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Research Article

# Chemical Characterization and Antibacterial Activities of Bio-oil from Durian Shell Pyrolysis

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**Abstract**

Foodborne bacteria cause food spoilage, usually *Staphylococcus aureus* and *Escherichia coli*. Thus, synthetic preservatives are employed in food preservation to prevent food spoilage caused by microorganisms. Excessive use of synthetic preservatives can cause disease. Bio-oil has become a natural preservative because of its high phenolic content. However, bio-oil still requires purification because the initial bio-oil (grade 3) still contains carcinogenic compounds that are dangerous for consumption. Therefore, this study aims to determine the components of the bio-oil compound after purification and its effectiveness as an antibacterial. Durian shell (DS) is pyrolyzed in a heating reactor without oxygen at a temperature of 330–600ºC (flow rate 6ºC/minute) with a 2-3 cm material size. Furthermore, bio-oil purification includes stages of filtration using activated zeolite, fractional distillation at 70–200ºC (grade 2), and filtration using activated charcoal (grade 1). Bio-oil purification includes stages of filtration using active zeolite and activated charcoal (grade 2), and fractional distillation at a temperature of 150–200ºC (grade 1). Based on Gas Chromatography-Mass Spectrometry (GC-MS) analysis, grade 2 and grade 1 contain the major compounds 1,4-dimethyl-1h-imidazole and acetic acid. The research showed that bio-oil grades 1 and 2, when used at a 30% concentration, exhibit antibacterial strong effects against Staphylococcus aureus and Escherichia coli. These findings suggest that bio-oil grades 1 and 2 could be valuable natural preservatives.

**Keywords**: antibacterial; bio-oil; durian shell; preservatives; pyrolysis

## 1. INTRODUCTION

Extensive research efforts have intensified to meet the growing demand for natural food preservatives, driven by the need to preserve perishable foods effectively [1,2]. Foodborne infections pose a significant challenge for consumers, the food industry, and food safety authorities. Several foodborne pathogens, including Staphylococcus aureus, Bacillus cereus, and Escherichia coli, are particularly worrisome for food safety [2,3]. Hence, there is a need for natural antimicrobial compounds to inhibit the growth of bacteria and fungi, enhancing food quality and prolonging shelf life [2]. The adoption of natural antimicrobials for food preservation is a trend embraced by both consumers and producers [1]. As the demand for minimally processed products, particularly those incorporating natural additives continues to increase, the use of natural antimicrobials is expected to grow steadily. Harmful preservatives, which include chemicals like sodium nitrate, sodium benzoate, trans fat, and sulfur dioxide, have numerous adverse effects on human health. These chemicals pose significant health risks when consumed in food containing such preservatives [4]. Preservatives are added to food to prevent spoilage and maintain freshness, but their dangerous chemical components can lead to diseases such as heart disease and diabetes [5].

The industry's focus has shifted to using natural preservatives that are innovative, economically efficient, environmentally friendly, or as natural alternatives to synthetic preservatives, which are potentially risky to human health [6,7]. Researchers have consistently innovated and generated novel and efficient antioxidant and antimicrobial compounds from natural sources for safe food constituents [8–10]. Recently, the use of natural compounds, such as plant extracts, traditional herbs, and spices, in preserving food has come to the fore [6,11–13]. Since ancient times, additives have been incorporated into food not only to improve taste but also for their medicinal properties and ability to preserve freshness. These additives, including polyphenolic compounds and essential oils, act as antioxidants, bacteriostatic, or bactericidal agents [8,13–18]. In addition to their preservative function, these compounds are known to provide health benefits to consumers. Traditional herbs, spices, essential oils, and plant extracts, among others, demonstrate various protective effects against diseases, making them promising and environmentally friendly alternatives to food additives, preservatives, and dietary supplements [19–24]. These compounds are abundant in various plants, with varying concentrations.

Food preservatives require environmentally friendly compounds and fewer chemicals. Control of chemical preservatives has been successful in preventing fungal and bacterial attacks and extending shelf life [25,26]. However, chemical preservatives have been limited due to concerns regarding their toxicity and risks to human health and the environment [26,27]. Bio-oil, a liquid product resulting from the pyrolysis of biomass material like lignocellulosic biomass, is an alternative approach [28–31]. It can potentially source natural chemicals through a thermochemical or pyrolysis process [29,30,32]. Pyrolysis goes through high-temperature carbonization (300–700 $^{o}$C) without or low oxygen [33,34]. Due to its complex structure and chemical composition, bio-oil from lignocellulosic biomass can be a potential source of antioxidant [35], antimicrobial [36], antifungal [37], germicidal [38], and insecticidal [39]. We obtain lignocellulosic biomass bio-oil from various sources, such as crops, algal biomass, municipal waste, and agricultural and forestry by-products. Bio-oils are rich in phenolics, tannins, and other volatile compounds such as acids, aldehydes, furans, and furan derivatives; therefore, they also have the potential as preservatives [38,40–43].

Durian, scientifically known as *Durio zibethinus* Murr., is a trendy tropical fruit and a vital economic fruit crop in Southeast Asia and neighboring islands. It generates substantial amounts of lignocellulosic biomass waste. Durian is now spread in several Southeast Asian countries [44,45]. Durian remains a promising export commodity. Indonesia, a significant producer, exported durians worth USD 232,000 in 2020 to several countries, including the Middle East [46]. The rise in durian fruit production is directly linked to an increase in the resulting processing residue, specifically durian shells (DS). The DS is generally the most significant part produced from the durian fruit. So far, durian fruit can only be used for its flesh, or about 20-35 percent of the entire durian section. At the same time, waste accounts for the remaining 65-80%. The DS waste has characteristics that are difficult to decompose, so it can potentially become a biological waste that can cause environmental pollution.

The composition of the DS includes cellulose (60.5%), hemicellulose (13.1%), and lignin (15.45%) [47]. Kumar et al. (2019) and Hoang et al. (2021) have outlined that this lignocellulosic biomass holds the potential for conversion into various chemical components, such as hydrocarbon mixtures, carbon-rich solid residues, and bio-oil, using diverse methods, including pyrolysis [48,49]. The pyrolysis method offers additional benefits compared to alternative conversion methods, including swift conversion, cost-effectiveness, the absence of chemical solvents, and high extraction rates [49,50]. Biomass pyrolysis yields bio-oil, charcoal, and gasses ($H_2$, $CO_2$, CO, and $CH_4$). Bio-oil, a liquid produced from the condensed vapor during the decomposition of biomass, contains various organic compounds. These include organic acids such as acetic, formic, and propionic acids, carbonyl compounds, and hydroxy carbonyl compounds like aldehydes and ketones. Bio-oil also contains sugars and anhydrous sugars, including levoglucosan, and phenolic compounds like pyrolytic lignin and phenols resulting from the thermal degradation of lignocellulosic constituents.

Additionally, bio-oil contains various polycyclic aromatic hydrocarbons (PAHs), which lack organoleptic properties but possess carcinogenic characteristics that pose health risks. The group of PAHs that are carcinogenic are organic compounds with two or more fused aromatic rings, namely some of the benzene group or containing nitrogen. Bio-oil produced through pyrolysis is often referred to as crude bio-oil. Bio-oil is a dark brown, viscous liquid with a pungent odor in its crude form. For various applications, purification is essential to eliminate or minimize carcinogenic elements like PAHs and reduce water content from source materials and dehydration processes in biomass pyrolysis. Various methods for purifying bio-oil include distillation, filtering, adsorption, extraction, evaporation, and crystallization. Researchers frequently employ filtration, adsorption, or distillation, individually or in combination, to purify bio-oil**.** Pedersen et al. (2018) stated that the distillation method is a way to separate a complex bio-oil mixture into fractions containing compounds of similar chemical structure based on their boiling points. The filtration method separates the remaining harmful substances to produce clear and odorless bio-oil due to the influence of zeolite and activated charcoal [51]. Following purification, researchers categorize crude bio-oil into three groups: grade 3, grade

2, and grade 1 bio-oil. Color, pH, density, Total Phenolic Content, compound content (GC-MS analysis), and antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli* bacteria depict the attributes of bio-oil grades 3, 2, and 1. The outcomes of these characteristics aid researchers in recognizing the antimicrobial advantages of the bio-oil group, thereby enabling its potential application as a natural preservative, contingent upon the required proportion.

## 2. RESEARCH METHODS

### 2.1 Process of pyrolysis

The sample used in this study was a durian shell from Kendari City, West Kendari District, Southeast Sulawesi. A quantity of 500 grams of dried DS was measured and introduced into a pyrolysis reactor featuring a 20 cm diameter and 50 cm height, possessing a total capacity of 0.157 $dm^3$. The pyrolysis process occurs without or low oxygen and any additional catalyst within a temperature range from 330–600ºC, with a heating rate of 6ºC per minute [32]. An automatic system manages the heating procedure. An independent thermocouple, positioned directly at the charge's center, measures the charge's temperature. The heating process was halted after reaching the maximum temperature, and observations continued until condensation was complete.

### 2.2 Purification of crude bio-oil

Bio-oil is collected after it reaches a heating temperature of 150 to 200ºC. The crude bio-oil is filtered in stages with activated zeolite and carbon at a 5:1 (w/w) ratio. Grade 2 bio-oil is the term used for this filtrate. Subsequently, the distillation stage follows. To purify Grade 2 bio-oil, the fractional distillation process is employed. Bio-oil collection occurs once the heating temperature of 150 to 200ºC is attained. Bio-oil resulting from distillation is called grade 1 bio-oil. This research is a modified method from previous research by Mashuni et al. (2019) [32] and Pedersen et al. (2020) [51].

### 2.3 Total phenolic content and compounds of bio-oil

The Folin-Ciocalteu (FC) method, as described in Mashuni's 2019 research, was used to measure the total phenolic content (TPC) [65]. It involved using a UV-Vis spectrophotometer at a maximum wavelength of 765 nm, with gallic acid as a standard solution. The concentration of TPC was quantified in units of g/L.

GC-MS analysis was conducted using a Thermo Scientific Trace 1300 GC/ISQ instrument for compound analysis. One µL of bio-oil (grades 1 and 2) was introduced into the GC-MS system, utilizing Electron Impact (EI) ionization at 70 eV. The injector and detector temperatures were set at 290°C, while the column temperature ranged from 70 to 280°C. The column, with a length of 30 m and a diameter of 25 mm, had a controlled temperature increase of 5°C per minute. Helium, with a 60 mL/min flow rate, was used as the carrier gas.

### 2.4 Antibacterial activity and fish preservative test

Antibacterial activity testing against Staphylococcus aureus and Escherichia coli bacteria follows the test method in Mashuni's research (2022) [30]. This test uses grade 1 and 2 bio-oil in a concentration of 30%, tetracycline as a positive control, and distilled water as a negative control. The fish preservative test uses the Total Plate Count (TPC) method by counting the number of colonies that grow on the test media. This research used raw fish samples, namely flying fish, obtained from the Kendari fish auction market. The TPC method is based on the method in Mashuni's research (2019) [65].

## 3. RESULTS AND DISCUSSION

### 3.1 Yield of Durian Shells pyrolysis

Pyrolysis is a thermal decomposition process that converts lignocellulosic biomass into carbon-rich solids and liquids without oxygen. The biomass material is heated in a reactor at 330-600ºC without oxygen in this procedure. The pyrolysis reactor tube is linked to the condenser, allowing most of the smoke produced during the heating process to condense into a liquid and tar mixture. The most essential component for pyrolysis products is temperature. The pyrolysis temperature employed in this investigation was 330-600ºC. According to Mashuni et al. (2017) research, the maximum temperature yielding the most bio-oil volume is 600ºC [52]. Figure 1 depicts DS pyrolysis results at temperatures ranging from 330 to 600ºC.

The constituent components of the biomass used strongly influence the production of bio-oil. Different types of biomasses as the primary material for bio-oil production can give different results because each type of biomass has various constituent components. The biomass's water, cellulose, lignin, and hemicellulose content will affect the yield and percentage of the resulting bio-oil compounds [53]. Figure 1 shows an average yield of DS bio-oil of 44.30%. To obtain maximum pyrolysis results in the form of liquid products, high operating temperatures (300-600ºC) with low heating rates are the required pyrolysis operating conditions [54]. Mainly, the composition of the chemical compounds within bio-oil determines their quality, as these compounds serve as quality criteria for characteristic taste and aroma. Testing the quality of bio-oil consists of testing the properties of bio-oil physically and chemically [55].

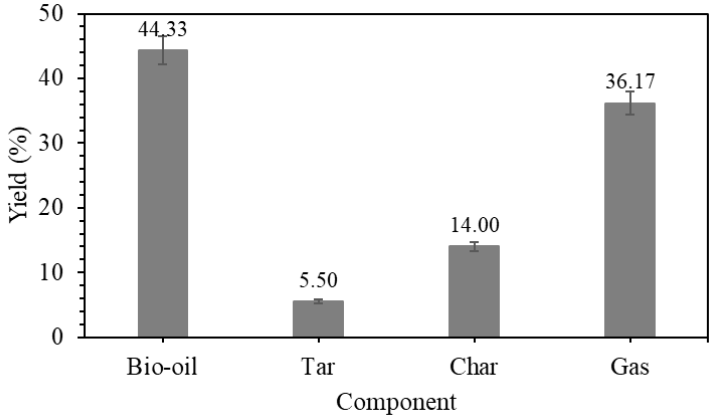


**Figure 1**. Pyrolysis products yield of durian shell

**Table 1**. Physical properties of DS bio-oil

| Parameters | Bio-oil of DS | | |
|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 |
| Total Phenolic Content (g/L) | 0.41 | 0.44 | 2.17 |
| pH | 3.02 | 2.98 | 2.72 |
| Density (g/cm$^3$) | 0.975 | 0.979 | 1.007 |
| Color | Clear | Yellow | Dark brown |
| Flavor | no smell | Slightly smoky | Smokey |

### 3.2 Properties of Physical and Chemical Bio-oil

The composition of its chemical compounds primarily determines the quality of bio-oil, as these compounds serve as quality criteria for the characteristic taste and aroma of the smoke. Testing the quality of bio-oil consists of testing its physical and chemical properties.

#### 3.2.1 The color and flavor of bio-oil

Observers noted a color change in the bio-oil throughout the refining process. Crude bio-oil (grade 3) was dark brown to yellow (grade 2) through filtration. Then, the distillation process produces clear bio-oil (grade 1). The filtration process with zeolite and activated carbon aims to remove tar residue and obtain bio-oil utterly free of harmful substances [56]. According to Supatro (2018), this filtering process causes harmful compounds such as benzopyrene and tar, which are still in the bio-oil, to be adsorbed by the zeolite [57]. Meanwhile, activated carbon can reduce/eliminate the smell of light smoke and not sting [57]. During distillation, high phenolic and carbonyl compounds are separated from carcinogenic compounds like polycyclic aromatic hydrocarbons (PAHs), and tar occurs [58,59]. The color changes that occur are visible in Figure 2 and Table 1. Limitations in the filtration and distillation processes cause a reduction in the yield produced. Also, imperfect separation can occur during pyrolysis due to the temperature used.

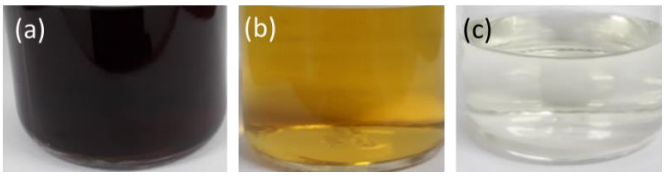


**Figure 2**. Type of bio-oil (a) grade 3. (b) grade 2. (c) grade 1

#### 3.2.2 The pH value of bio-oil

One of the quality parameters of the bio-oil generated is the pH value. If the bio-oil has a low pH value, the quality of the bio-oil generated is high since it affects the shelf life and organoleptic qualities of the bio-oil product [60,61]. Table 1 depicts a fall in the resulting pH value as more components dissolve and generate acidic chemical compounds in the durian skin. The pH value of bio-oil from the pyrolysis process is 3 for wood sources (pine sawdust, mesquite sawdust and wheat shell) [62]. The pH value obtained from the analysis ranged from 2.72 to 3.02. The created bio-oil is of high quality, particularly as an antibacterial and preservative. Microbes and bacteria cannot live and reproduce well in low-pH environments [63].

#### 3.2.3 Density of bio-oil

Density is the ratio between the weight of a sample and its volume. Observing the density of bio-oil from several grades indicates that the sample type does

not change significantly from the density value of bio-oil. The densities of the three bio-oil samples can be seen in Table 1, showing values that are not much different, ranging from 0.975, 0.979, and 1.007 g/cm$^3$. The results were like those of studies using bagasse raw materials with density values of 1.09 g/cm$^3$ grade 3 and 1.05 g/cm$^3$ grade 2 [62].

### 3.2.4 Total Phenolic Content of bio-oil

The oxidation reaction of the hydroxyl groups of phenolic compounds, which react with FC reagent to form a blue complex compound. In Table 1, the highest Total Phenolic Content was in grade 3 bio-oil of 2.173 g/L. Then, there was a decrease in grade 2 and grade 1 bio-oil, possibly because the time required to obtain phenol compounds was not maximized during the fractional distillation process [64]. The one that affects the distillation process is distillation time. Like the theory of evaporation, heating the liquid can increase the volume of space for the liquid to move so that the bonds between the molecules of the liquid become weak. It will make it easier for the liquid molecules to escape from the group, which is detected as evaporation. So, the longer the operating time, the more steam contained.

## 3.3 Compounds in bio-oil

The results in Table 2 show that the chemical compounds produced by each bio-oil are derivatives of pyrimidine, pyridine, triazole, and carboxylic acid compounds. Grade 1 bio-oil contains 12 main organic compounds, and grade 2 bio-oil contains 17 organic compounds. Bio-oil compounds belong to a group or class of acid compounds, pyridine, pyrimidine, and phenolic compound derivatives. Bio-oil grades 1 and 2 produce the main compounds, namely acetic acid, and 1,4-dimethyl-1h-imidazole. The difference in the compounds produced is probably due to the compounds being absorbed during the filtration process and condensing during the distillation process. Sapruto et al. (2018) stated that activated carbon has a large carbon surface and a porous structure because of its granular shape [57]. During filtration, aromatic compounds with molecular sizes equal to or smaller than the pores have the potential to be absorbed. This mechanism enables the bio-oil to release specific compound components and smoke smell. Within bio-oil grades 1 and 2, numerous compounds, such as pyridine, furan, furfural, acetic acid, and cyclotene, are recognized as contributors to the aroma of bio-oil. Additionally, the chemical composition of the produced bio-oil can be influenced by the temperature and duration of distillation [64].

According to Agustina et al. (2017), a material's organic acids and phenols will determine its potential activity as an antibacterial [65]. Based on research by Suresh et al., the furfural group of compounds can inhibit bacterial growth [66,67]. In addition, a nitrous acid compound, cyclohexyl ester, is an ester compound of cyclohexanol and nitric acid that can be antibacterial [68]. Acetic acid compounds are more potent in inhibiting microbial growth than phenolic compounds [69]. According to Guimaraes and Venancio (2022), acidic compound derivatives significantly influence fungi

**Table 2**. Compounds of grade 1 and grade 2 bio-oil

| No | Compound Name | Molecular Formula | Area % of Bio-oil | |
|---|---|---|---|---|
| | | | Grade 1 | Grade 2 |
| 1. | 1,4-dimethyl-1h-imidazole | $C_5H_8N_2$ | 11.91 | 11.70 |
| 2. | 1,4-pentadien-3-one | $C_5H_6O$ | 1.35 | - |
| 3. | 1,1'-bicyclobutyl | $C_8H_{14}$ | 1.28 | - |
| 4. | 2-cyclohexen-1-one | $C_6H_8O$ | 3.40 | - |
| 5. | 1-cyclopropyl-2-propen-1-one | $C_6H_8O$ | 11.14 | 2.55 |
| 6. | 2-amino-3-hydroxypyridine | $C_5H_6N_2O$ | 2.08 | 1.03 |
| 7. | 5-methyl-pyrimidine | $C_5H_6N_2$ | 6.35 | - |
| 8. | 6-methyl-3-heptyne | $C_8H_{14}$ | 3.13 | 6.83 |
| 9. | 5-methyl-3-heptyne | $C_8H_{14}$ | 1.00 | 3.63 |
| 10. | 2-ethylidene cyclohexanone | $C_8H_{12}O$ | 1.62 | 4.21 |
| 11. | 8-methylene-bicyclo [5.1.0] octane | $C_9H_{14}$ | 1.00 | 0.52 |
| 12. | acetic acid | $C_2H_4O_2$ | 10.37 | 19.24 |
| 13. | propanoic acid | $C_3H_6O_2$ | - | 3.24 |
| 14. | pyridine | $C_5H_5N$ | - | 3.97 |
| 15. | methylene-cyclopentane | $C_6H_{10}$ | - | 8.44 |
| 16. | morpholine | $C_4H_9NO$ | - | 5.21 |
| 17. | 2-methyl-3-hexyne | $C_7H_{12}$ | - | 0.80 |
| 18. | 5-hydroxypyrimidine | $C_4H_4N_2O$ | - | 6.53 |
| 19. | 6-methyl-4(1h)-pyrimidinone | $C_5H_6N_2O$ | - | 4.88 |
| 20. | 3,4-dimethyl-2-cyclopenten-1-one | $C_7H_{10}O$ | - | 1.57 |
| 21. | 2,2-dimethyl-3-heptyne | $C_9H_{16}$ | - | 10.52 |

**Table 3**. Antibacterial activity test

| Sample name | Inhibition zone (mm) | | Category |
|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Escherichia coli*** | |
| *Grade* 1 bio-oil | 10 | 11 | Moderate; Strong |
| G*rade* 2 bio-oil | 12 | 17 | Strong; Strong |
| Positive control | 18 | 23 | Strong; very strong |
| Negative control | 0 | 0 | Not inhibit |

because the more significant the concentration of acidic compounds, the more effectively they inhibit fungi [70]. These compounds could be further studied and potentially used as active ingredients in natural preservatives for food and other applications. The research likely identifies specific compounds in bio-oil from durian shells that exhibit antibacterial activity. These findings contribute to the growing body of research on natural preservatives and could pave the way for developing new and effective safe, sustainable, and environmentally friendly preservatives.

### 3.4 Effectiveness Grade 1 and 2 Bio-oil as antibacterial

#### 3.4.1 Activity against Staphylococcus aureus and Escherichia coli bacteria

Antibacterial testing of bio-oil using diffusion method. The inhibition zone indicates the effectiveness of antibacterial substances. The inhibition zone appears as an explicit or clean area surrounding the wells where the antibacterial substance in the bio-oil diffuses. Table 3 presents the quantitative analysis of the apparent zone diameter of grade 1 and 2 bio-oil against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Table 3 displays the results of the antibacterial activity test. Grade 1 bio-oil, with a 30% dilution concentration, inhibited Staphylococcus aureus bacteria growth, resulting in a 10 mm inhibition zone categorized as moderate. For Escherichia coli bacteria, the inhibition zone was 11 mm, categorized as strong. Grade 2 bio-oil, also diluted at 30%, produced larger inhibition zones than grade 1 bio-oil. Specifically, it yielded a 12 mm inhibition zone categorized as strong against Staphylococcus aureus bacteria and a 17 mm inhibition zone categorized as potent against Escherichia coli bacteria. Categorized inhibition as extreme when the zone diameter exceeded 20 mm, strong for diameters falling within the 10-20 mm range, moderate for diameters ranging from 5-10 mm, and weak for diameters less than 5 mm [71]. The negative control, which utilized sterile distilled water as the solvent, showed no inhibition in this antibacterial effectiveness test. In the positive control, tetracycline at a concentration of 1000 ppm demonstrated an 18 mm inhibition zone for Staphylococcus aureus bacteria and a 23 mm zone for Escherichia coli. Tetracycline is known for its broad-spectrum antibiotic properties, encompassing Gram-positive and Gram-negative bacteria. Its mechanism of action involves inhibiting protein synthesis in bacterial ribosomes [72].

Antibacterial compounds exhibit a varied and complex mechanism of action. In addition to damaging bacterial cell walls, carbonyl and phenol compounds can denature proteins, leading to cell death. Furthermore, acetic acid in bio-oil is vital as an antibacterial agent [73]. The cytoplasmic membrane of specific bacterial structures consists of proteins and fats. This instability in the cell wall and cytoplasmic membrane disrupts selective permeability, active transport, and the overall control of bacterial cell structure. Consequently, disrupting cytoplasmic integrity in bacteria releases macromolecules and ions from the cell, leading to bacterial cell shape distortion and lysis [74]. However, the damage primarily involves a loss of integration and damage to the cell envelope structure [75]. The antibacterial mechanism mainly targets the bacterial cell structure. Antibacterial attacks the cytoplasmic membrane, resulting in the destabilization of protons and electrons and the coagulation of cell components [76]. Pasqua et al.'s research (2007) concluded that a reaction occurs between antibacterial and bacterial cells, affecting the structure and shape of bacterial cells [77].

#### 3.4.2 Effectiveness as a fish preservative

The microbial population in food significantly impacts the rate of food ingredient deterioration. According to Yuliana (2015), the primary cause of fish spoilage is the presence of microbes on the gills, skin surface, and surrounding environment [78]. From a microbiological perspective, the rate of fish spoilage depends on the growth of present microbes, particularly spoilage bacteria. Bacterial growth involves an increase in cellular constituents or mass, followed by cell division, increasing the number of cells. TPC is used to determine the number of microbes present in the test material. We conducted this test because bio-oil contains acid, carbonyl, and phenolic compounds, which serve as antimicrobial agents. Antimicrobial substances are

**Table 4**. Total Plate Count

| Sample name | TPC on days (CFU/g) | | |
|---|---|---|---|
| | **1** | **3** | **5** |
| P1 | $3.2 \times 10^5$ | $3.5 \times 10^5$ | $1.88 \times 10^6$ |
| P2 | $4.2 \times 10^5$ | $3.9 \times 10^5$ | $1.65 \times 10^6$ |
| P3 | $1.34 \times 10^6$ | $1.29 \times 10^6$ | $1.08 \times 10^6$ |
| P4 | cannot be calculated | | |

*P1= bio-oil and storage in the cooler; P2= bio-oil; P3= without bio-oil and refrigerated storage; and P4 = without treatment

biological or chemical compounds that can inhibit the growth and activity of microbes; these substances can be bactericidal (kills bacteria), bacteriostatic (inhibits bacterial growth), fungicidal (kills mold), and fungistatic (inhibits mold growth), inhibits the germination of bacterial spores.

In this study, we are testing the activity of bio-oil as a fish preservative using grade 2 bio-oil, which is typically employed as a food preservative. According to research by Anggraini and Yuniningsih (2017), bio-oil grade 2 has a longer shelf life (2 days) than without bio-oil [79]. The shelf life of fish in this study used controls of 1, 3, and 5 days, with several types of treatment. The total bacteria in the process of using grade 2 bio-oil as a fish preservative are in Table 4.

Table 4 shows that the Total Plate Count (TPC) increased from the first to the fifth day across all treatments. Samples stored in the cooler and those with the addition of grade 2 bio-oil exhibited notably lower TPC compared to samples with grade 2 bio-oil but not stored in the cooler, as well as other treatments during storage. This suggests that a 30% grade 2 bio-oil has a natural inhibitory effect on microbial growth. Significantly, the TPC values for samples P1 and P2, even up to the fifth day, did not exceed the government-mandated maximum bacterial colony threshold for fish, specified by SNI number 01–2332–3–2006, which is $5 \times 10^5$ CFU/g. Fish remains categorized as fresh when bacterial counts stay below this maximum threshold. However, sample P3 (without bio-oil and refrigeration) did not meet the predetermined maximum threshold value for the first and third days. This occurred because the TPC levels in the sample were $1.34 \times 10^6$ CFU/g on the first day and $1.29 \times 10^6$ CFU/g on the third day, surpassing the established threshold. On the other hand, the TPC value for the P4 sample (without any treatment) suggested that the colony count could not be calculated from the first to the fifth day.

According to Husni et al. (2015), the number of bacteria in fish meat during initial storage is similar but will increase with the length of storage [80]. Susanto et al. (2011) discussed that while low temperatures can hinder bacterial development, they cannot halt bacterial growth completely [81]. This is because bacteria must adapt to their environment before they reproduce. Bacteria that grow at low-temperature storage are psychrophilic because they grow well at temperatures of –7ºC to 10ºC [82]. This is based on research by Anggraini and Yuniningsih (2017) explaining that the substances contained in bio-oil include formaldehyde, acetaldehyde, carboxylic acids (formic, acetic, and butyric acids), phenol, cresol, primary and secondary alcohols, ketones. etc., can inhibit the activity of bacteria (bacteriostatic) [79]. The test results generally show good effectiveness against bacteria and preservatives in fish. However, the chemical composition of bio-oil contains a complex mixture of compounds, including phenolics, aldehydes, and organic acids, some of which may be toxic at specific concentrations. In addition, the effect of bio-oil is concentration-dependent, with its toxicity varying based on the amount used and the length of exposure. Higher concentrations or doses may cause adverse effects, although lower doses are considered safe.

## 4 CONCLUSIONS

Bio-oil resulting from the pyrolysis of durian shell biomass is purified using filtration and distillation methods. It contains compounds that exhibit antibacterial properties. The antibacterial activity of bio-oil grades 1 and 2 against Staphylococcus aureus and Escherichia coli at a 30% concentration falls into the category of strong inhibition. They suggest that bio-oil grades 1 and 2 serve as natural preservatives. However, grade 2 bio-oil is typically used as a preservative for raw food ingredients. Grade 2 bio-oil is effective as a natural preservative, inhibiting bacterial growth in fish samples.

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