turned brownish yellow (Figure 6). Positive secondary metabolite compounds contained in cucumber leaves have the potential to be developed in the treatment of acne because of their bioactivity as an antibacterial and anti-inflammatory agent.

This is because in the treatment of acne, antibacterial agents are needed to inhibit the growth of *P. acnes* bacteria, and anti-inflammatory agents to prevent acne inflammation

Interaction of Cucumber Leaf Active Compounds with KSI and JNK1 Proteins as Acne Causing Agents using Molecular Docking

Molecular docking studies were carried out to confirm the bond between the active compounds of cucumber leaves and the target proteins KSIII and JNK1. The results of molecular docking obtained Binding efficiency and bonds with amino acids (Table 1). Binding Affinity indicates the energy required by the ligand to be able to bind to the receptor and the amino acid bond indicates the location or part of the receptor that is bound by the ligand.

The amino acid bonds in the control were used as a comparison to conclude the inhibitory activity of the active compounds in KASI and JNK1 proteins.

Table 7. Results of Binding Affinity of Cucumber Leaf Active Compounds with KAS III and JNK1 as Target Proteins

No	KASS III (6A9N)			Active Compound	JNK 1 (3V3V)		
	Active Compound	*Binding affinity* (kcal/mol)	Interaction amino acid	Bond type		*Binding affinity* (kcal/mol)	Interaction amino acid	Bond type
1	Glycerol (Kontrol)	-3,4	Thr:18, Arg:75, Asp:299	hidrogen	3,5,6,7-Tetrahydroxy-2-(3,4-Dihydroxyphenyl)-4h-Chromen-4-One (Kontrol)	-8,7	**Ile:32, Val:40,** Ala:53, Ala:113, **Leu:168,** Asp:169	**Pi-sigma Pi-sigma** Pi-Alkyl hidrogen **Pi-sigma** hidrogen
2	Saponarin	-7,4	Asn:57, Thr:60, His:98, Lys:151, Trp:204,	hidrogen hidrogen hidrogen hidrogen Pi-Pi Stacked	Saponarin	-7,1	Leu:241, Thr:243, Pro:244, Lys:300	hidrogen hidrogen hidrogen Pi-sigma
3	Isovitexin	-7,4	Asn:57, Lys:151, Ser:153, Lys:213, His:215, Leu:217	hidrogen hidrogen hidrogen hidrogen Pi-Alkyl Pi-Sigma	Isovitexin	-8,2	**Ile:32, Val:40,** Lys:55, Met: 108, Met:111, Asp:112, Asn:114, Val:158, **Leu:168**	**Pi-Sigma Pi-Sigma** hidrogen Pi-sulfur hidrogen hidrogen hidrogen hidrogen **hidrogen**
4	Cucurbitacin B	-8,3	His:93, His:94	hidrogen	Cucurbitacin B	-6,8	Pro:244, Cys:245, Leu:302	hidrogen hidrogen hidrogen
5	Cucurbitacin C	-7,9	His:93, His:94	hidrogen	Cucurbitacin C	-6,4	Gly:244, Ser:299, Leu:302	hidrogen hidrogen unfavorable acceptor-acceptor

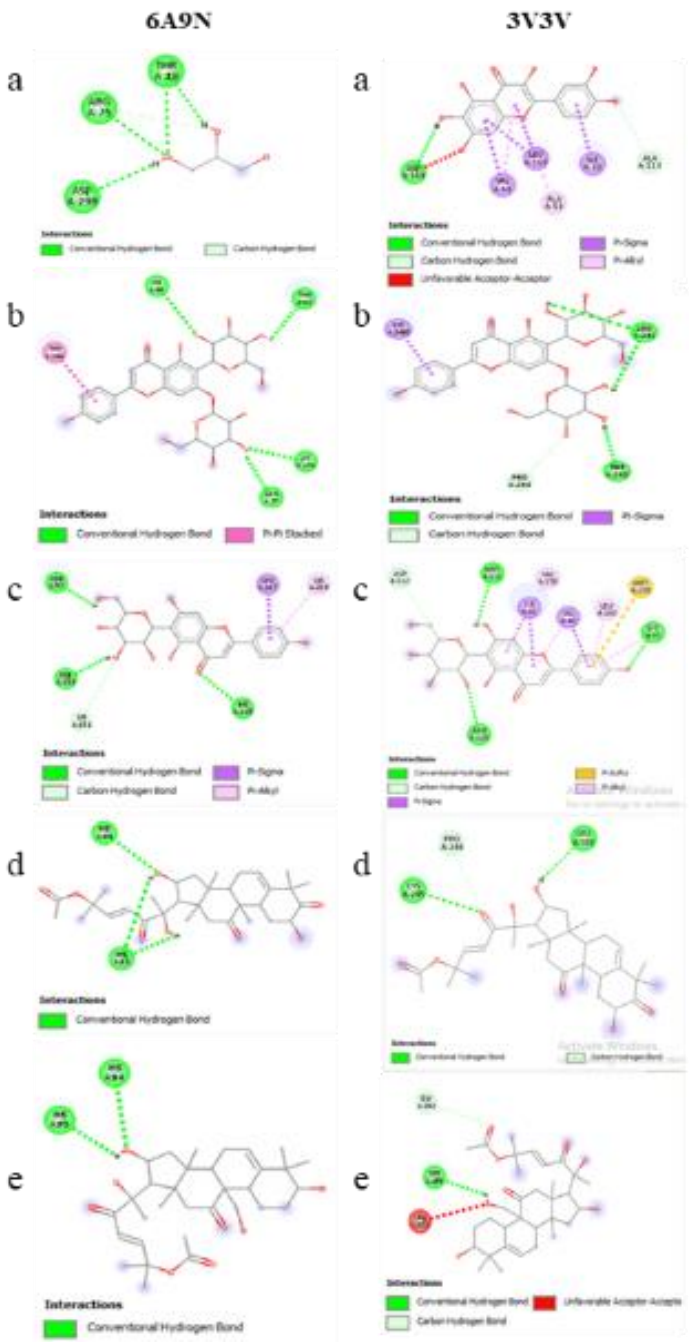

Figure 8. Docking Results Visualization of Cucumber Active Compounds with KASI protein (6A9N) and JNKI protein (3V3V), (a) native ligand, (b) Saponarin, (c) Isovitexin, (d) Cucurbitacin B, (e) Cucurbitacin B

β-ketoacyl acyl carrier protein (ACP) synthase III (KAS III) is an enzyme involved in the synthesis of fatty acids. Inhibition of KAS III activity is necessary to prevent the synthesis of fatty acids, where the synthesis of these fatty acids is necessary for the survival of P. acnes [21], based on the binding affinity value of KASI, the lowest was Cucurbitacin B, Cucurbitacin C, saponarin, and isovitexin with values of -8.3, -7.9, -7.4, and -7.4 kcal/mol respectively. Based on the results of amino acid interactions, the four active compounds did not have the same amino acid bonds as the control. So it is predicted that the active compounds in cucumber leaves cannot inhibit the KSIII protein. 2D interaction images can be seen in Figure 7.

Whereas in JNK1 the lowest values were Isovitexin, saponarin, Cucurbitacin B, and Cucurbitacin C, namely -8.2, -7.1, -6.8, and -6.4 kcal/mol. When viewed from the similarity of interactions with controls, only isovitexin in

JNK1 has the same amino acid bonds, namely Ile:32, Val:40, and Leu:168 (Figure 8). In addition, there are also hydrogen bonds with the amino acid residues Met:111 and Asn:114. Based on the results of previous studies, the amino acids Met:111 and Asn:114 are inhibitory sites on the JNK1 protein. The active compounds that have been bound are then predicted to inhibit the induction of proinflammatory cytokines, namely IL-1a, IL-1b, IL-8, and TNF-alpha through TLR2 which then prevents JNK1 activation. JNK1 which is inhibited will result in the inflammatory process being inhibited so that acne inflammation can be prevented therefore, based on the results of molecular docking, only isovitexin is predicted to inhibit JNK1 activation in forming acne inflammation but does not have the potential to inhibit KASI in fatty acid synthesis. Meanwhile, other active compounds did not have the potential to inhibit both KASI and JNK1.

Based on previous data in silico screening results, starting from the analysis of physicochemical properties (Lipinski), pharmacokinetic properties (data didn't show), and analysis of interactions with molecular docking, it can be seen that of the four active compounds in cucumber leaves, isovitexin is the drug candidate which is predicted to have the most potential in inhibiting acne inflammation because based on Lipinski's criteria:

(a) isovitexin has good physicochemical properties, (b) isovitexin has good absorption, distribution, metabolism, and excretion and is not toxic to be used as a candidate. oral medication, and (c) isovitexin can interact with JNK1 protein with a fairly good binding affinity.

However, from this study, it is known that isovitexin can only inhibit the JNK1 protein so that it can reduce acne inflammation, but cannot reduce oil production on the face because of its limitations, namely it cannot inhibit the KASI protein which plays a role in the synthesis of fatty acids which causes excessive oil on the face. Therefore, it is predicted that Isovitexin can still be used in the development of acne drugs, but it needs to be accompanied by other active compound components so that the performance in treating acne can be better.

4. CONCLUSION

This research can conclude that histochemical analysis showed cucumber leaves positively contain secondary metabolites of tannins, terpenoids, flavonoids, phenols, and alkaloids. These results also confirm the four active compounds isovitexin and saponarin which are phenol groups and confirm cucurbitacin B and C which are flavonoid compounds. Based on the molecular docking analysis, isovitexin can only inhibit the JNK1 protein so that it can reduce acne inflammation and its potential to develop candidate drug acne.

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REFERENCES

[1] A. C. L. Katzman M, "Acne vulgaris: nutritional factors may be influencing psychological sequelae," *Journal of Med Hypotheses*, vol. 69, no. 5, pp. 1080-4, 2007.

[2] D. P. K. J. J. L. A. W. L. A. R. S. E. C. S. D. M. T. A. S. V. V. K. A. B. C. K. S. R. B. John S Strauss, "Guidelines of care for acne vulgaris management," *Journal of American Academy of Dermatology*, vol. 56 , no. 4, pp. 651-63, 2007.

[3] J. K. D. J. Dasom Cheon, "Target Proteins of Phloretin for Its Anti-Inflammatory and Antibacterial Activities Against Propionibacterium acnes-Induced Skin Infection," *Journal of Molecules*, vol. 2, no. 7, p. 1319, 2019.

[4] E. A. Tanghetti, "The Role of Inflammation in the Pathology of Acne," *J Clin Aesthet Dermatol*, vol. 6, no. 9, p. 27–35., 2013.

[5] S. S. A. F. Y. U. Pratika Viogenta, "Fraksi Kloroform Ekstrak Buah Mentimun (Cucumis sativus L.) Sebagai Anti Bakteri terhadap Staphylococcus epidermidis," *Jurnal Kesehatan*, vol. 8, no. 2, pp. 165-169, 2017.

[6] A. A. M. Ammar Adnan Tuama, "Phytochemical screening and in vitro antibacterial and anticancer activities of the aqueous extract of Cucumis sativus," *Saudi Journal of Biological Sciences*, vol. Volume 26, no. 3, pp. 600-604, 2019.

[7] S. F. F. H. W. Rajasree, "Phytochemicals of Cucurbitaceae Family – A Review," *International Journal of Pharmacognosy and Phytochemical Research*, vol. 8, no. 1, pp. 113-123, 2016.

[8] F. N. A. H. D. A. T. A. Balqis, "Efikasi Mentimun (Cucumis sativus L.) Terhadap Percepatan Penyembuhan Luka Bakar (Vulnus ombustion) Derajat IIIB Pada Tikus Putih (Rattus norvegicus)"," *Jurnal Medika Veterinaria* , vol. 10, no. 2, pp. 90-93, 2016.

[9] S. A. S. Novelina, "Morfologi dan Histokimia Kelenjar Mandibularis Walet linchi (Collocalia linchi) Selama Satu Musim Berbiak dan Bersarang," *Jurnal Kedokteran Hewan*, vol. 4, no. 1, pp. 194-202, 2010.

[10] L. Krovat, "Recent Advances in Docking and Scoring," *Journal of Computer*, vol. 1, no. 1, pp. 93-102, 2005.

[11] D. F. W. Thomas Nogrady, Medicinal Chemistry: A Molecular and Biochemical Approach, New York: Oxford University Press, 2005.

[12] M. Rudrapal, "Molecular Docking, Drug-likeness Studies and ADMET Prediction of Quinoline Imines for Antimalarial Activity," *J. Med. Chem*, vol. 2, no. 1, 2019.

[13] F. D.-V. A. V.-J. José Luis Velázquez-Libera, "LigRMSD: a web server for automatic structure matching and RMSD calculations among identical and similar compounds in protein-ligand docking," *Journal of bioinformatics*, vol. 36, no. 9, pp. 12-2914., 2020.

[14] N. N. Y. C. R. O. Andriya, "nalisis Struktur Anatomi dan Histokimia Tiga Varietas Kumis Kucing (Orthosiphon aristatus (Blume) Miq.)," Rostiana, Otih, 2016. [Online]. Available: http://repository.ipb.ac.id/handle/123456789/83929.

[15] D. R. D. D. Rupa, "dentifikasi Struktur Sekretori Dan Analisis Histokimia Serta Fitokimia Tumbuhan Obat Anti-Infeksi Di Kawasan Taman Nasional Bukit Duabelas Jambi.," IPB University Scientific Repository, 2015. [Online]. Available: http://repository.ipb.ac.id/handle/123456789/78946.

[16] A. K. S. S. Gono Semiadi, "Morfologi, Anatomi dan Uji Histokimia Rimpang Curcuma aeruginosa Roxb, Curcuma Longa L. dan Curcuma heyneana Valeton dan Zijp," *Jurnal Ilmu-ilmu Hayati*, vol. Volume 17 , no. Nomor 2, 2018.

[17] B. A. W. I. N. S. N. Dorly, "Struktur Sekretori dan Uji HistokimiaTumbuhan Obat Anggota Suku Asteraceae di Hutan Pendidikan Gunung Walat," in *Seminar Nasional XII Pendidikan Biologi FKIP UNS.*, 2015..

[18] T. R. A. H. Khoiria Anisa, "Profil Metabolit Skunder Daun Tin (Ficus carica) melalui Analisis Histokimia dan Deteksi Flavonoid dengan Metode Kromatografi Lapis Tipis (KLT)," *Jurnal SAINS ALAMI*, vol. Vol. 1 , no. No. 1 , 2018.

[19] D. I. A. R. L. Nery Sofiyanti, "Kajian Anatomi-Histokimia Tangkai Daun dan Karakteristik Epidermis Pucuk Merah (Syzygium myrtifolium Walpp. – Myrtaceae)," *Journal of Buletin Anatomi dan Fisiologi* , vol. 7, no. 2, 2022.