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Test liquid smoke toxicity for cocoa skin (*Theobroma Cacao-L*) with the BSLT method at different pyrolysis temperatures

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Abstract. Cocoa is one of the leading plantation sector commodities in West Sumatra. Cocoa skin waste is still of little use. One of the uses of cocoa pods can be used as raw material for making liquid smoke. The purpose of this study was to determine the toxicity properties of liquid smoke of cocoa pod skin with the BSLT method at different pyrolysis temperatures. Testing the toxicity of liquid smoke of cocoa skin using the *Brine Shrimp Lethality Test* (BSLT) method. The results LC₅₀ number of cocoa shell liquid smoke at pyrolysis temperature (200°C at 10% water content) was 11,858.58 ppm, pyrolysis temperature (200°C at 15% water content) at 13,094.23 ppm, pyrolysis temperature (200°C at 20 water content %) of 13,373.94 ppm, pyrolysis temperature (200°C at 25% water content) of 15,703.52 ppm. The next a pyrolysis temperature of 300°C 10% water content of 11,604,26 ppm, pyrolysis temperature of 300°C 15% water content of 11,673,05 ppm, pyrolysis temperature of 300°C 20% water content of 13,373,94 ppm, pyrolysis temperature of 25% water content of 13,373.94 ppm. Furthermore, at a pyrolysis temperature of 400°C at a level of 10% at 9,213.73 ppm, a pyrolysis temperature at 400°C at a level of 15% at 13,094237 ppm, a pyrolysis temperature at 400°C at a level of 20% at 13,373.94 ppm, a pyrolysis temperature at a level of 25% at 12,493.63 ppm. All the results of the process of making liquid cocoa skin smoke at different pyrolysis temperatures show the results of liquid smoke with toxic properties classified as non-toxic.

Keywords: water content, toxicity, liquid smoke, cocoa skin, pyrolysis

1. Introduction

Cocoa skin is a cacao fruit processing waste that is very abundant and has not been utilized. This abundant amount of waste has the potential to pollute the surrounding environment [1]. Cocoa pods contain secondary metabolites such as phenolic compounds and flavonoids [2]. The polyphenol content includes cinnamic acid, tannin, pyrogallol, quercetin, resorcinol, and epicatechin-3-galat [3]. The major compounds found in the cocoa peel are epicatechin and hydroxybenzoic acid [4]. Based on the content of these active compound components, the skin of cocoa fruit can be used as a raw material for making liquid smoke. Liquid smoke is the result of the condensation process of steam from combustion directly or indirectly using materials that contain lignin, cellulose, hemicellulose, and hydrocarbon compounds [5]. Several studies have used cocoa pod husks as raw material for making liquid smoke, such as research [6], producing liquid cocoa pod smoke, and conducting



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antibacterial tests on *Escherichia coli* and *Staphylococcus aureus* bacteria. [7] tested the chemical content of cocoa shell liquid smoke using the GC-MS method.

In addition to the active compounds above, liquid smoke produces chemical components that can be formed in the manufacture of liquid smoke, namely Polycyclic Aromatic Hydrocarbons (PAH) and their derivatives. Some of these components are carcinogenic [8]. Benzo [a] pyrene is one of the PAH compounds that are known to be carcinogenic and commonly found in smoking products [9]. Based on this statement, it is necessary to test the safety of liquid smoke of cocoa pods before applying them to food products. One method commonly used to determine the toxicity of an ingredient is by using the Brine Shrimp Lethality Test (BSLT) method. Toxicity testing with the BSLT method can be done quickly, cheaply, and easily, so it is widely used as an initial stage (screening) in the screening of extracts of active ingredients [10].

This study uses different pyrolysis temperatures, 200, 300, and 400 oC. This study aims to determine the toxicity properties of liquid smoke from the skin of pyrolysis cocoa fruit. Toxicity values are calculated based on LC50 values. This study aims to optimize the use of cocoa skin as a raw material for making liquid smoke, the author has observed the toxicity test of the liquid smoke of cocoa pod skin on *Artemia salina* Leach larvae.

2. Material and Methods

2.1. Plant materials and research location

The material used in this study was cocoa shells obtained from Lubuk Minturun Village, Padang City that had been adjusted according to the water content. Then, the pyrolysis process was carried out until liquid smoke of cocoa pods, DMSO, seawater, and *Artemia salina* Leach larvae were obtained. The equipment used is a series of pyrolysis devices, dropper pipettes, micropipettes, goblets, vial bottles, magnifying glass, aquariums, water pumps, 10-watt lamps, cabinet dier.

The research was conducted at the Agricultural Product Technology Laboratory of Ekasakti University. This study was an explorative research design using the *Artemia salina* Leach larvae. Liquid smoke can be obtained by using a pyrolysis tool from the raw material of cocoa pods with different levels of water content.

2.2. Making liquid smoke of cocoa pod skin [11]

Cocoa skin is weighed 15 kg and then reduced in size. Drying under the sun to reach the water content with the treatment that is 10-25%. Then put into a pressure cooker with an initial temperature of 27°C and closed tightly until it is airtight. The pyrolysis process is carried out using temperatures according to the treatment namely 200, 300, and 400°C. During the pyrolysis process, make sure that enough water flows and inundates the condenser spiral pipe so that the condensation process is complete.

2.3. The toxicity test of the Brine Shrimp Lethality Test [12]

Shrimp eggs are hatched in dark and bright vessels. The dark zone is where the egg and the aerator are, while the bright zone is placed with lights to provide lighting in the hatching and separating between cysts. In the vessel filled with \pm 50-100 mg of shrimp eggs to be hatched, then the vessel is divided into 2 parts of the dark zone and the light zone which is given a lamp that is turned on for 48 hours. Then the larvae piped as many as 10 tails in 2500 μ L of seawater. For the sample to dissolve, add 2 drops of DMSO (dimethyl sulfoxideda). The extracts to be tested were made in concentrations of 100, 1000, 2000, 5000, and 10000 ppm. Next in the pipette the sample solutions to be tested were 2.5 ml or 2500 μ L each and adjusted to 5 ml or 5000 μ L. For each concentration, 3 repetitions were performed. For the control carried out without the addition of the sample only added 2 drops of DMSO as a negative control. The solution was left for 24 hours, then counted the number of dead and surviving larvae from each vial then counted by probit analysis to determine LC50.

3. Results and Discussion

3.1. Results

In addition to producing antimicrobial compounds and antioxidants, liquid smoke can produce other chemical components, namely Polycyclic Aromatic Hydrocarbons (PAH) and their derivatives. Some of these components are carcinogenic [8]. Benzo [a] pyrene is one of the PAH compounds that are known to be carcinogenic and commonly found in smoking products [13] [9] [8]. Based on the results of research conducted, differences in the water content level of cocoa pod skin raw material and pyrolysis temperature obtained LC50 values of liquid cocoa pod smoke contained in Table 1.

Table 1. LC₅₀ values of liquid smoke of cocoa pod husks

Treatment (Water content)	Temperature (Pyrolysis)(°C)	Equation	x	LC ₅₀ (ppm)	Information
10%	200	$y = 2,1186x - 3,6096$	4,063815727	11,852.85	Non Toxic
15%		$y = 2,1481x - 3,8439$	4,11708021	13,094.23	Non Toxic
20%		$y = 2,144x - 3,8467$	4,126259328	13,373.94	Non Toxic
25%		$y = 2,1184x - 3,8888$	4,195996979	15,703.52	Non Toxic
10%	300	$y = 2,2254x - 4,0454$	4,064617597	11,604.26	Non Toxic
15%		$y = 2,1246x - 3,6387$	4,067184557	11,673.05	Non Toxic
20%		$y = 2,0691x - 3,6692$	4,126259328	13,373.94	Non Toxic
25%		$y = 2,144x - 3,8467$	4,126259328	13,373.94	Non Toxic
10%	400	$y = 2,2607x - 3,9624$	3,964435794	9,213.73	Non Toxic
15%		$y = 2,2788x - 4,1761$	4,026724592	10,634.64	Non Toxic
20%		$y = 2,2254x - 4,0454$	4,064617597	11,604.24	Non Toxic
25%		$y = 2,0809x - 3,5248$	4,096688933	12,493.63	Non Toxic

3.2. Discussion

Based on the previous data in the table, it can be seen that the LC₅₀ value of liquid smoke of cocoa pods ranged from 9,213.73 - 15,703.52 ppm. The highest LC₅₀ value is at the 200oC pyrolysis temperature treatment at 25% raw material moisture content with a value of 15,703.52 ppm, while the lowest LC₅₀ value is at 400oC pyrolysis temperature treatment at 10% raw material moisture content with 9,213.73 ppm. Based on the results of the study it can be seen that the higher the pyrolysis temperature the more extracted PAH compounds are characterized by the smaller LC₅₀ value produced. The value of the liquid smoke toxicity of cocoa pods belongs to the non-toxic category. LC₅₀ is a concentration of the substance that can cause 50% of the population of tested animals to die. A compound has the potential for acute toxicity if it has an LC₅₀ value of fewer than 1000 µg / Ml [14]. Table 1 shows that the liquid smoke of cocoa pods has a low content of benzo [a] pyrene which is indicated by the high LC₅₀ value produced. The content of benzo [a] pyrene in liquid smoke is generally very low, even according to [13] the use of liquid smoke makes it possible to produce smoke products that do not contain benzo [a] pyrene and other carcinogenic compounds. Also, liquid smoke used in this study is the result of condensation of smoke originating from the skin of cocoa pods at the highest pyrolysis temperature of 400oC. Factors that cause the formation of PAH compounds are the fuming temperature and benzo [a] pyrene are not formed if the pyrolysis temperature is below 425 oC [13] [8]. The results of the liquid smoke toxicity test of cocoa pods at different levels of water content and pyrolysis temperature are shown in figure 1,2,3,4,5,6,7,8,9,10,11,12 below.

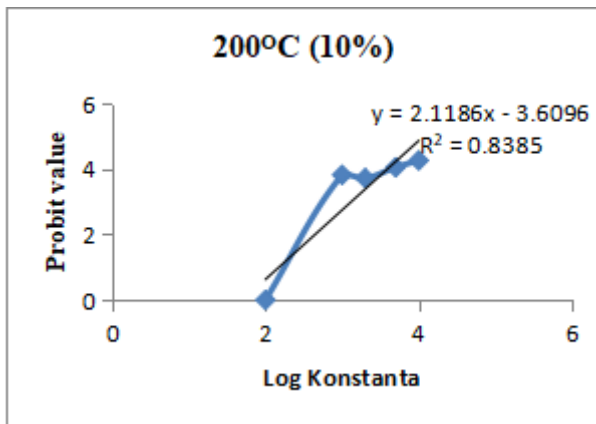


Fig.-1: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 200°C, water content 10% raw material

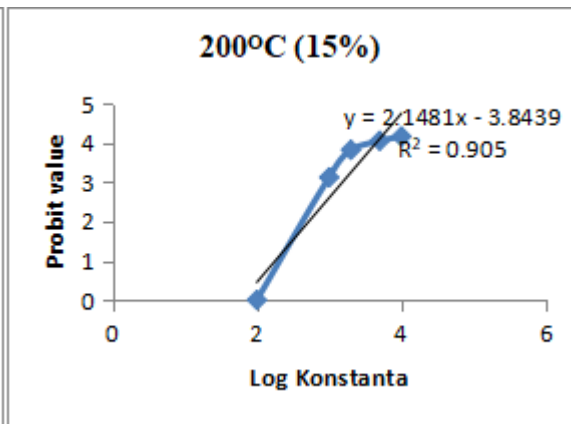


Fig.-2: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 200°C, water content 15% raw material

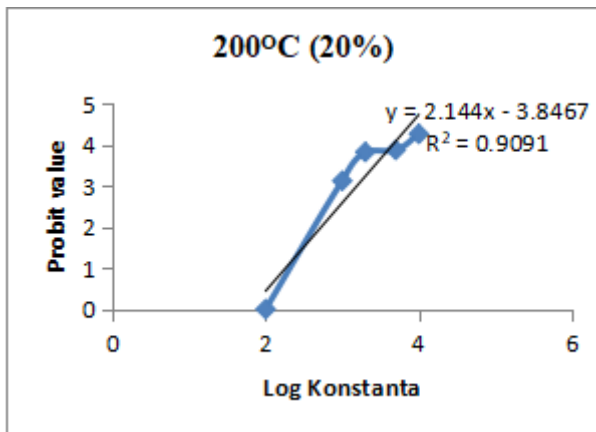


Fig.-3: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 200°C, water content 20% raw material

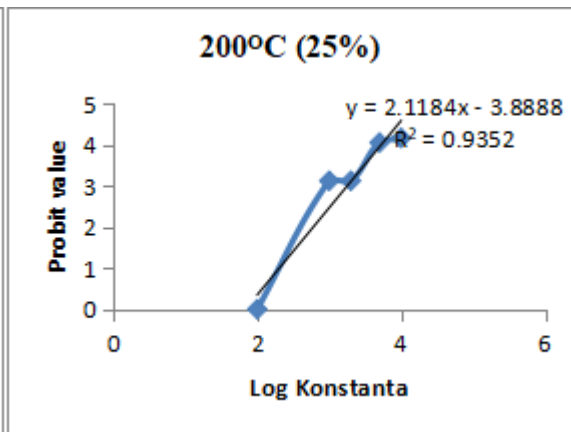


Fig.-4: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 200°C, water content 25% raw material

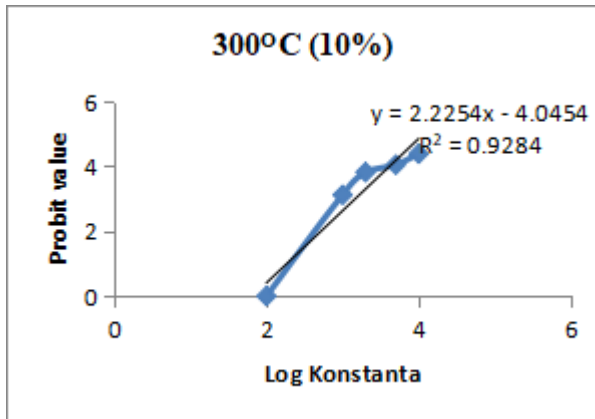


Fig.-5: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 300°C, water content 10% raw material

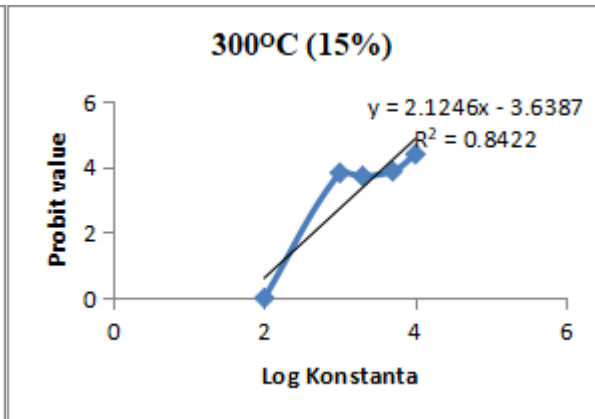


Fig.-6: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 300°C, water content 15% raw material

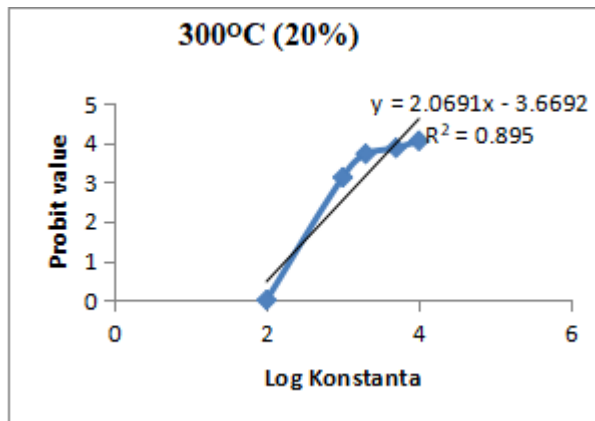


Fig.-7: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 300°C, water content 20% raw material

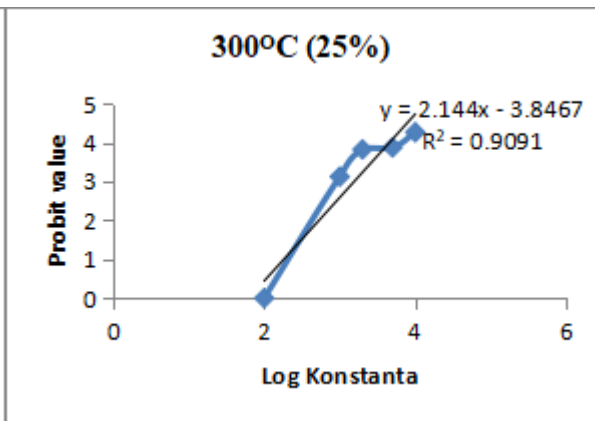


Fig.-8: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 300°C, water content 25% raw material

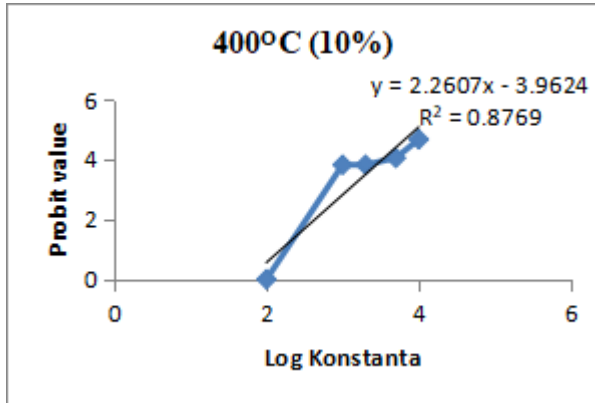


Fig.-9: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 400°C, water content 10% raw material

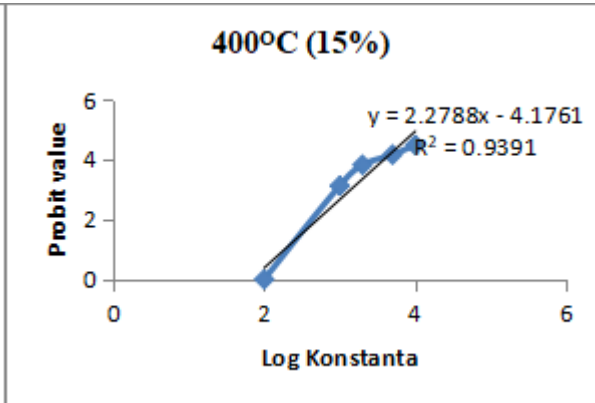


Fig.-10: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 400°C, water content 15% raw material

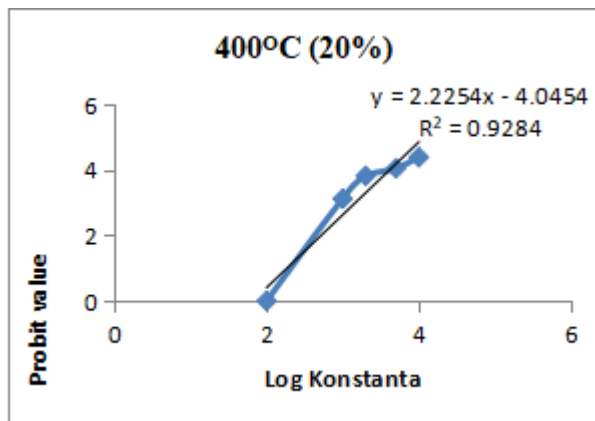


Fig.-11: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 400°C, water content 20% raw material

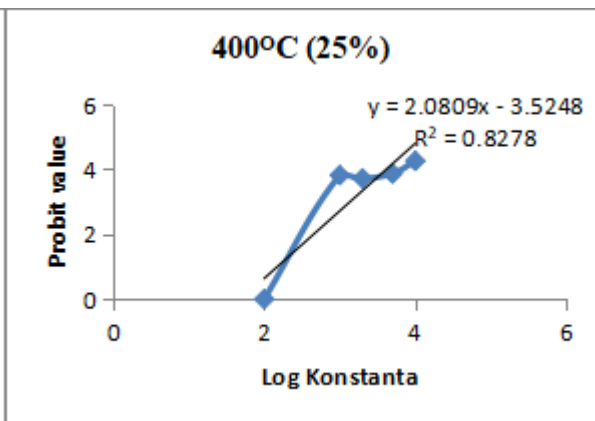


Fig.-12: Curve probit value for brown skin liquid smoke at pyrolysis temperature of 400°C, water content 25% raw material

4. Conclusion

Pyrolysis temperature affects the LC50 value of liquid smoke of cocoa pods produced. The higher the pyrolysis temperature, the lower the LC50 value produced, it is suspected that more PAH compounds are extracted during the pyrolysis process. All treatments showed that the cocoa pod's liquid smoke produced was not toxic to the shrimp larvae of *Artemia salina* leach. This means that liquid smoke of cocoa pod husk can be applied to food products with a certain concentration.

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References

- [1] T. Harsini and Susilowati, "Pemanfaatan kulit buah kakao dari limbah perkebunan kakao sebagai bahan baku pulp dengan proses organosolv," *J. Ilm. Tek. Lingkungan.*, vol. 2, no. 2, pp. 80–89, 2010.
- [2] A. Jusmiati, R. Rusli, and L. Rijai, "Aktivitas antioksidan kulit buah kakao masak dan kulit buah kakao muda," *J. Sains dan Kesehat.*, vol. 1, no. 2, pp. 34–39, 2016. DOI: 10.25026/jsk.v1i1.7
- [3] S. . Fapohunda and A. Afolayan, "Fermentation of Cocoa Beans and Antimicrobial Potentials of the pod Husk Phytochemicals," *J. Physiol. Pharmacol. Adv.*, 2012.
- [4] M. Arlorio, J. D. Coison, F. Travaglia, F. Varsaldi, G. Miglio, G. Lombardi, and A. Martelli, "Antioxidant and biological activity of phenolic pigments from Theobroma cacao hulls extracted with supercritical CO₂," *Food Res. Int.*, vol. 38, pp. 1009–1014, 2005.
- [5] S. A. Kondo, W. Gunawan, and C. Rizke, "Pengaruh pemberian asap cair pada berbagai konsentrasi terhadap pertumbuhan streptococcus sanguis penyebab gingivitis," *JKD*, vol. 6, no. 1, pp. 106–113, 2017.
- [6] I. K. Budaraga and D. P. Putra, "Liquid Smoke Antimicrobial Test of Cocoa Fruit Peel Against Escherichia Coli and Staphylococcus Aureus Bacteria," *ICATES*, vol. 365, pp. 1–10, 2019.
- [7] M. M. Wijaya, M. Wiharto, and M. Anwar, "Kandungan kimia asap cair kulit kakao dengan metode GC-MS," *J. Kim. dan Pendidik. Kim.*, vol. 2, no. 3, pp. 191–197, 2017.
- [8] A. Stołyhwo and Z. E. Sikorski, "Polycyclic aromatic hydrocarbons in smoked fish - A critical review," *Food Chem.*, vol. 91, no. 2, pp. 303–311, 2005.
- [9] N. Kazerouni, R. Sinha, C. H. Hsu, A. Greenberg, and N. Rothman, "Erratum: Analysis of 200 food items for benzo(a)pyrene and estimation of its intake in an epidemiologic study," *Food and Chemical Toxicology*. 2002.
- [10] N. D. Fajarningsih, H. I. Januar, M. Nursid, and T. Wikanta, "Potensi Antitumor Ekstrak Spons Crella papilata Asal Taman Nasional Laut Kepulauan Seribu," *J. Pascapenen dan Bioteknologi Kelaut. dan Perikan.*, vol. 1, no. 1, p. 35, 2006. DOI: 10.15578/jpbkp.v1i1.229
- [11] I. K. Budaraga, Arnim, Y. Marlida, and U. Bulanin, "Liquid Smoke Production Quality from Raw Materials Variation and Different Pyrolysis Temperature," *Int. J. Adv. Sci. Eng. Inf. Technol.*, vol. 6, no. 3, pp. 306–315, 2016.
- [12] A. W. Ningdyah, A. H. Alimuddin, and A. Jayuska, "Uji toksisitas dengan metode BSLT (Brine Shrimp Lethality Test) terhadap hasil fraksi ekstrak kulit buah tampoi (Baccaurea macrocarpa)," *JKK*, vol. 4, no. 1, pp. 75–83, 2015.
- [13] M. D. Guillen, P. Sopolana, and M. A. Partearroyo, "Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations," *J. Agric. Food Chem.*, vol. 48, pp. 5083–6087, 2000.
- [14] J. L. Carballo, Z. L. Hernández-Inda, P. Pérez, and M. D. García-Grávalos, "A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products," *BMC Biotechnol.*, vol. 2, no. 17, 2002.

