ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 27 (3) (2024): 145-150

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

The Characteristics of Urease Enzyme of Green Bean Seeds (*Vigna radiata* L.) and Its Activity as An Antifungal Against *Candida albicans*

Niken Istikhari Muslihah [1,*], Zusfahair [1], Dian Riana Ningsih [1], Fajar Nuradha [1]

[1] Department of Chemistry, Mathematics and Natural Science Faculty, Jenderal Soedirman University, Purwokerto 53122, Indonesia

* Corresponding author: niken.istikhari@unsoed.ac.id

https://doi.org/10.14710/jksa.27.3.145-150

Article Info

Article history:

Received: 14th October 2023
Revised: 28th March 2024
Accepted: 01st April 2024
Online: 8th April 2024

Keywords:
Candida albicans; enzyme; green bean seeds; urease

Abstract

Urease is an enzyme responsible for catalyzing the hydrolysis reaction of urea into CO_2 and NH_3. The urease isolated in this study came from green bean seeds. The urease enzyme was then tested for its antifungal activity against *Candida albicans*. The research aims to extract and characterize the urease enzyme from green bean seeds and explore its potential use as an antifungal agent. Green bean seeds were smoothed with a mortar and pestle, followed by homogenization using a stirrer and cold centrifugation. The crude extract of the urease enzyme was assessed for its activity through the Nessler method and measured by employing a UV-Vis spectrophotometer at a wavelength of 500 nm. The well-diffusion method was conducted to determine the antifungal activity of rough enzyme extracts against *C. albicans*. Positive controls were 100% ketoconazole, and negative controls used a pH buffer of 7. The characterization of the urease enzyme from green bean seeds revealed the optimum urease activity at a concentration of 0.25 M, pH level of 7, and an incubation temperature of 35°C with a value of 32.115 U/mL. Maximum reaction rate (V_{max}) and Michaelis-Menten constant value (K_M) were obtained at 56.497 U/mL and 0.215 M, respectively. Antifungal tests of *C. albicans* resulted in strong inhibitory activity at a concentration of 100% crude urease extract of 12 mm inhibition zone. The inhibitory concentration value grows at least 0.5% by 0.25 mm and positive control of 19.802 mm.

1. Introduction

Infection is a condition when microorganisms such as bacteria, viruses, or fungi enter the body and begin to reproduce. Infections can happen to anyone and anywhere, including in tropical areas like Indonesia, where humidity levels are high enough for the microorganisms to grow well. The most common cause of infection is a fungal infection. *Candida albicans* is a common pathogenic fungus that causes human infections [1]. The infectious condition caused by the fungus *C. albicans* is known as candidiasis, a fungal disease of the skin, nails, respiratory tract, and gastrointestinal tract of an acute and subacute [2].

Nistatin, clotrimazole, myconazole and ketoconazole are topical drugs used to treat candidiasis. The drug has shortcomings in its use, including hypersensitivity to the skin, such as allergies, the onset of toxic reactions, poor penetration into the skin tissue, and fungal resistance appearance [2]. Therefore, there is a need for exploration of natural antifungal drugs and the discovery that canatoxins are ureases that are capable of inhibiting the radial growth of filament fungus [3]. The fungitoxic activity of urease occurs at submicromolar doses, making this protein 2-3 times more potent than other plant-derived antifungal proteins. The fungitoxic activity of these ureases results in damage to cell walls and cell membranes and plasmolysis. Therefore, it can be concluded that the urease enzyme has potent antifungal activity.

Urease enzymes can be isolated from bacteria, fungi, and plants that have an essential function within the nitrogen cycle in nature [4, 5, 6]. Beans such as peanuts and long beans are often used as sources of enzyme producers of urease [7]. Green bean seeds belong to the family Fabaceae, which produces seeds. The protein

content of 24% in green bean seeds indicates a high level of enzymes. Therefore, the urease enzyme from green bean seeds can be used as an antifungal.

One way to test antifungal activity is by using a series dilution technique to determine the minimum amount of antifungal substances needed to inhibit the growth of microorganisms in vitro, which is called Minimum Inhibitory Concentration (MIC). MIC is determined by variations in the sample concentration that can inhibit the growth of *C. albicans*. Good antifungal activity is when MIC occurs at low sample concentrations, but the impedance is high [8]. In this study, urease enzyme extraction was performed using the maceration method of green bean seeds with distilled water. The crude extract resulted from an antifungal test against *C. albicans*, characterized by variations of concentration, pH, temperature, and determination of MIC.

2. Experimental

This research started with sampling green bean seeds from Wage Market, Purwokerto. The seeds were macerated with distilled water, smoothed with a mortar and pestle, and homogenized using a stirrer and cold centrifugation. Crude urease extract was obtained for further characterization. The activity of the urease enzyme was calculated with a linear regression equation from the standard curve of ammonium sulfate.

2.1. Materials and Equipment

The materials used in this study included green bean seeds, distilled water, ammonium sulfate (Merck), sodium dihydrogen phosphate dihydrate (Merck), sodium hydrogen phosphate dihydrate (Merck), urea (Merck), sulfuric acid (Merck), Na-Wolframite (Merck), Nessler reagent (Merck), buffer citrate (Merck), buffer tris-hydrogen chloride (Merck), Sabauraud dextrose broth (SDB) (Merck), Sabauraud dextrose agar (SDA) (Merck) and *Candida albicans* fungi (ATCC 10231) from Biological Laboratory, Bogor Agricultural Institute.

The equipment used in this research included glasses commonly used in biochemical laboratory, analytical balance (ACIS), filler (Glasfirn), autoclave (Hirayama), pH meter (Lutron), vernier caliper (Krisbow), UV-Vis spectrophotometer (Shimadzu 1800), incubator thermometer (Memmert), centrifuge (O'haus), refrigerator (Panasonic), crock bor, Drigalski spatula, and petri dish.

2.2. Extractions

About 10 g of green beans were measured and soaked in distilled water for 6 hours. The seeds were subsequently smoothed with mortar and pestle. The green bean seeds were then soaked in 40 mL of phosphate buffer pH 7 at 4°C and mixed with a stirrer for 3 hours, resulting in two distinct layers. The first layer was a filtrate, and the second was a suspension. The filtrate was separated using a muslin fabric. The resulting filtrate was centrifugated at 12,000 rpm at 4°C for 15 minutes. The crude extract was tested for its activity and characterized by substrate concentration, pH, and temperature variations.

2.3. Standard Curve Determination

The standard curve was determined using a standard ammonium sulfate solution with 10, 11, 12, 13, and 14 ppm concentrations. The standard solution was taken as much as 1.5 mL plus 250 µL of the Nessler reagent. The absorption value was measured at 500 nm wavelength

2.4. Urease Activity Test of Crude Extract

A total of 1 mL of 0.25 M urea was inserted into the sample tube plus 1.9 mL pH 7 phosphate buffer solution and 0.1 mL enzyme crude extract. One mL of 0.25 M urea was inserted into the sample tube and added with 2 mL pH 7 phosphate buffer solution as a blank tube. Both tubes were incubated for 15 minutes at 35°C for further cooling with ice. The solution in the sample tube and the blank tube, respectively, was added 1 mL 2/3 N H_2SO_4 to stop the activity of the enzyme urease and added with 1 mL Na- Wolframite to complete the reaction of H_2SO_4. Each tube went through 15 minutes centrifugation process. The sample tubes and blanko solutions were taken in 1.5 mL, respectively, followed by 250 µL of Nessler reagent. The solution then measured its absorption using a UV-Vis spectrophotometer at 500 nm wavelength. The urease enzyme activity of green bean seeds was determined using the standard ammonium sulfate curve [9].

2.5. Determination of Optimum Substrate Concentration

The determination of urease activity on the variation of the substrate concentration was done using a procedure similar to the activity test. However, the substrate concentrations were varied at 0.15, 0.2, 0.25, 0.3, and 0.35 M and then incubated for 15 minutes using the specified substrate concentration variation. The data was used to determine the maximum reaction rate (V_{max}) and Michaelis-Menten constant (K_M).

2.6. Determination of pH Optimum

The urease activity under different pH conditions followed the same procedure as the urease enzyme activity test. However, the pHs of the substrate were varied at pH levels of 6, 6.5, 7, 7.5, and 8 in 0.2 M buffer solution at optimal substrate concentrations. Their activities were tested at pH 6, 6.5, 7, and 7.5 with phosphate buffer, whereas pH 8 with tris-hydrogen chloride buffer.

2.7. Determination of Optimum Temperature

The same procedure was used to determine urease activity at different temperatures as for the enzyme activity test. However, it used a range of incubation temperatures of 25, 30, 35, 40, and 45°C. The urease enzyme was controlled at the ideal substrate concentration and pH. The reaction time was 15 minutes, with the determined temperature variation, and its activity was measured.

2.8. Antifungal Activity Test

The initial phase involved regenerating *C. albicans* by inoculating a fungal culture into 15 mL of SDA medium. This was achieved by transferring one loopful of *C.*

albicans onto the SDA medium and then incubating it at 37°C for 18-24 hours. The tool and material were previously sterilized in an autoclave for 30 minutes. The insulated suspension was measured with a UV-Vis spectrophotometer at λ 600 nm until a transmission value of 25% was obtained; if this threshold was not met, the suspension was gradually diluted with distilled water.

A total of 15 mL of SDA media at ±40°C was poured into a petri dish and left to solid. A total of 100 µL of *C. albicans* culture was spread over the surface of a solid SDA medium. The SDA medium was perforated with a ±6 mm-diameter drill crock and inserted into which 50 µL crude extract of urease enzyme with a 100% (v/v) extract concentration, then incubated at 37°C for 24 hours, with positive control of ketoconazole and negative control of pH 7 phosphate buffer. The visible clear zone around the hole indicated the antifungal activity. The visible clear zone was measured using the vernier caliper.

2.9. Minimum Inhibitory Concentration (MIC)

Following the discovery of antifungal activity, the crude urease enzyme extract from green bean seeds was subsequently subjected to the Minimum Inhibitory Concentration (MIC) test against *C. albicans*. The method used was the same as that used to test antifungal activity by performing variations in sample concentration. Each 50 µL of different concentrations of crude urease enzyme extract from green bean seeds (100, 50, 25, 12.5, 6.5, 3, 1.5, 1, 0.5, and 0.25% (v/v)) was tested by inserting into the hole of SDA medium previously inoculated with *C. albicans* and incubated at 37°C for 1×24 hours. The antifungal MIC was obtained by measuring the clear zone around the sample hole using the vernier caliper for each concentration.

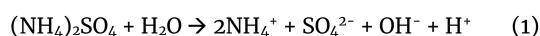
3. Results and Discussion

3.1. Urease Enzyme Activity

The urease enzyme activity test was quantitatively determined using UV-Vis spectrophotometry to measure the hydrolyzed urea levels using the Nessler method. Nessler method frequently implemented in analytical practice was ammonia reaction with Nessler reagent (tetraiodomercurate in potassium hydroxide alkali solution). The reaction between NH_3 and the Nessler reagent, which follows Beer-Lambert's Law, results in complex compounds with a yellow tint. The apparent color intensity and ammonia concentration in the samples were directly proportional linked. Urea solution without any enzyme addition controls the enzyme activity test to ensure that ammonia is not produced. The solution had a clear color when the Nessler reagent was introduced. The apparent color intensity's absorbance was determined at a wavelength of 500 nm. The Nessler approach is used because it is simple, has great sensitivity, is a low-cost reagent, and is noticeably faster [7].

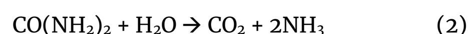
The first step in this study was to smooth green beans soaked with distilled water for 6 hours. Green bean seeds, which had been smoothly hydrolyzed with phosphate buffer pH 7 at 4°C and mixed with a stirrer for 3 hours, reveal two layers of filtrate and suspension. The filtrate was filtered using a muslin fabric. The resulting filtrate was centrifugated at 12,000 rpm at 4°C for 15 minutes. The superfood produced was called a crude extract. The urease activity test of crude extract was measured by the amount of the formed ammonia. Urease enzyme activity was expressed in units per milliliter (U/mL). One unit of activity was expressed as the quantity of ammonia formed in micrograms per milliliter per minute from the hydrolysis of urea catalyzed by the urease enzyme in the sample.

In this research, the standard curve used ammonium sulfate because the ammonium sulfate in the water would produce the same ammonia ion as the hydrolysis result of urea [10]. The reaction of ammonium sulfate in water can be seen in Reaction (1).



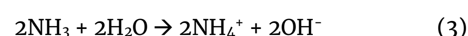
$$(NH_4)_2SO_4 + H_2O \rightarrow 2NH_4^+ + SO_4^{2-} + OH^- + H^+ \quad (1)$$

Hydrolysis reaction of urea by urease is shown in Reaction (2).



$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3 \quad (2)$$

The nitrogen in the ammonia will be in two molecules and two forms when dissolved in water, namely ammonium (NH_4^+) and ammonia (NH_3) (Reaction (3)) [11].



$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (3)$$

The ammonium ion produced during the reaction was quantified using the Nessler method and UV-Vis spectrophotometer at 500 nm wavelength. The concentration curve of ammonium sulfate against absorption produces a linear curve, as seen in Figure 1.

The relation between concentration and absorption values was either straight or linear. This was in line with Lambert-Beer's law; as the concentration of a solution was higher, the absorption value of the solution was also higher [12]. Ammonium sulfate concentrations ranging from 10 to 14 ppm were utilized to generate standard curves. These concentrations were selected to achieve wavelengths with high and consistent absorption values within the range of 0.2 to 0.8, as the Lambert-Beer law is applicable within this range. From Figure 1, a linear equation was derived: y = 0.105x – 0.924. With an R^2 value of 0.998 for this regression equation, it can be concluded that the standard curve of ammonium sulfate exhibited a correlation coefficient close to 1. This indicates a strong correlation between the variables involved [13].

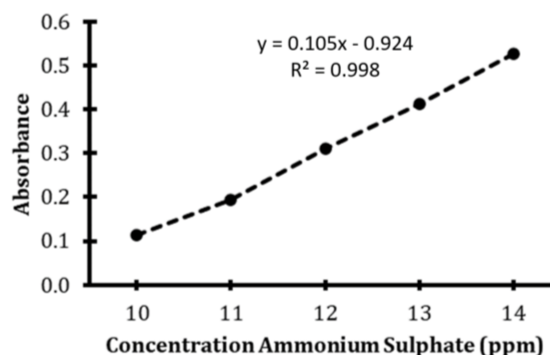


Figure 1. Standard curve of ammonium sulfate

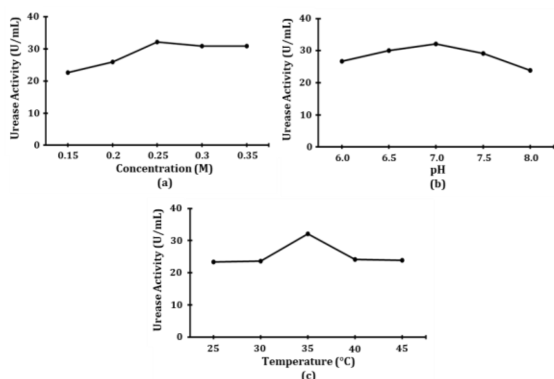


Figure 2. The urease enzyme activity with variation in (a) concentration of substrate, (b) pH level, and (c) temperature

The enzyme activity of urease was evaluated using Nessler's reaction, resulting in the formation of a complex yellow-brown-colored compound. The color absorption was measured with a UV-Vis spectrophotometer. Based on the results obtained, the value of the activity of the urease enzyme produced was 32.086 U/mL.

3.2. Characterization of Urease Enzyme

Urease enzyme activity was influenced by several factors, including substrate concentration, pH, and temperature. The optimal substrate concentration was 0.25 M (Figure 2a), with the highest urease activity value of 32.115 U/mL. Substrate was able to bind to the active side of the enzyme so that it could produce a product. Urease activity increases at a concentration of 0.15-0.25 M and then decreases at a concentration of 0.3-0.35 M. At low substrate concentrations, the enzyme has many active sites available for binding to the substrates. As a result, the activity of enzymes increases as the substrate concentration increases. However, after reaching the optimal concentration, all the active sites may already be filled with the substrates, and the enzyme has reached a saturation point. Therefore, adding more substrates will not increase the enzyme activity further or even inhibit enzymes [14].

Urease enzymes from green bean seeds had optimal activity at pH 7 (Figure 2b) with urease enzyme activity values of 32,065 U/mL. Under optimum pH conditions, enzymes had the active side conformation that corresponds to the substrate so that the collision between the enzyme and the substrates occurs very effectively and facilitates the formation of complex enzyme substrates, as a result of which more and more products were formed and produce high values of enzymatic activity [15]. At a pH of 6-7, urease activity increases because, in this stage, many of the functional groups of amino acids in the enzymes (such as carboxylic and amino acid groups) were in ionic form. This allowed for the formation of ionic bonds between these groups and the ionic groups on the substrate, enhancing the interaction between the enzyme and the substrate. At a pH of 7.5-8, urease enzyme activity decreased because of the increased alkalinity [16]. This happened because, under non-optimal pH conditions, the enzyme undergoes conformational changes, leading to alterations in its structure and subsequent loss of activity.

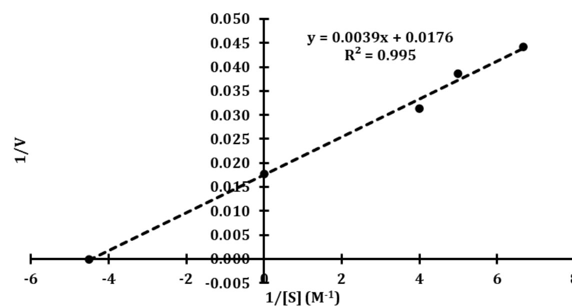


Figure 3. Diagram between 1/[S] and 1/V

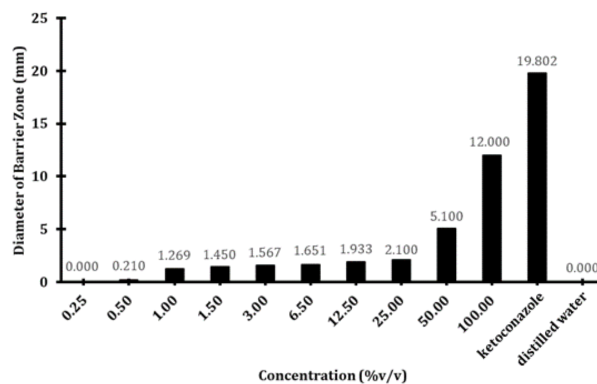


Figure 4. Relationship between concentration of crude enzyme extract and diameter of the barrier zone

Urease enzyme activity in green bean seeds increased at 25-35°C. It reached the optimal temperature at the incubation temperature of 35°C (Figure 2c) with the urease enzyme activity value of 32,115 U/mL. Increased enzyme activity indicates the increased rate of reaction of enzymes as the kinetic energy of the enzymatic process rises [17]. The enzyme was denatured at 40 to 45°C, rendering it unable to interact with the substrate effectively and consequently reducing urease activity. Previous research found that the urease enzyme isolated from choro beans had an optimal temperature of 35°C [18].

The determination of enzymatic kinetics could be seen through the relationship between 1/V and 1/[S] based on the Lineweaver-Burk equation (Figure 3). The maximum rate of reaction (V_{max}) of the urease enzyme from the green bean seed to hydrolyze its substrate was 56,497 U/mL. Substrate concentration at the catalytic velocity of enzymes reached half of its maximum speed, which could be expressed by the Michaelis-Menten constant (K_M). The K_M value obtained is 0.215 M. The larger K_M value indicates that the enzyme had a low affinity to the substrates, so the reaction balanced toward E+S [19]. The enzyme requires a lower substrate concentration to maximum activity when K_M is small. This indicates that enzymes had a higher affinity to their substrates or could interact with substrates very effectively at lower concentrations.

3.3. Minimum Inhibitory Concentration (MIC)

MIC was conducted to determine the minimum concentration of the sample that could inhibit the growth of *C. albicans*. Antifungal activity was considered high when MIC occurred at low sample concentrations but had

a large barrier force. MIC was determined by testing several concentrations of samples produced by dilution. The concentrations used in this study were 100, 50, 25, 12.5, 6.5, 3, 1.5, 1, 0.5, and 0.25% (v/v). The results of the MIC test can be seen in Figure 4.

The barrier zone for crude urease enzyme extraction from green bean seeds grew as concentrations rose. Figure 4 shows that the higher the extract concentration, the higher the active substance, such as hydroxyl groups. It disrupts organic components and nutrition transport, leading to toxic effects and inhibiting harmful spores in fungi, resulting in enhanced antifungal activity [20, 21]. The antifungal activity continues to be classified according to the diameter of the barrier zone from the weak to the strong. The diameter of the barrier zone of antifungal activity was classified as follows [22]: The barrier zone diameter > 20 mm has a very strong fungal growth barrier response; the barrier zone diameter 11–20 mm has a strong fungal growth barrier response; the barrier zone diameter 5–10 mm has a moderate fungal growth barrier response; the barrier zone diameter < 5 mm, weak fungal growth barrier response.

A positive control (ketoconazole) and a 100% urease enzyme crude extract with a sequential barrier zone of 19.802 and 12 mm. MIC was formed on a 0.5% urease enzyme crude extract in concentration and a barrier area of 0.205 mm. Using the pH 7 buffer as a negative control did not form a barrier zone. This indicates that the pH 7 buffer did not impact the urease extract itself but rather served solely as a solvent to dilute the extract. Therefore, it did not influence the outcome of activity tests conducted against the growth of *C. albicans*. Ketoconazole was a fungicide, an antifungal that could eliminate fungi [23].

Ketoconazole exerts its antifungal effect against *C. albicans* by disrupting cell membranes. This disruption occurs through the inhibition of ergosterol synthesis, a process facilitated by interacting with C-14 alpha demethylase—an enzyme reliant on the P-450 cytochrome system. This inhibition prevents the conversion of ergosterol to its thinner form, rendering the fungi structurally unstable. Consequently, the fungus may form pseudohyphae or hyphae, leading to cell death [24].

4. Conclusion

Urease enzymes from green bean seeds had optimum activity at a substrate concentration of 0.25 M, pH 7, and at an incubation temperature of 35°C with an activity value of 32.115 U/mL. Crude urease enzyme extracts from green bean seeds could inhibit *C. albicans* antifungal activity at concentrations of 100% with a barrier zone of 12 mm and a growing barrier concentration at a minimum of 0.5% with a diameter of a barrier zone of 0.205 mm.

Acknowledgment

The authors gratefully acknowledge the Biochemistry Team, Mathematics and Natural Science Faculty, and Jenderal Soedirman University for supporting our work by providing necessary lab facilities during research.

References

[1] Hartanti Dian Ikawati, Aktivitas Antidermatofitik Ekstrak Daun Urang-aring (*Eclipta alba* (L.) Hassk) terhadap *Trichophyton mentagrophytes*, *Jurnal Kefarmasian Indonesia*, 4, 1, (2014), 27-32

[2] E. Jawetz, J. L. Melnick, E. A. Adelberg, *Mikrobiologi Kedokteran*, 23rd ed., E. Nugroho, R.F. Maulany, Salemba Medika, Jakarta, 2007,

[3] Melissa Postal, Anne H. S. Martinelli, Arlete B. Becker-Ritt, Rodrigo Ligabue-Braun, Diogo R. Demartini, Suzanna F. F. Ribeiro, Giancarlo Pasquali, Valdirene M. Gomes, Celia R. Carlini, Antifungal properties of *Canavalia ensiformis* urease and derived peptides, *Peptides*, 38, 1, (2012), 22-32 https://doi.org/10.1016/j.peptides.2012.08.010

[4] Laras Dwi Maharani, Sasangka Prasetyawan, Chanif Mahi, Optimasi amobilisasi urease dari *Schizzosaccharomyces pombe* menggunakan matrik Ca-alginat, *Kimia Student Journal*, 2, 1, (2013), 421-427

[5] Shaela Pervin, Sarowar Jahan M. G., Masud Rana A. Y. K. Md., N. K. Sana, Habibur Rahman, R. K. Shaha, Effects of some environmental variables on urease in germinating chickpea (*Cicer arietinum* L.) Seed, *Journal of Stress Physiology & Biochemistry*, 9, 3, (2013), 345-356

[6] Burak Koçak, Importance of urease activity in soil, *International Scientific and Vocational Studies Congress–Science and Health (BILMES SH 2020)*, 2020

[7] Mohamed E. El-Hefnawy, Mohamed Sakran, Ali I. Ismail, Eman Fahmy Aboelfetoh, Extraction, purification, kinetic and thermodynamic properties of urease from germinating *Pisum Sativum* L. seeds, *BMC Biochemistry*, 15, (2014), 15 https://doi.org/10.1186/1471-2091-15-15

[8] Dian Riana Ningsih, Mantari D. Zusfahair, Diyu Mantari, Ekstrak daun mangga (*Mangifera indica* L.) sebagai antijamur terhadap jamur *Candida albicans* dan identifikasi golongan senyawanya, *Jurnal Kimia Riset*, 2, 1, (2017), 61 https://doi.org/10.20473/jkr.v2i1.3690

[9] Zusfahair Zusfahair, Dian Riana Ningsih, Amin Fatoni, Darul Santri Pertiwi, Pemurnian Parsial dan Karakterisasi Urease dari Biji Kacang Panjang (*Vigna unguiculata subsp sesquipedalis* L.), *ALCHEMY Jurnal Penelitian Kimia*, 14, 1, (2018), 72-83 https://doi.org/10.20961/alchemy.14.1.13000.72-83

[10] S. Senthil Balan, Fazila Fathima, S. Jayalakshmi, Characterization of urease enzyme from marine bacterium Klebsiella species, *African Journal of Microbiology Research*, 6, 30, (2012), 5914-5923

[11] Aswin Lim, Dary Aulia Muhammad, Anastasia Sri Lestari, Studi Eksperimental Kemampuan Biosementasi Bakteri Lokal pada Tanah Pasir Lepas, *Jurnal Teknik Sipil*, 26, 2, (2019), 129-138

[12] Reuben Alexander Day, Arthur Louis Underwood, *Analisis kimia kuantitatif*, I. Sopyan, Penerbit Erlangga, Jakarta, 1989,

[13] J. N. Miller, J. C. Miller, *Statistics and Chemometrics for Analytical Chemistry*, Pearson/Prentice Hall, 2005,

[14] Nisha Kumari, Veena Jain, Sarla Malhotra, Purification and characterization of extracellular acidophilic α-amylase from *Bacillus cereus* MTCC

10205 isolated from soil, *African Journal of Microbiology Research*, 7, 48, (2013), 5440-5448 https://doi.org/10.5897/AJMR12.1371

[15] Fadeli Muhammad Habibie, Agustin Krisna Wardani, Mochamad Nurcholis, Isolasi dan Identifikasi Molekuler Mikroorganisme Termofilik Penghasil Xilanase dari Lumpur Panas Lapindo, *Jurnal Pangan dan Agroindustri*, 2, 4, (2014), 231-238

[16] Sujoy Banerjee, Aparna Aggarwal, Isolation, partial purification, characterization and inhibition of urease (E.C. 3.5.1.5) enzyme from the *Cajanus cajan* seeds, *Asian Journal of Bio Science*, 7, 2, (2012), 203-209

[17] Nies Suci Mulyani, Mukhammad Asy'ari, Heru Prasetiyoningsih, Penentuan Konsentrasi Optimum *Oat Spelts Xylan* pada Produksi Xilanase pari *Aspergillus niger* dalam Media PDB *(Potato Dextrose Broth*), *Jurnal Kimia Sains dan Aplikasi*, 12, 1, (2009), 7-13 https://doi.org/10.14710/jksa.12.1.7-13

[18] Aldy Ramadhan Susanto, Ekstraksi dan Karakterisasi Enzim Urease dari Biji Kacang Koro (*Canavalia ensiformis* (L.) DC.) serta Uji Antibakteri terhadap *Staphilococcus aureus*, Kimia, Universitas Jenderal Soedirman, Purwokerto, 2023

[19] Dyah Ayu Saropah, Akyunul Jannah, Anik Maunatin, Kinetika Reaksi Enzimatis Ekstrak Kasar Enzim Selulase Bakteri Selulolitik Hasil Isolasi dari Bekatul, *ALCHEMY Journal of Chemistry*, 2, 1, (2013), 34-45 https://doi.org/10.18860/al.v0i0.2297

[20] J. M. Ritter, R. J. Flower, G. Henderson, Y. K. Loke, D. MacEwan, H. P. Rang, *Rang & Dale's Pharmacology*, Elsevier Health Sciences, 2018,

[21] Hartini Hartini, Uji aktifitas antifungi ekstrak sarang lebah dari Luwu Utara terhadap *Candida albicans*, *Bioedukasi UNS*, 10, 2, (2017), 44-46

[22] Fryano Kandoli, Uji Daya Hambat Ekstrak Daun Durian (*Durio zybethinus*) Terhadap Pertumbuhan *Candida albicans* Secara In Vitro, *PHARMACON*, 5, 1, (2016), 46-52

[23] Cut Ria Fitri, Sri Peni Fitrianingsih, Suwendar Suwendar, Evaluasi Potensi Aktivitas Antifungi Ekstrak Etanol Daun Pandan Wangi (*Pandanus amaryllifolius* Roxb.) terhadap *Candida albicans* Secara In Vitro, *SPeSIA (Seminar Penelitian Sivitas Akademika Unisba)*, 2016

[24] A. B. Becker-Ritt, A. H. S. Martinelli, S. Mitidieri, V. Feder, G. E. Wassermann, L. Santi, M. H. Vainstein, J. T. A. Oliveira, L. M. Fiuza, G. Pasquali, C. R. Carlini, Antifungal activity of plant and bacterial ureases, *Toxicon*, 50, 7, (2007), 971-983 https://doi.org/10.1016/j.toxicon.2007.07.008