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ANTIBACTERIAL ACTIVITY OF BIO-OIL FROM OIL PALM EMPTY FRUIT BUNCHES AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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ABSTRACT. Empty fruit bunches (EFB) rich in lignocellulose are produced as waste in the palm oil processing industry. Although abundant, they have not been fully utilized. EFB waste has the potential to be used as a chemical feedstock for bio-oil production through pyrolysis. Bio-oil contains phenolic compounds that are antimicrobial and have economic value. This study aimed to investigate the effects of variations in pyrolysis temperature and time on the phenol content in bio-oil, evaluate the antimicrobial activity of bio-oil against bacteria, and qualitatively identify the chemical compounds that make up bio-oil using GC-MS. The pyrolysis process was varied at temperatures of 300, 350, and 400°C, with times of 45 and 60 min. The highest bio-oil yield was obtained at 400 °C for 60 min (33.51±7.18%), whereas the highest total phenolic content was obtained at 300 °C for 45 min (15.06±4.90%). Antibacterial activity varied with pyrolysis conditions and bio-oil concentration. Pyrolysis at 400°C for 45 min and 60 min were identified as the potential conditions, as they produced bio-oil with the largest significant antimicrobial activity against E. coli (inhibition zone of 8.08 mm) and S. aureus (inhibition zone of 8.56 mm). GC–MS analysis identified phenols, alcohols, esters, ketones, aldehydes, carboxylic acids, and ethers as major compound groups. These findings demonstrate that pyrolysis conditions strongly influence the yield and chemical composition of EFB bio-oil and its antibacterial activity

INTRODUCTION

Oil palm is a leading primary plantation commodity in Indonesia. The main products of oil palm plants are crude palm oil (CPO) and palm kernel oil (PKO), both of which have higher economic value than other plantation commodities. Oil palm plays a fundamental role in Indonesia, with a total plantation area by province of approximately 16 million hectares in 2024 and an oil palm production yield of 47.5 million tons per (*Direktorat Statistik Tanaman Pangan, Hortikultura and Perkebunan*, 2025). The palm oil industry can support national plantation development and create employment opportunities that enhance welfare of the community.

In the process, the palm oil industry produces solid waste in the form of empty palm fruit bunches (EFB) from fruit harvesting stations, fibers, and shells from fruit peeling, and liquid waste in the form of sludge from oil refining stations, as well as air waste produced from boiler chimneys or generator machine exhausts (Azzahro *et al*., 2022). Each ton of fresh fruit bunch (1 ton of FFB) of oil palm yields approximately 228 kg of the main product, crude palm oil (CPO), while the rest produces 384 L (38%) of liquid waste palm oil mill effluent (POME), 178 kg (18%) of FFB, 95 kg (9.5%) of fiber, and 54 kg (5.4%) of palm kernel shell waste. Increasing palm oil productivity increases the amount of unused biomass waste that can pollute the environment. An FFB capacity of 802,800 tons/year is estimated to produce 561,100 tons/year of solid and liquid palm oil waste, or approximately 1,537.26 tons/day (Yusuf *et al*., 2025). Empty palm fruit bunches are the largest solid waste from the palm oil industry and contain high levels of organic compounds such as cellulose, lignin, holocellulose, hemicellulose, water, and other extractive substances. If not properly processed, these compounds can cause foul odors in the

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environment due to the formation of NH_3, H_2S, CH_4, and other odorous gases (Suprihatin *et al*., 2024).

Pyrolysis is a thermochemical conversion process in which biomass (C, H, and O) undergoes thermal decomposition into liquid, solid, and gaseous products at high temperatures in an oxygen-limited environment to prevent combustion of the biomass. The liquid product, known as bio-oil or bio-crude, is a dark brown, viscous fluid with a relatively high density and is composed of a complex mixture of oxygenated organic compounds (Gasification *et al*., 2020).

Bio-oil is a distillation liquid obtained through a high-temperature heating process, followed by vapour condensation to produce a liquid. Bio-oil can be produced from the pyrolysis of biomass, such as coconut shells, coconut fibers, coconut trunks, wood sawdust, empty palm fruit bunches, and other biomass. Bio-oil contains phenol and acetic acid, which can function as antioxidants, antiseptics, and antibacterial agents through mechanisms involving the denaturation of cell wall proteins and disorganization of the cytoplasmic membrane. Phenolic compounds with hydroxyl (−OH) groups can interact with membrane lipids and proteins, disrupting cellular structural stability and reducing selective permeability. These interactions can result in intracellular leakage, a decrease in the proton gradient, and disruption of metabolic processes. This ultimately leads to cell lysis and bacterial death (Mendel and Philip, 2003; Oulahal and Degraeve, 2022).

The objectives of this study are (1) to determine the effect of pyrolysis temperature (300°C, 350°C, and 400°C) and pyrolysis time (45 min and 60 min) on empty oil palm fruit bunches (EFB) on bio-oil yield and quality (colour and total solids), (2) to evaluate the effectiveness of pyrolysis bio-oil against *Staphylococcus aureus* and *Escherichia coli* bacteria through testing variations in phenol concentration, and (3) to identify the content of bioactive compounds using GC-MS analysis.

The novelty of this research lies in the integrated analysis of the chemical composition and biological activity of bio-oils. This study quantified the total phenolic content and correlated the phenolic compounds identified by GC–MS with antibacterial inhibition against Staphylococcus aureus and Escherichia coli. This study differs from those of Farina Nury *et al*. (2022) and Rezki *et al*. (2023), which focused on the yield and physicochemical characterization of bio-oil without evaluating its biological activity. Therefore, this study contributes to the literature by demonstrating the potential of empty palm fruit bunches as a source of active phenolic compounds for the development of natural antibacterial agents.

MATERIAL AND METHODS

Time and Place of Research

This study will be conducted from November 2024 to May 2025. The sampling was conducted in PKS, Banten, Indonesia. Bio-oil production was performed at the Surfactant and Bioenergy Research Center (SBRC)-IPB Bogor. Total phenolic testing and chemical compound analysis were conducted at the Chemical Engineering and DIT laboratories of IPB in Bogor. GC-MS analysis was performed at the Biofarmaka Laboratory of IPB University.

Research Equipment and Materials

The equipment used in this research included an autoclave, aluminium cups, porcelain cups, desiccators, a Shimadzu GC-MS with an HP-5MS UI Agilent column (30 m × 0.25 mm, i.d., film thickness 0.25 μm) using helium as the carrier gas with an injector temperature of 250 °C, and a set of pyrolysis machines, chemical analysis, and microbiological testing equipment.

The materials used were distilled water, alcohol, bio-oil samples, boric acid, Cu2+ catalyst, empty palm fruit bunches (EFB), dichloromethane, filter paper, Folin-Ciocalteu solution, gallic acid, hexane, $K_2Cr_2O_7$, NaOH, Na_2S_2O3 solution, HCl solution, Na_2CO_3, syringe, injection filter, nutrient agar nutrient broth, PP indicator, spiritus, sulfuric acid, Whatman No. 41 and 42 filter paper.

Data Sources

Primary data were obtained directly from laboratory experiments, whereas secondary data were obtained from various publications and official agencies, including *Direktorat Statistik Tanaman Pangan, Hortikultura, dan Perkebunan* (2025) and Yusuf *et al*. (2025). The compounds were identified using GC-MS data based on the NIST Mass Spectral Library accessed via PubChem (Medicine, 2024).

Experimental Design

A completely randomized design with a 3 × 2 factorial arrangement was used, with pyrolysis temperature (300, 350, and 400 °C) and residence time (45 and 60 min) as the experimental factors. Each treatment was conducted in three independent replicates. Antibacterial activity was evaluated using a

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completely randomized design with bio-oil concentration as the treatment factor, followed by Tukey's HSD test when appropriate. Statistical significance was set at $p < 0.05$.

Data Analysis

The data analysis in this study consisted of two stages: (1) bio-oil production from oil palm empty fruit bunches (OPEFB) through pyrolysis, analyzed using a Completely Randomized Design (CRD) with a two-way factorial arrangement, and (2) antibacterial activity testing against Staphylococcus aureus and Escherichia coli using a one-way CRD followed by Tukey's HSD post hoc test. Statistical significance was set at $p < 0.05$.

Research Implementation

The stages of the research are presented in Figure 1.

Bio-Oil Production Using the Pyrolysis Method

The prepared EFB sample was placed in a pyrolysis reactor purged with nitrogen gas (5 bar) to maintain an inert atmosphere. The reactor was heated to target temperatures of 300, 350, and 400°C, and maintained for 45 or 60 min. The condensable vapours were collected in the condenser as bio-oil, while the solid residue (bio-char) remained in the reactor. After finishing, the system was cooled under nitrogen flow, and the bio-oil and bio-char yields were measured.

$$\text{Bio Oil Yield or Bio Char} = \frac{\text{biochar weight (g) or bio oil weight (g)}}{\text{sample weight (g)}} * 100\%$$

Antibacterial Assay of *S. aureus* and *E. coli*

The disc diffusion method was used, which paper discs were impregnated with a 70% alcohol solution and bio-oil solutions (phenol concentrations: 1%, 1.5%, and 2%) for 15 min. Then, put the discs on agar media inoculated with *S. aureus* or *E. coli*. The samples were incubated for 24 hours at 37°C, and the inhibition zones were observed to determine the size of the inhibition zones formed. This was performed to determine the sensitivity or resistance of the test organisms to the antibacterial agents. The test was performed in triplicate, and the average inhibition zone was determined. The diameter of the inhibition zone was measured in millimeters.

RESULTS AND DISCUSSION

The Effect of EFB Pyrolysis Temperature and Time on Bio-char, Bio-oil, and Total Phenol Yields

Temperature greatly influences the conversion of products resulting from pyrolysis. In this study, a low pyrolysis temperature of 300°C was used because, at this temperature, EFB powder is mainly converted to biochar, whereas at temperatures below 300°C, cellulose has not yet degraded into biochar. The differences in the degradation temperature stability of cellulose, hemicellulose, and lignin have been described by Kalina *et al*. (2022). Hemicellulose degrades at temperatures of 220–315°C, cellulose at 315–400°C, while lignin, with its complex structure, undergoes gradual degradation over a wider temperature range, specifically 160–900°C. A temperature of 400°C was chosen as the maximum pyrolysis temperature because increasing the temperature and pyrolysis residence time tends to increase gas production (CO, CO_2, H_2, C_1-C_4) or cause polymerization into tar/liquid char, thereby decreasing the yield of bio-char and bio-oil (Hyeongtak Ko *et al*., 2025).

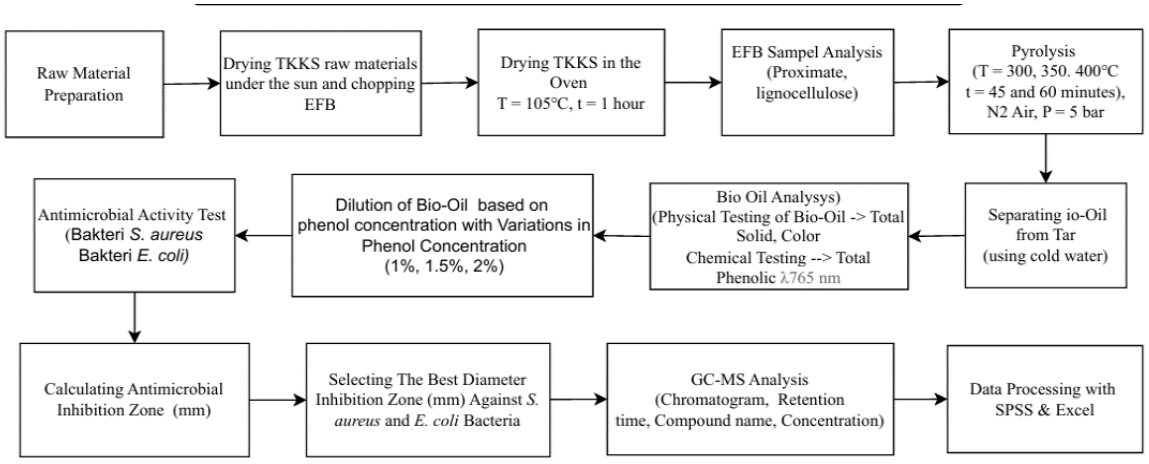

Figure 1. Flow chart of bio-oil production process from EFB Waste

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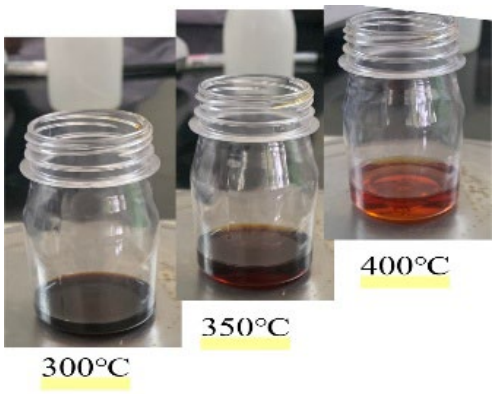

Figure 2. Physics of Bio-oil

This finding is in line with research conducted by Saipanya *et al*. (2022), who reported that bio-oil reached its optimum point at a temperature of 400°C, and then decreased again at higher temperatures owing to secondary gasification reactions. The pyrolysis residence time was set at 45 min because bio-char formed at the minimum time, and bio-oil production stopped after 60 min of relatively decreased char. In the EFB pyrolysis results, bio-oil with the lowest pyrolysis temperature of 300°C appears dark brown because at low pyrolysis temperatures, the degradation process occurs slowly, and the biomass has not been completely decomposed by heat, resulting in the main by-products of bio-char, more tar, and other heavy substances, with an average calculated solid content of 9.32%. The dark black color tended to fade at a pyrolysis temperature of 350°C, with a solid content of 8.79%. At 400°C. The bio-oil produced at 400°C contained less tar, with a dark brown, clear oil color, and a significant decrease in the total solid content to only 5.91%.

As shown in Table 2, the highest bio-oil yield was produced at a temperature of 400°C for 60 min, whereas the lowest yield of 33.51%, while the lowest yield was produced at a temperature of 300°C for 45 minutes, with a yield of 12.29%. This trend is shown in Figure 3, where higher pyrolysis temperatures result in increased bio-oil yields, whereas the bio-char yields decrease. These results are consistent with the study by Rezki *et al*. (2023), where the highest bio-oil yield was produced at 400°C, and the highest bio-char yield was produced at 300°C. The total phenolic content was analyzed using the Folin–Ciocalteu method with a UV-Vis spectrophotometer at a maximum wavelength of 765 nm. This technique is widely applied to measure the total phenolic content in products derived from lignocellulosic biomass, such as bio-oil, owing to its ability to comprehensively measure phenolic compounds through a redox reaction between the Folin and Ciocalteu reagents and aromatic hydroxyl groups, forming a stable blue complex.

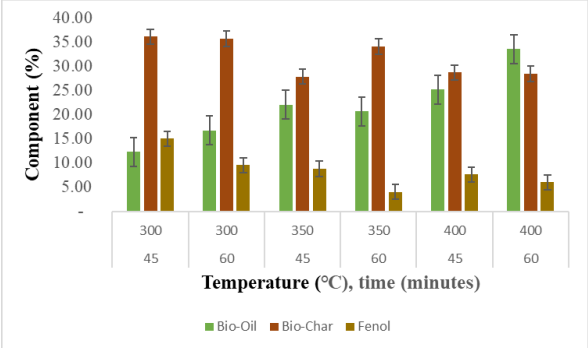

Figure 3. Effect of EFB pyrolysis temperature and time on bio-char, bio-oil, and total phenol yield

The wavelength of 765 nm was chosen because the molybdenum blue complex produced showed maximum absorbance at this wavelength, providing optimum measurement sensitivity and accuracy. The Folin-Ciocalteu method is rapid, produces minimal error (<5.8%), and has low uncertainty (±1.1%) at a 95% confidence level, yielding results comparable to those obtained with the liquid-liquid extraction method e.

Table 2. Effect of pyrolysis temperature and time on bio-oil, bio-char, and phenol yield

Temperature (°C)	Time (minutes)	Bio-Oil (%)	Bio-Char (%)	Phenol (%)
300	45	12.29±0.91	36.09±0.31	15.06±4.90
300	60	16.75±2.63	35.63±0.97	9.57±3.06
350	45	22.06±5.02	27.87±5.33	8.81±1.00
350	60	20.68±1.20	34.10±2.02	4.05±1.17
400	45	25.19±2.71	28.70±2.16	7.63±252
400	60	33.51±7.18	28.45±1.20	6.06±1.15

Note: The results presented are the averages of three replicate pyrolysis treatments.

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The Tukey HSD test results showed that temperature had a significant effect on the total phenol content (p = 0.010), as did pyrolysis time (p = 0.010), where higher temperatures and longer pyrolysis times resulted in lower total phenol levels. However, the interaction between temperature and pyrolysis time had no significant effect (F interaction temperature × time = 1.268, p = 0.316), indicating that the effect of pyrolysis temperature on total phenol content remained stable at both 45 min and 60 min of pyrolysis time. The pyrolysis time did not increase the phenol content; rather, it played a role in further degradation, which was influenced by the pyrolysis temperature. The interaction between temperature and time, which did not have a significant effect, can be interpreted as meaning that the optimization of phenol content is determined by the selection of temperature and time separately, without the need for their combination. These results are in line with similar studies on other palm kernel shells, where extending the pyrolysis time accelerated the conversion of phenols into non-phenolic aromatic products. The highest total phenol content was produced at a temperature of 300℃ for 45 min (15.06%), whereas the trend decreased significantly, with the lowest value at a pyrolysis temperature of 350℃ for 60 min (4.05%).

The data indicate that an increase in pyrolysis temperature from 300℃ to 350℃ can significantly reduce the phenol content (p = 0.012), while the results return to stable or slightly increase (p = 0.830) at a pyrolysis temperature of 400℃ for 45 min (7.63%) and 60 min (6.06%). The sharp decrease in total phenol content is thought to be due to secondary reactions in the vapour phase in the form of cracking/deoxygenation of phenolic compounds formed from the lignin depolymerization process, which increases significantly at a temperature of 350℃, resulting in the formation of light gases such as CO, CO_2, and H_2O, as well as simple non-phenolic aromatics (Waters *et al*., 2017).

This difference indicates that phenol is relatively stable at low pyrolysis temperatures and further decomposes into several derivatives, such as guaiacol and cresol, at higher temperatures than those of the other two. Treatment for 45 min tended to produce higher total phenol levels than 60 min, regardless of the pyrolysis temperature.

Bacterial Inhibition Test

The total phenolic concentration was quantitatively measured from bio-oils using a spectrophotometer because phenol is the main product of lignin decomposition. The measured total phenol concentration was then used to calculate the phenol concentration dilution before conducting the bacterial inhibition test. The total phenol concentration dilution is necessary because at low concentrations (0.1–1% as bacteriostatic and 1–2% as bactericidal), phenol exhibits effective classical antimicrobial properties. In contrast, at a concentration of 5%, it can kill spores. (Juszkiewicz, *et al.,* 2019). In addition to its effectiveness, a phenol concentration of 1–2% was selected for dermal screening. According to the National Institute for Occupational Safety and Health (NIOSH, 2011). Exposure to phenol concentrations exceeding 2% can increase skin permeability, disrupt the protective epidermal barrier, and potentially cause chemical burns in humans. Therefore, a natural antiseptic formulation containing 1–2% phenol is considered to achieve an optimal balance between antimicrobial efficacy and dermal screening. *Escherichia coli* and *Staphylococcus aureus* were selected as representative Gram-negative and Gram-positive bacteria, respectively, for microbiological tests to provide different antimicrobial responses (Lv *et al*. 2024).

Table 3. Inhibition test of bacteria

Pyrolysis Temperature (℃)	**Pyrolysis Time (Minutes)**	**BO's Inhibition Effect (mm) on *E. coli* with Phenol Concentration (%)**			**BO's Inhibition Effect (mm) on *S. aureus* with Phenol Concentration (%)**		
		1.0	1.5	2.0	1.0	1.5	2.0
300	45	2.00±0.54	3.13±0.87	2.81±0.94	2.49±0.92	2.78±1.07	3.44±0.44
300	60	2.20±1.20	2.29±1.36	3.39±0.68	1.44±0.31	3.06±0.56	4.83±0.92
350	45	3.19±1.10	4.03±0.28	5.14±0.95	2.17±0.31	3.13±0.37	4.25±1.00
350	60	3.29±0.68	5.56±0.31	7.08±1.00	2.75±0.25	3.88±1.48	4.83±1.24
400	45	1.63±0.50	3.42±0.21	8.08±1.25	4.50±1.57	6.63±0.10	8.50±4.50
400	60	3.38±0.89	4.88±0.89	4.63±0.62	3.58±0.96	2.81±0.69	8.56±1.80

Note**:** Data represent mean inhibition zone diameters (mm) from three experimental replicates (n = 3).

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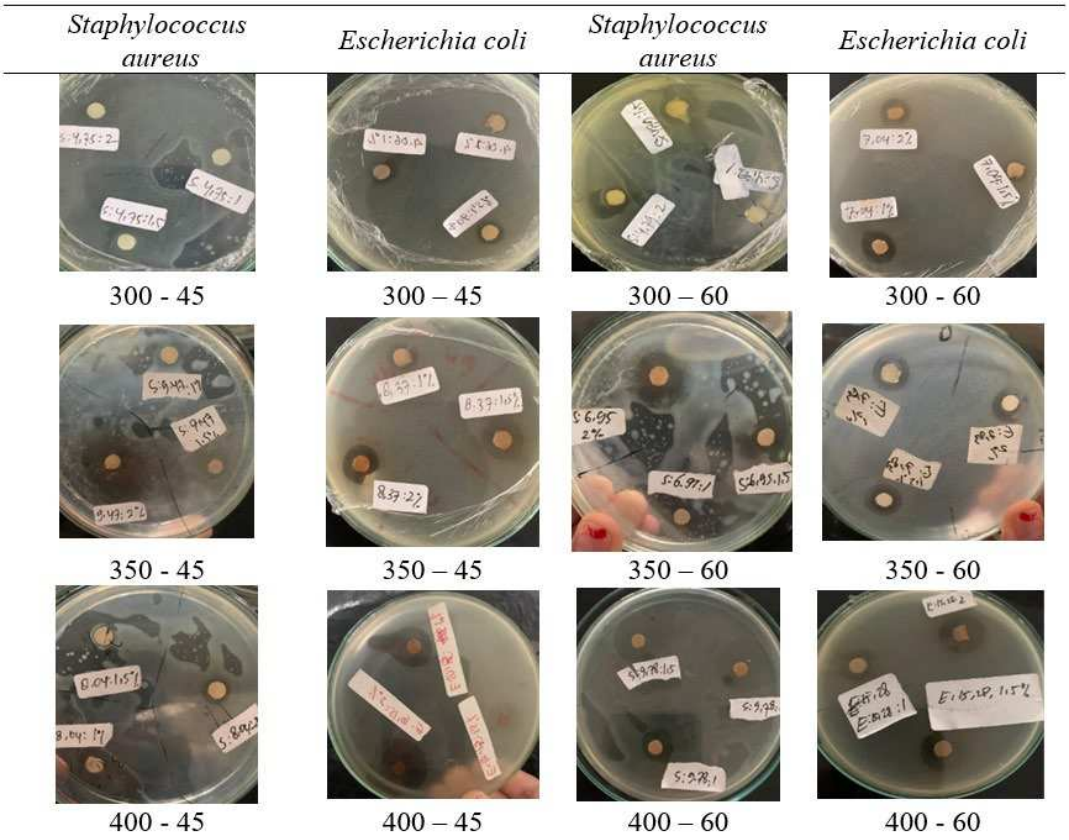

Figure 4. Visual Representation of bacterial inhibition zones at different pyrolysis temperatures (T = °C) - Times (t = minutes)

The dilution of the total phenol concentration was calculated from the total phenol concentration obtained from spectrophotometer measurements (Table 2), then diluted using the formula V1*C1 = V2*C2 with sterile distilled water as the diluting medium to concentrations of 1%, 1.5%, and 2%. The total phenol from the diluted bio-oil was then tested for inhibitory activity against *E. coli* and *S. aureus* bacteria on Nutrient Agar medium

The results of the bacterial inhibition test are shown in Figure 4. Figure 4 presents representative inhibition zones for *S. aureus* and *E. coli* obtained from bio-oil produced under various pyrolysis conditions. Larger inhibition zones were observed at higher pyrolysis temperatures, particularly at 400°C for 45 min, indicating stronger antibacterial activity. The bio-oil inhibition test was conducted at phenol concentrations of 1%, 1.5%, and 2%. The results of the bio-oil inhibition test showed that the highest inhibition against *E. coli* was 8,08, which came from a bio-oil sample with a phenol concentration of 2% at a pyrolysis temperature of 400°C for 45 min, while for *S. aureus*, the inhibition value was 8.56 mm at a temperature of 400°C for 60 min. Based on the analysis of variance with Tukey HSD, it was found that the inhibition test against *E. coli* with a 1% bio-oil concentration was significantly different from 1.5% (p = 0.017) and 2% (p = 0.000), and 1.5% was also significantly different from 2% (p = 0.002).

Meanwhile, the results of the inhibitory test against *S. aureus* showed that the 1% phenol concentration was not significantly different from 1.5% (p = 0.409), but was significantly different from 2% (p = 0.001). The 1.5% was also significantly different from 2% (p = 0.024). The results of the inhibition test with varying phenol concentrations showed different responses for Gram-positive and Gram-negative bacteria. The higher the concentration, the wider the inhibition zone for both *S. aureus* (a Gram-positive bacterium) and *E. coli* (a Gram-negative bacterium).

These results also indicate that the effectiveness of phenol as an antibacterial agent in EFB bio-oil depends on its concentration. After conducting inhibition tests with varying phenol concentrations, four samples with the experimental replicates' inhibition values for *S. aureus* and *E. coli* were selected for further analysis. The selected samples were derived from the pyrolysis of EFB bio-oil at 400°C for 45 min (sample replicates A1, A2) and 400°C for 60 min (sample replicates B1, B2).

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Table 4. Antibacterial test values of four selected samples

	Sample	*E. coli* Bacteria	*S. aureus* Bacteria
Inhibition Zone (mm)	A1	10.50	13.00
	A2	5.17	4.00
	B1	4.13	9.88
	B2	5.63	7.25

Note: A1 and A2 are replicates of the treatment carried out at 400 °C for 45 min ($\bar{x}$ *E. coli* =7.83 mm, $\bar{x}$ *S. aureus* = 8.50 mm), whereas B1 and B2 are replicates of the treatment carried out at 400 °C for 60 min ($\bar{x}$ *E. coli* = 4.88 mm, $\bar{x}$ *S. aureus* = 8.56 mm).

On average, the bio-oil inhibition zone against *E. coli* was greater than that against *S. aureus*; however, in some individual treatments, the results were the opposite (Table 3, where *S. aureus* had a greater inhibition zone than *E. coli*). This difference is likely due to a combination of factors, such as physiological variations (bacterial growth phase) and differences in the composition of bio-oil between the samples subjected to pyrolysis. Antibacterial activity does not always correlate directly with the total phenolic content in bio-oil under all pyrolysis conditions. Pyrolysis temperature plays a major role in the production of phenols and their derivatives, and the composition and relative abundance of these phenolic compounds influence the antimicrobial activity.

Bio-oil is a complex liquid containing a mixture of phenols, aldehydes, ketones, esters, ethers, and alcohols (Figure 2), the combined activity of which can affect bacterial inhibition. Therefore, compositional analysis (GC-MS) is necessary to identify compounds with antimicrobial activity against Gram-positive or Gram-negative bacteria. Table 4 shows that the inhibition zone values for *S. aureus* were higher than those for *E. coli*. In bio-oil samples resulting from pyrolysis at 400 °C for 45 min (B1 and B2), the average inhibition zone for *S. aureus* was 8.50 mm, which was greater than that for *E. coli* (7.83 mm). The inhibition zone under these conditions was also more effective than under other pyrolysis conditions, producing an average inhibition zone of 8.56 mm for *S. aureus* and 4.88 mm for *E. coli.*

The difference in antibacterial activity correlated positively with the total phenol content of the bio-oil samples (Figure 5). Samples B1 and B2 had a higher phenol content (35.6%) than A1 and A2 (32.76%), indicating that an increase in phenol content has the potential to increase the effectiveness of inhibition against both test bacteria. This phenomenon may be due to the fact that not only does the total phenol content play a role, but also specific types of phenol in sample A1 that are more active. The GC-MS analysis results showed that sample A1 contained phenol, 2-methoxy- (4.66%) > B1 (4.03%), phenol-2,6-dimethoxy/syringol A1 (4.91%), B1 (6.24%), and creosol A1 (0.26%), whereas B1 did not contain creosol. Based on the GC-MS data, sample A1 contained phenolic compounds and their derivatives with a more diverse range of active phenols. Phenols and their derivatives, such as creosol, syringol, and guaiacol, can damage cell membranes, denature proteins, and inhibit enzymes (Oulahal and Degraeve, 2022; Tang *et al.,* 2022). These compounds are generally effective against Gram-positive bacteria, such as *S. aureus*, because they lack an outer membrane layer and have a thick but more permeable peptidoglycan cell wall, allowing phenolic compounds to enter, diffuse, and damage the cell more easily (Friedman *et al.,* 2003). Inhibition tests with *E. coli* yielded relatively smaller inhibition zone values compared to almost all other data points, because *E. coli* can increase its tolerance to phenolic compounds through changes in membrane fatty acid composition. Gram-negative bacteria have a cytoplasmic membrane surrounded by a thinner peptidoglycan and an outer membrane consisting of a hydrophilic lipopolysaccharide (LPS) layer, which acts as a barrier that can resist and inhibit the diffusion of hydrophobic phenolic compounds (Keweloh *et al.,* 1991). These results indicate that *S. aureus* is more sensitive to phenol-based bio-oil than *E. coli*.

GC–MS analysis was conducted only on bio-oil samples obtained at 400 °C for 45 and 60 min, as these conditions produced the highest bio-oil yield with clearer liquid quality and the lowest tar content (5.31%), allowing for easier application without the need for complex filtration. These samples also exhibited the most effective antibacterial activities. A temperature of 400 °C is the optimal point for lignin degradation, resulting in a higher proportion of phenolic compounds, such as guaiacol, syringol, and cresol, which possess strong antimicrobial properties (Rover and Brown, 2013). Additionally, this condition was selected for its cost efficiency and to focus the study on the qualitative identification of the dominant phenolic compounds. The phenolic profiles identified by GC–MS were subsequently used to reinforce the relationship between the higher total phenolic content and enhanced antibacterial effectiveness observed in both samples.

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Identification of Compound Groups from GC-MS Analysis

The bio-oil samples (A1, A2, B1, and B2) were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS, Agilent 7890A coupled with Agilent 5975C MS detector) to qualitatively identify the compound classes. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm; 5% phenyl–95% methylpolysiloxane) with helium as the carrier gas (1.0 mL min^{-1}). The injection was performed in split mode (20:1) at 250 °C, with the MS transfer line at 280°C. The oven temperature was programmed to 40°C (3 min), then raised to 150°C at 2°C min^{-1}, followed by a rise to 280°C at 10°C min^{-1} and held for 3 min. Ionization was conducted using EI at 70 eV with a scan range of m/z 35–550. Dichloromethane (CH_2CL_2) was selected to dissolve and extract organic compounds from the bio-oil because of its moderate polarity and low boiling point, which allows efficient vaporization without residue. Compound identification was performed using the NIST library with a 1000 ppm alkane standard for the % match and retention index determination. Seven dominant groups, namely phenols, alcohols, ketones, carboxylic acids, aldehydes, esters, and ethers, were classified based on the identified compounds. As shown in Figure 5, sample B1 (39.1%) contained the highest amount of total phenolic compounds and their derivatives, whereas sample B2 (26.42%) had the lowest amount.

PCA Analysis

Principal Component Analysis (PCA) was used to reduce data complexity and identify the dominant chemical groups responsible for the variation in samples (Table 5). PCA was used to identify the distribution patterns of compounds in the four samples, allowing the determination of which compound groups contributed most to the differences in the characteristics of each sample. In summary, Principal Component Analysis (PCA) reduces the complexity of multivariate data by transforming them into a few principal components, thereby facilitating a clearer visualization of the relationships among samples and the contribution of dominant compound groups. PCA analysis produced two principal components (PC1 and PC2) that explained the sample, with PC1 having a variance proportion (Initial Eigenvalues) of 56.85%, while PC2 was 30.97%, so the total data diversity was 87.82%. The PC1 bi-plot presents the differences in the composition of phenols, carboxylic acids, and alcohols (positive quadrant on the right axis), whereas ester compounds are in the negative quadrant on the left. This indicates that the PC1 quadrant (56.85%) is the main axis distinguishing the composition of phenols, carboxylic acids, and alcohols from esters. The PC2 quadrant (30.97%) was influenced by ketones and ethers and was opposite to aldehydes on the lower side. PC2 represents the difference between bio-oil with a high content of ketones and ethers and bio-oil containing many aldehydes. The combined result of 87.82% shows that the distribution of samples and variables in the two quadrants is sufficiently representative to illustrate the differences in the chemical composition between the bio-oil samples.

Table 5. PCA result variation values

Component	Standard Deviation	Proportion of Variance (%)
PC1	3.980	56.85
PC2	2.168	30.97

The PCA bi-plot in Figure 6 shows a clear separation of the chemical groups. On PC1 and PC2. Phenols, alcohols, and carboxylic acids clustered in the positive quadrant, whereas esters, ethers, and ketones clustered in the negative quadrant.

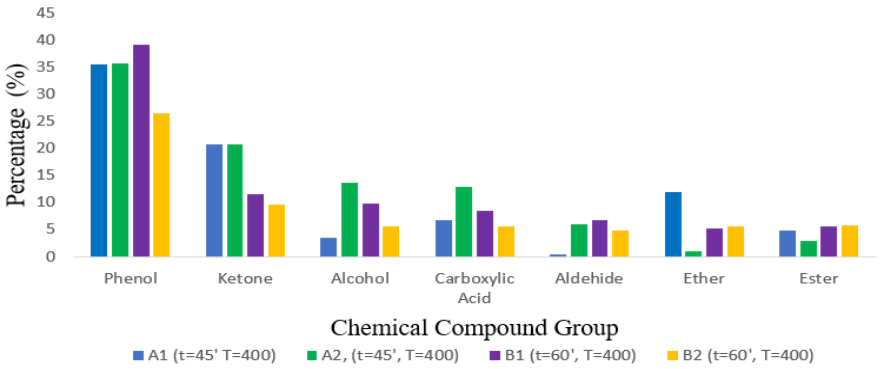

Figure 5. Profile of compound groups resulting from GC-MS Analysis

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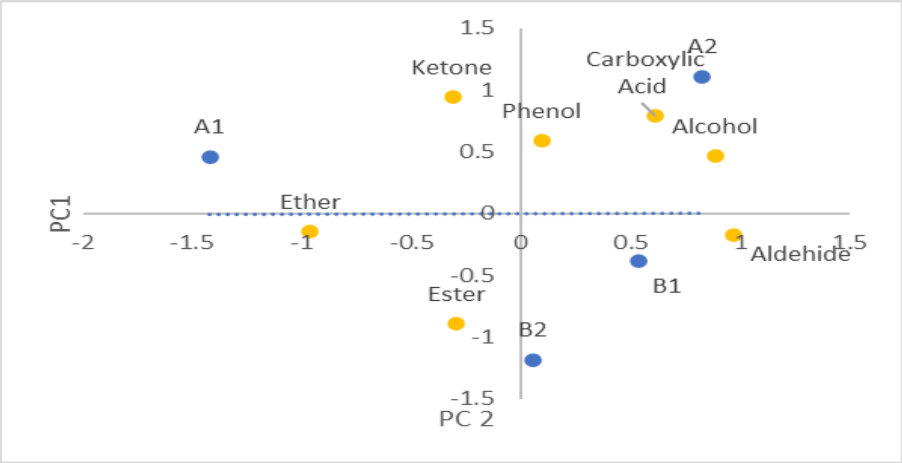

Figure 6. PCA Bi-plot sample vs variables

Table 1. Matrix correlations

Chemical Compound Group		Phenol	Alcohol	Ester	Carboxylic Acid	Eter	Ketone	Aldehide
Correlation	Phenol	1.00	0.39	-0.29	0.51	-0.00	0.43	0.13
	Alcohol	0.39	1.00	-0.66	0.91	-0.91	0.16	0.79
	Ester	-0.29	-0.66	1.00	-0.90	0.49	-0.93	-0.07
	Carboxylic	0.51	0.91	-0.90	1.00	-0.71	0.56	0.45
	Eter	-0.00	-0.91	0.49	-0.71	1.00	0.13	-0.87
	Ketone	0.43	0.16	-0.79	0.56	0.13	1.00	-0.48
	Aldehide	0.13	0.79	-0.07	0.45	-0.87	-0.48	1.00

This illustration highlights the differences in the chemical characteristics of the products formed during pyrolysis. Biochemically, phenolic and alcohol groups tend to form from the depolymerization of lignin at higher temperatures, whereas esters and ethers form under conditions of partial degradation of lignin. This consistency is evident in the correlation matrix, where compounds associated with the same degradation pathway exhibit strong positive correlations (e.g., carboxylic acids and alcohols and alcohols and aldehydes), whereas compounds from opposing reaction pathways exhibit negative correlations (e.g., alcohols and ethers). The PCA bi-plot confirmed this pattern, where positively correlated compounds appeared close together, whereas compounds with negative correlations were in opposite directions.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Based on the results of this study, the following conclusions were drawn: (1) The pyrolysis conditions that produced bio-oil with the best antibacterial activity differed for each test organism. For *S. aureus*, the largest inhibition zone of 8.56±1.80 mm was obtained at 400°C for 60 minutes, whereas for *E. coli*, the largest inhibition zone of 8.08±1.25 mm was obtained at the same temperature for 45 minutes. Both treatments were evaluated using a bio-oil inhibition assay with a 2% phenol concentration; (b) GC–MS analysis indicated that phenols, alcohols, carboxylic acids, esters, ketones, aldehydes, and ethers were major compound groups in the selected bio-oil samples. Overall, the antibacterial activity of EFB bio-oil cannot be attributed solely to total phenolic content and may be influenced by the composition and relative abundance of individual compounds.

Recommendations

Further research is recommended using large-scale or pilot plant tests to assess the feasibility of pyrolysis for mass-balance bio-oil production. Furthermore, future research should focus on fractionating the dominant compounds based on GC-MS analysis results and conducting inhibition tests for each fraction. A potential phenol concentration formulation (2%) can be developed as a natural antiseptic and alternative to 70% alcohol. Future studies should focus on the

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fractionation of phenolic compounds and the evaluation of their in vivo antibacterial efficacy and cytotoxicity or irritation testing where relevant. The presence of active phenolic compounds with proven antibacterial activity supports their prospective use in the development of eco-friendly antiseptics, disinfectants, and natural preservatives derived from renewable biomass.

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