

Microwave Assisted Extraction of Banana Stem Waste and its Antifungal Activity against *Fusarium Oxysporum*

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Abstract: Banana stem (BS) waste is obtained in large quantities in banana production. In this work, microwave assisted extraction (MAE) from BS was used, and the solvent concentration parameters were 50%, 60%, 70%, 80%, 96% (v/v) ethanol, and 5% HCl with a sample weight of 10 g each. The influence of solvent, time, temperature, and solid: the liquid ratio was evaluated on the extraction results. Total phenolic content (TPC) and its functional performance against *Fusarium oxysporum*. The effect of a high ethanol concentration on BS extraction was observed because the higher ethanol solvent concentration resulted in higher extraction results and TPC. Optimal MAE conditions were determined at 200 watts for 20 minutes, with 80% ethanol showing better TPC results than other ethanol concentrations, namely 0.7171 mg g⁻¹. The results of chemical content analysis using gas chromatography-mass spectrometry (GC-MS) of BS extract of 80% ethanol obtained phenolic compounds, carboxylic acids, aldehydes, esters, ketones, and furans. BS extract of 80% ethanol showed antifungal activity of *Fusarium oxysporum* with an apparent zone diameter of 9.7 mm (moderate category). It can be concluded that MAE is an efficient technique for releasing bioactive molecules from BS with TPC and antifungal applications in the agricultural industry.

Keywords: Antifungal, banana, phenolic, microwave

1. Introduction

The banana (*Musa paradisiaca*) is a plant from Southeast Asia widely spread worldwide, including Indonesia. Almost all areas of Indonesia are suitable for growing banana plants (Uda et al., 2020). Banana plants are distributed from the lowlands to the highlands and cultivated on particular land, gardens, or yards. Almost every yard in Indonesia has banana plants. The plants produce fruit quickly and are easy to plant and maintain. Indonesia is one of the countries known as banana producers in the world. Bananas

are tropical fruit that is already popular in the community. Bananas are also a leading commodity and contribute the most to national fruit production (Sulistyowarni et al, 2020; Uda et al., 2020). Banana fruit production is ranked first in the agricultural industry in the world (Sulistyowarni et al., 2020).

The fresh weight ratio between BSs, leaves, and fruit is 63%, 14%, and 23%, respectively (Arianto et al., 2022; Ouattara et al., 2023). The BS has a specific gravity of 0.29 g/cm with a fiber length of 4.20 - 5.46 mm and has as many constituent components as cellulose, hemicellulose, and lignin (Laoubol et al, 2022; Thi Thuy Van et al., 2022). Lignin produces aromatic chemical compounds in the form of phenolics. Phenolics have an aromatic ring bonded to one or more hydroxy groups (OH-) and other accompanying groups (Kim et al., 2020; Shu et al., 2020). Numerous natural phenolic compounds have known structures, including flavonoids, simple monocyclic phenols, polyphenols (lignin, melanin, tannins), and phenolic quinones (Shu et al., 2020). These phenolic compounds have antifungal and antibacterial properties against several pathogenic and carcinogenic bacteria (Danish et al., 2020; Kalogianni et al., 2020).

According to Liu and Siemińska-Kuczer, phenolic compounds will interact with the cell membrane proteins of microorganisms through an adsorption process by binding to the hydrophilic part of the cell membrane (Liu et al., 2020; Siemińska-Kuczer et al., 2022). BS contains active flavonoid compounds, which are included in the group of phenolic compounds with glycoside bonds (Liu et al., 2020; Lobiuc et al., 2023). Phenolic compounds will then enter the cell membrane and cause cell protein precipitation (Nicolás-García et al., 2021; Rocchetti et al., 2022). This disrupts the permeability of cell membranes so that cell membranes can undergo lysis (Lobiuc et al., 2023; Nicolás-García et al., 2021; Rocchetti et al., 2022), and it is expected that the growth of fungi such as *Fusarium oxysporum* fungi can be inhibited by phenolic compounds in BSs.

One type of post-harvest mushroom commonly found is the mushroom species *Fusarium oxysporum* (Giménez-Santamarina et al., 2022; Khan et al., 2022). *Fusarium oxysporum* is a pathogenic fungus that causes various diseases such as wilting and rotting of fruit in plants (Aiello et al., 2021). Various plant species such as cocoa, banana, coffee, oil palm, cotton, pepper, mango, tomato, and vanilla are affected by this pathogenic fungus (Khan et al., 2022). Efforts are made for farmers to control pests and diseases, which are obstacles to increasing productivity and quality of crops to use pesticides (Javaid et al., 2023; Rehman et al., 2022). Farmers generally use synthetic pesticides, which pose a risk to health and the environment, can have negative impacts, and are relatively expensive in terms of costs (Rani et al., 2021).

Natural pesticides are developed, which can be degraded, and negative impacts can be eliminated so that efforts to control pests and diseases in plants can be carried out safely and environmentally friendly. One way to deal with plant diseases caused by the fungus *Fusarium oxysporum* is by using natural pesticides as bio fungicides made from natural ingredients found in nature as inhibitors for the fungus *Fusarium oxysporum*. The cell wall of *Fusarium oxysporum* is composed of 39% chitin, 29% glucan, 7% protein, and 6% fat (Giménez-Santamarina et al., 2022). Most of the fungal hyphae's ECM (Extracellular Material) consists of proteins and carbohydrates (Aiello et al., 2021).

Various methods can extract phenolic compounds. One of the methods used to extract phenolic compounds from BS is the microwave method (Drevelegka & Goula, 2020; Mashuni et al., 2020; Oroian et al., 2020; Rodsamran & Sothornvit, 2019). Microwave-based extraction is a technique using microwave heating in an extraction system and a method used to extract

active compounds from vegetable materials and vegetable waste. In general, microwave performance depends on the operational level of heating, for example, constant power heating and intermittent power heating (Drevelegka & Goula, 2020; Li et al., 2019). MAE techniques are becoming famous for extracting phenolics compared to conventional solvent extraction because this technique reduces the use of solvents and is fast and efficient (Khan et al., 2020; Mashuni et al., 2020). The ethanol solvent is used in the extraction process. The ethanol solvent is a safer compound from a toxicological point of view compared to other types of solvents, such as acetone and methanol (Mashuni et al., 2020; Rizkiyah et al., 2023; Viñas-Ospino et al., 2023). In addition, this solvent is a cheap alternative and can be produced by fermentation with various biological materials using simple technology. Based on this background, the phenolic content of BS waste extract was determined based on the microwave method and the inhibitory activity test against the fungus *Fusarium oxysporum*

2. Methods

2.1 Materials

BS waste, ethanol, sodium carbonate, gallic acid, Folin Ciocalteu reagent were obtained from Merck (Darmstadt, Germany), Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were bought from Sigma Aldrich (St. Louis, MI, USA).

2.2 Microwave Assisted Extraction

Dried BS were dried and crushed (80 mesh). A sample of 10 g of delicate banana stems was put into an Erlenmeyer and added with 50% (v/v) ethanol-water solvent (Mashuni et al., 2020). Then, the mixture was homogenized, and 5 mL of 37% HCl was added. Next, heat in the microwave at 200 watts for 20 minutes. Treatments were carried out for variations in ethanol solvent concentrations of 60%, 70%, 80%, and 96% (v/v). The resulting extract was filtered, and the filtrate was taken and then stored at room temperature.

2.3 Determination of Total Phenolic Content

Gallic acid standard solutions were prepared at 0.5, 1, 3, 5, 7, and 9 mg L⁻¹ concentrations. The phenolic content of banana stem extract was tested by taking 3 mL of the extract and adding 15 mL Folin-Ciocalteu 10% reagent. The solution was homogenized and allowed to stand for 3 minutes. Then, add 12 mL of 7.5% sodium carbonate solution and homogeneous. Allow it to stand at room temperature for 60 minutes, then measure the absorbance at a wavelength of 765 nm using a UV-Vis spectrophotometer. The phenolic concentration was obtained through the gallic acid standard calibration curve ($y = ax + b$). The TPC of the extract is determined through the equation, and the measurement results are expressed as mg g⁻¹.

2.4 Analysis of GC-MS

The chemical compound analysis of BS extract content was carried out using GC-MS instrument. As much as 1 µL of each extract sample was injected into a Thermo Scientific GC-MS Trace 1300 GC/ISQ, with ionizing type EI (Electron Impact) 70 ev, injector and detector temperature 290°C, column temperature 70°C to 280°C, 30 m column length, 25 mm diameter in column, 5°C temperature rise per minute, 100 kPa Helium carrier gas, flow rate of 60 ml/min. Compounds were identified by comparing their retention times to well-characterised materials. The chemical content characteristics of extract BS were identified using the GC-MS

instrument and the chemical compound structures were determined using the NIST MS software standard (Mashuni et al., 2019).

2.5 Antifungal Activity Test of *Fusarium oxysporum*

Antifungals using the well-diffusion method. The well method used two layers: solid PDA media and semisolid PDA media. Solid PDA is poured into a petri dish as a base layer to cover the surface of the petri dish, and after solidifying, a buffer/mold is installed; semisolid PDA media, which is still liquid, is then added to the test fungus *Fusarium oxysporum* as much as one ose then added 10 mL of distilled water and then homogenized with a vortex. Then the semisolid PDA containing the test fungus was poured over the solid base layer of solid PDA media. Then it is allowed to solidify; after it has solidified, a scavenger is taken to make a well.

The wells formed were then filled using the test compound BS extract and 80% ethanol as a negative control and Armure with the active ingredients difenoconazole (150 g/mL) and propiconazole (150 g/mL) as positive controls each of 15 μ L, 10% dilution and then incubated for 48 hours, at room temperature. After the incubation period, the inhibition zones formed were measured to determine the results of the antifungal inhibition test.

3. Results and Discussion

3.1 Total Phenolic Content

The BS samples were extracted using the MAE method. MAE is a method that utilizes microwave radiation to accelerate selective extraction by heating the solvent quickly and efficiently (Mashuni et al., 2020) which combines ethanol and HCl. The addition of HCl functions as a lignocellulosic hydrolyzer to separate lignin, cellulose, and hemicellulose, and the resulting heat effect will degrade the lignin structure so that it becomes a phenolic compound (Viñas-Ospino et al., 2023). The choice of ethanol solvent for extracting banana stem samples is because this solvent is a safer compound from a toxicological point of view compared to other types of solvents, such as acetone, methanol, and other organic solvents (Li et al., 2019). In addition, this solvent is a cheap alternative solvent and can be produced by fermentation of various biological materials using simple technology. In this study, the target compounds to be analyzed were phenolics which tended to be polar, so in this extraction, the ethanol solvent was very suitable for use because it is polar and quickly penetrates the cell membrane to BS extract (Kayaputri et al., 2014).

During microwave irradiation, heating occurs as a direct result of the interaction of the microwaves with the solvent and solid matrix. Heating is affected by two phenomena: ionic conduction and dipole rotation (Kaderides et al., 2019). As a result of changes in the electric field from the emitted microwaves, electrophoretic migration of molecules will occur. As a result of the resistance of the solution causing, collisions between molecules will generate heat energy and increase the surrounding temperature (Kaderides et al., 2019; Khan et al., 2020). The phenomenon of dipole rotation is a rearranging phenomenon of dipole molecules (polar solvents) due to a fast-changing electric field (Khan et al., 2020). Dipole rotation occurs when polar molecules attempt to align themselves with an alternating electric field in a medium produced by microwaves (Bhatt et al., 2020; Vernès et al., 2019). The oscillation of these polar species leads to collisions between them and the surrounding molecules, generating heat (Vernès et al., 2019), so that the heat generated by the microwaves will help the mass transfer of the bioactive compounds to be extracted from solids to ethanol and water as the solvents used.

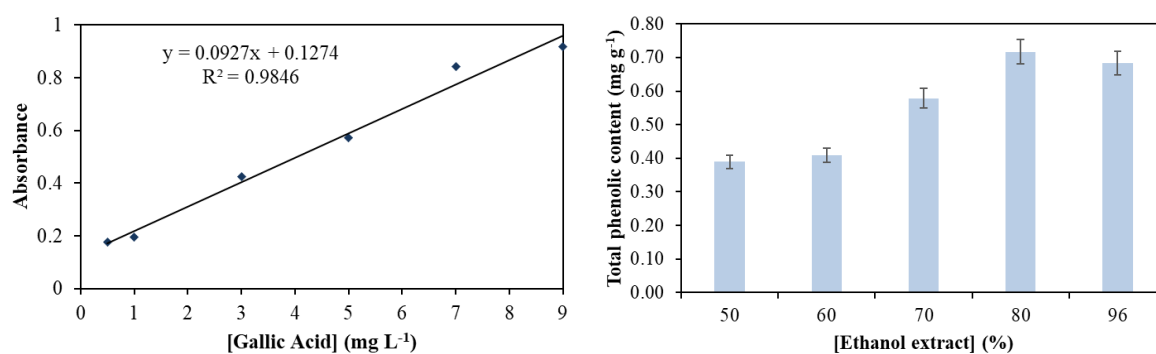


Figure 1. (a) Gallic acid standard solution calibration curve and (b) Total phenolic content of BS extract

According to Delgado and Mashuni, the TPC standard selection is adjusted to the type of phenolic compound in the sample (Delgado et al., 2019; Mashuni et al., 2020). However, gallic acid is the recommended standard to obtain reliable results. Gallic acid is known to have relatively high reactivity to the Folin-Ciocalteu reagent compared to other phenolic compounds so that it can be used as a standard in determining total phenolic content (Delgado et al., 2019). The results of the study of the absorbance measurement of the calibration curve of the gallic acid standard solution are presented in Figure 1.

Based on the standard solution's absorbance measurement results, a calibration curve equation was created to explain the relationship between absorbance and gallic acid concentration (Figure 1a). The principle of the Folin-Ciocalteu method is the formation of a blue complex which can be measured at a wavelength of 765 nm (Mashuni et al., 2020). Determination of phenol content using the Folin-Ciocalteu method was left at a range of operating time of 60 minutes. Settling in the operating time range aims to determine when the reaction between the sample and the reagent is at its optimum condition. A relatively stable absorbance value indicates the optimum reaction. At the beginning of the reaction, the absorbance of the colored compounds will continue to increase until, at a specific time, a stable absorbance is obtained. However, the longer the measurement time, there is a possibility that the colored compound will be damaged, causing the color intensity to decrease and the absorbance to decrease (Mashuni et al., 2020; Pallawagau et al., 2019). Determination of phenolic ions formed through proton dissociation of phenolic compounds, this reaction can only occur under alkaline conditions, so sodium carbonate is used as a base (Pallawagau et al., 2019) (Figure 2). The phenolic ion reacts with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex which can be detected with a spectrophotometer. The resulting blue color describes the complex formed, so the higher of TPC in an extract, the darker the blue color is produced (Pallawagau et al., 2019; Rizkia et al., 2022).

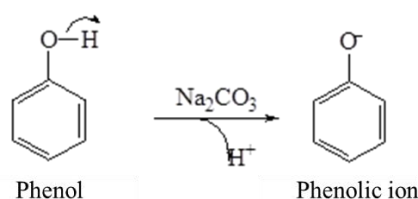


Figure 2. Formation of phenolic ions in alkaline conditions

The concentration of the sample was determined by entering the absorbance value of the sample into the gallic acid calibration curve regression equation $y = 0.0098x + 0.0431$. The

results obtained were expressed as mg g⁻¹. The TPC highest was at 80% ethanol concentration with total phenolic of 0.7171 mg g⁻¹, then decreased at concentrations of 96%, 70%, 60%, and 50%, namely 0.6836; 0.5790; 0.4085 and 0.3895 mg g⁻¹. The effect of concentration during extraction is that the greater the concentration used, the TPC greater obtained, but after reaching the optimum point, the phenol content decreases. It is presumably because the penetration of ethanol solvent into the material has decreased, so the components taken up in the material become less. So, 80% ethanol solvent has a better ability to extract compounds.

3.2 GC-MS Analysis

The results of the GC-MS analysis of banana stem extract using the MAE method obtained derivatives of phenolic compounds, carboxylic acids, aldehydes, esters, and furans, namely 4-(2-aminopropyl)-phenol; 2-ethyl heptanoic acid; 4-isopropylcyclohexanone; 1,2, 5,6 dianhydrogalactitol; tetradecanoic acid; tridecanoic acid; methyl ester tridecanoic acid; n-hexadecanoic acid; octadecanoic acid; nonadecanoic acid; 3,4,5-trimethoxy-a-methyl phenethylamine; methyl ester hexadecanoic acid; 1-tetradecanol; and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl cyclopenta[9]-2-benzopyran.

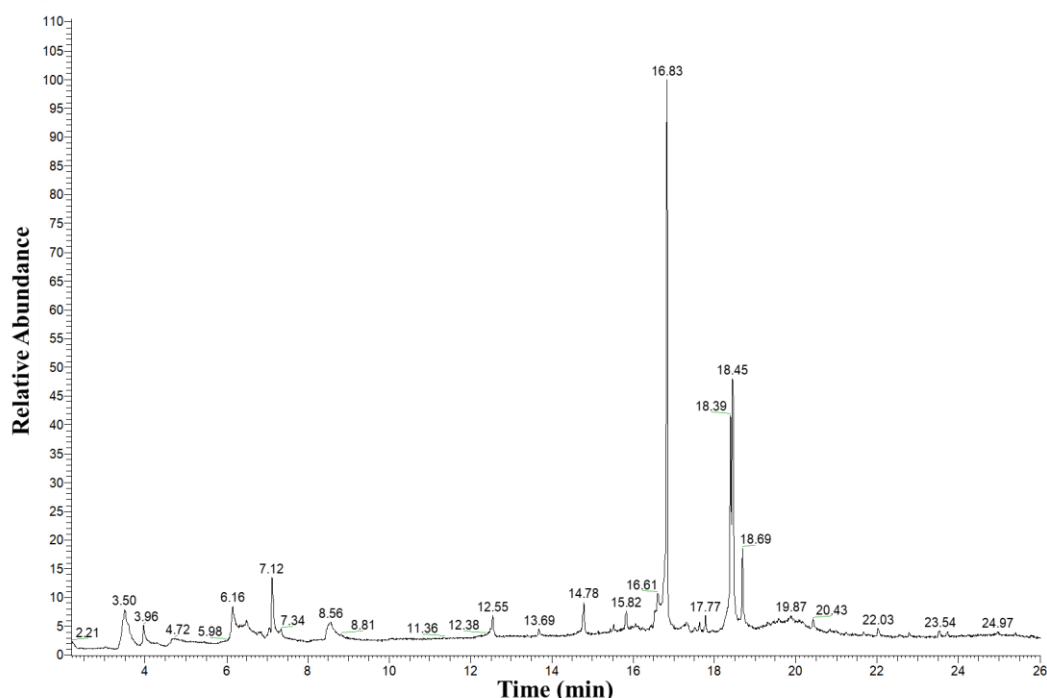


Figure 3. GC-MS spectrogram of BS extract from 80% ethanol extract

3.3 Antifungal Activity of *Fusarium oxysporum*

The antifungal test aims to determine the ability of BS extract to inhibit the growth of the fungus *Fusarium oxysporum*. Antifungal testing used BS extract with solvent concentrations of 50%, 60%, 70%, 80%, and 96%, respectively, and positive and negative control. The positive control is a compound that provides an obstacle to the growth of the fungus *Fusarium oxysporum*, so that it can be used as a comparison against the inhibitory power of BS extract. The negative control was 80% ethanol based on the highest phenolic concentration. The negative control is the solvent used as an extractor. The goal is that negative control does not affect the extract activity test.

The bioactivity test results in Table 1 show that microwaved banana stem extract is active in inhibiting the activity of the fungus *Fusarium oxysporum* as indicated by the formation of a clear zone around the wells at each sample concentration in Figure 4. The inhibition zone

produced at a concentration of 50%, 60%, and 70% and is included in the weak category in inhibiting the activity of *Fusarium oxysporum*. Meanwhile, concentrations of 80% and 96% respectively, which are categorized as moderate in inhibiting the fungus *Fusarium oxysporum*. These results indicate that the higher the total phenol content, the stronger the antifungal activity of the extract of a sample (Pallawagau et al., 2019).

Tabel 1. Diameter of inhibition zone of BS extract against *Fusarium oxysporum* fungus

No	[Ethanol extract] (%)	Inhibition Zone Diameter (mm)	Category
1.	50	3	weak
2.	60	4	weak
3.	70	6	weak
4.	80	10.7	moderate
5.	96	8	moderate
6.	Control positive (Armure)	16.5	strong
7.	Control negative (80% ethanol)	1	Not inhibit

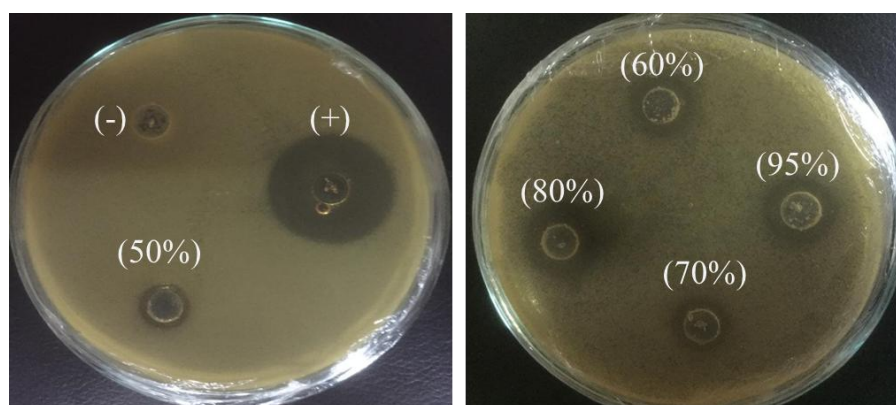


Figure 4. Inhibition zone of BS extract against *Fusarium oxysporum* fungus

The activity test of the negative control and positive control as a comparison of the results showed that 80% ethanol solvent as a negative control had activity in inhibiting as indicated by the diameter of the inhibition zone of 1 mm. This indicates that 80% ethanol solvent affects the inhibition of *Fusarium oxysporum*, so the banana stem extract with a concentration of 80% needs to be reduced by the diameter of the negative control inhibition zone to obtain a banana stem extract inhibition zone of 9.7 mm which is categorized as medium in inhibiting *Fusarium oxysporum* fungus as for the positive control armure with a chemical composition in the form of difenoconazole and propiconazole compounds made in a 10% dilution used as a comparison showing that armure has effectiveness against *Fusarium oxysporum* with an apparent zone diameter of 15.5 mm which is categorized as strong in fungal inhibiting activity so that it can be stated that banana stem extract concentration of 80% in inhibiting *Fusarium oxysporum* is still below the concentration of 10% armure.

The mechanism of action of an antimicrobial compound is by inhibiting the synthesis of the cell wall of microorganisms causing lysis, changing the permeability of the membrane so that it causes leakage of nutrients from inside the cell, causes denaturation of cell proteins and inhibits the action of enzymes in the cell (Mulia et al., 2023; Wang et al., 2017). Phenol compounds can diffuse through microbial membranes and enter cells, disrupting metabolic

pathways due to the disruption of the synthesis of ergosterol, glucan, chitin, protein, and glucosamine in mushrooms (Mulia et al., 2023; Rizkia et al., 2022).

When viewed from their structure, the antifungal mechanism of phenolic compounds has a hydroxyl group (OH) and a carbonyl group (C=O). The hydroxyl group in phenolic compounds can interfere with the permeability of the fungal cell membrane, where one of the constituents of the cell membrane is protein. The OH groups can damage the tertiary structure of proteins by forming hydrogen bonds to N and H atoms as constituents of proteins, thus damaging the nutrient transport pathways, eventually resulting in toxic effects on fungi. The mechanism of antifungal action of microwaved stem extract is not only due to phenolic compounds but due to the synergistic effect of several compounds present in banana stem extract so that they can adequately inhibit fungal growth. The same thing was also stated by that the mechanism of antifungal action is not only influenced by phenolic compounds but the presence of other compounds that help inhibit the growth of the fungus properly (Mashuni et al., 2020; Mulia et al., 2023; Pallawagau et al., 2019; Wang et al., 2017).

Conclusions

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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