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Microwave-assisted Extraction of Phenolic Compounds from Pineapple Peel against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Pineapple production is mostly marketed as a processed product, resulting in large amounts of industrial waste consisting of pineapple peel, core and crown. The total phenolic content and antibacterial activity of organic solvents used in microwave-assisted extraction (MAE) of phenolic compounds from pineapple peel were investigated in this work. The application of microwave technology in phenolic extraction provides a fast extraction process and produces higher yields. In addition, organic solvent ethanol is considered an environmentally friendly solvent characterized by low toxicity and increased capacity to extract bioactive compounds. The solvent compositions are ethanol (80%), ethanol-HCl (80%-5%), and ethanol-NaOH (80%-5%). A higher phenolic compound was obtained using ethanol-HCl (80%-5%) of 2.68 mg eq GA/g DW. The results of the antibacterial activity test against Staphylococcus aureus was 19.00 mm and Escherichia coli was 19.31 mm. These results indicate that pineapple peel extract from ethanol-HCl solvent can be an effective antibacterial agent.

Keywords: Antibacterial, microwave, phenolic, pineapple

1. Introduction

Pineapple stands out as an extensively traded tropical fruit globally, owing to its appealing sensory qualities and nutritional attributes. In 2018, there was an approximately 8% rise in global pineapple production. According to the most recent data provided by the Food and Agriculture Organization (FAO), the leading pineapple-producing region is Asia, with the Philippines, Thailand, India, China, Vietnam, Taiwan, and Malaysia being the primary contributors to the substantial increase in production [1]. Because of extensive damage, half of the world's pineapple supply is sold as processed fruit, such as canned fruit or juice. This manufacturing process generates a significant volume of industrial waste (50%), comprising predominantly of peel (30-40% w/w), core (9-10% w/w), stem (2-5% w/w), and crown (2-4% w/w) [2]. Therefore, it requires a sound waste management system. Otherwise, it will contribute to environmental pollution. [3]. Effective waste management is vital in harnessing bio-based processes and utilizing by-products to create value-added products, including chemicals, materials, and fuels. This thing, in turn, contributes to mitigating environmental issues and fostering economic growth [4].

Pineapple peel contains nutrients like those found in the pineapple flesh, encompassing fibres, pectins, proteins, sugars, water, vitamins, flavonoids, carotenoids, minerals, and phenolic compounds [3], [5-6]. Notably, it possesses antioxidant properties that are valuable for applications in health, cosmetics, and the chemical industries [3]. The phenolic compounds in pineapple and its by-products consist of ferulic acid, syringic acid, tannic acid, and p-coumaric acid [7]. These chemicals have a great ability to prevent the generation of free radicals, which has a good impact on health and antibacterial activity [4]. Given the escalating bacterial resistance to synthetic antimicrobials, phenol has been extensively explored as an antimicrobial agent. Within pineapple residue, specific phenolic structures such as linalool, α-terpineol, and furfural demonstrate inhibitory effects against *Escherichia Coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* [8-9].

Increasing research interest in natural phenolic compounds has spurred extensive research into the extraction of bioactive compounds using both traditional and unconventional methods. Each method comes with its own set of advantages and drawbacks. Combining these two approaches can yield superior outcomes. Enhancing the economic value of bioactive compounds, particularly those rich in phenolic compounds, may necessitate the adoption of more sophisticated extraction technologies. Microwave techniques have garnered attention due to their environmentally friendly nature, simplicity, and relatively low processing costs [10]. Pineapple peel waste proves conducive to non-conventional extraction methods, offering advantages such as reduced processing time, elimination of hazardous and costly solvents, and lower energy consumption [3]. Moreover, this approach enhances the overall extraction yield.

To extract phenolic components from pineapple peel, this study used the unconventional microwave-assisted extraction (MAE) technique. Previous studies have used hydrolysis [4] and MAE with conventional solvents (water, ethanol, and methanol) to extract phenolic compounds from pineapple waste. MAE represents an innovative technology for extracting dissolved compounds from diverse materials into liquids using microwave energy [11]. The process entails the application of microwave energy to expedite the transfer of solutes from the matrix to the solvent. In this investigation, the operational parameters in the MAE process, precisely the type of solvent employed in the extraction, were examined for their impact on the total phenolic content of pineapple peel. Additionally, the inhibitory activity of the extract against *Staphylococcus aureus* and *Escherichia Coli* was assessed.

2. Materials and Methods

2.1 Materials

Pineapple peel waste, ethanol (C_2H_5OH), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), Folin Ciocalteau reagent ($H_3PO_4(MoO_3)_{13}$) were obtained from Merck (Darmstadt, Germany), Sodium agar (NA) were bought from Sigma Aldrich (St. Louis, MI, USA), tetracycline, and distilled water:

Staphylococcus aureus and *Escherichia coli* bacteria from the Biochemistry Laboratory, Department of Chemistry, Halu Oleo University.

2.2 Microwave Assisted Extraction

Dried pineapple peel was dried and ground to 80 mesh size. A fine sample of 20 g was put into an Erlenmeyer flask, and the organic solvents ethanol 80% (v/v), hydrochloric acid 5% (v/v), and sodium hydroxide 5% (w/v) were added, according to Table 1. Then, The mixture was homogenized and heated in a microwave (Panasonic NN-ST342M) at 200 watts for 20 minutes. Treatments were carried out for varying concentrations of other solvents. The resulting extract was filtered, and the filtrate was taken and then stored at room temperature.

Tabel 1. Variation of solvent concentration

Code	Parameters
E_1	Ethanol 100 mL
E_2A_1	Ethanol 95 mL + hydrochloric acid 5 mL
E_2A_2	Ethanol 90 mL + hydrochloric acid 10 mL
E_3B_1	Ethanol 95 mL + sodium hydroxide 5 mL
E_3B_2	Ethanol 90 mL + sodium hydroxide 10 mL

2.3 Determination of Total Phenolic Content

A standard gallic acid (GA) solution was prepared with varying concentrations, referring to research by Mashuni et al. [12]. A UV-Vis spectrophotometer was used to measure the absorbance at a wavelength of 765 nm. Phenolic concentrations were obtained via a gallic acid standard calibration curve (y = ax + b). The total phenolic content of the extract was calculated using an equation and reported as mg eq of gallic acid/g dry weight (mg eq of GA/g DW).

2.4 Antibacterial activity test

The well-diffusion method was used to test the antibacterial activity. The well method uses two layers: solid NA media and semisolid NA media. The semisolid NA was added to 1000 μL of the test bacterial suspension and homogenized using a vortex. Then, pour it over the base layer of solid NA media that has solidified. The solidified NA media is then taken with a backup/mould to make three wells in one petri dish. The wells formed were then filled using 10 μL of extract, ethanol as a negative control and tetracycline as a positive control. Next, it was incubated for 24 hours at 37^{o}C. Make observations by using a screw micrometre to measure the diameter of the inhibitory zone created.

3. Results and Discussion

3.1 Total Phenolic Content

The pineapple peel extraction process uses MAE, namely extraction that utilizes microwave radiation to speed up selective extraction by heating the solvent quickly and efficiently. Microwave energy affects molecules directly through two processes: ionic conduction and dipole rotation. As a result, only polar materials may be heated using their dielectric constant. The heat from microwave radiation will penetrate the transparent material until it reaches the granular glands and vascular system. The cell walls will break, and the active substances in the plant cells will escape into the solvent used. Ethanol, hydrochloric acid 5, and sodium hydroxide are polar solvents, so they attract polar to semi-polar components more efficiently. Ethanol was chosen as the primary solvent because it is relatively non-toxic, not explosive when mixed with air and easy to obtain. Adding 5% hydrochloric acid and sodium hydroxide is a hydrolyzer for lignocellulose to separate lignin, cellulose and hemicellulose. The resulting heat effect will degrade the lignin structure into a phenol compound.

Figure 1 depicts the total phenolic content (TPC) represented in mg eq GA/g DW. The calculated values ranged from 2.03 to 2.68 mg eq GA/g DW.

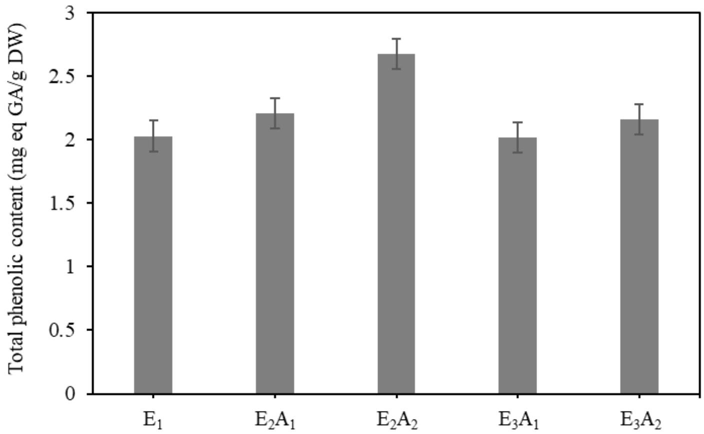

Figure 1. The effect of solvent on pineapple peel extract's total phenolic content

A solvent mixture of 90 ml ethanol and 10 ml hydrochloric acid yielded higher Total Phenolic Content (TPC) results than other treatments, precisely at 2.68 mg eq GA/g DW. Notably, there was no significant difference between the TPC obtained using this mixture and the conventional solvent, ethanol alone. The combination of ethanol and hydrochloric acid as a solvent produced results comparable to those of traditional solvents. This result is related to the greater interaction between phenolic compounds and the hydrogen bonds created, as well as the higher polarity in comparison to water and alcohol-based solvents. When employing environmentally friendly extraction techniques like MAE, instead of using alternative solvents such as water and ethanol-water mixtures [13-14], adding an acidic solvent contributes to a higher TPC. Regarding the extraction yield from pineapple peel, the choice of solvent did not result in any significant difference, remaining within the 70-75% range.

3.2 The Antibacterial Activity

The antibacterial activity test of pineapple peel extract was carried out using the well diffusion method by observing the clear zone formed. The wider the clear zone formed, the stronger the antimicrobial power [15]. The diameter of the inhibition zone resulting from the antibacterial activity test for *Staphylococcus aureus* and *Escherichia coli*, respectively, is presented in Figures 2 and 3 and Table 2.

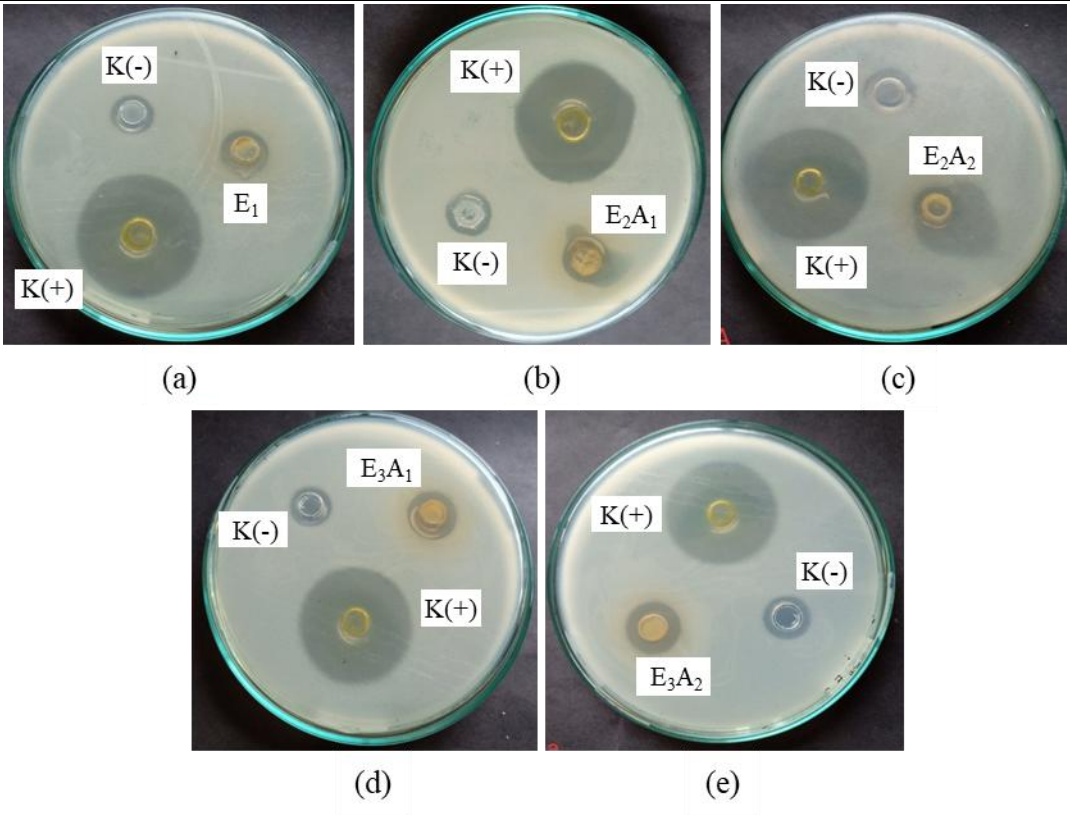

Figure 2. Antibacterial activity of *Staphylococcus aureus*

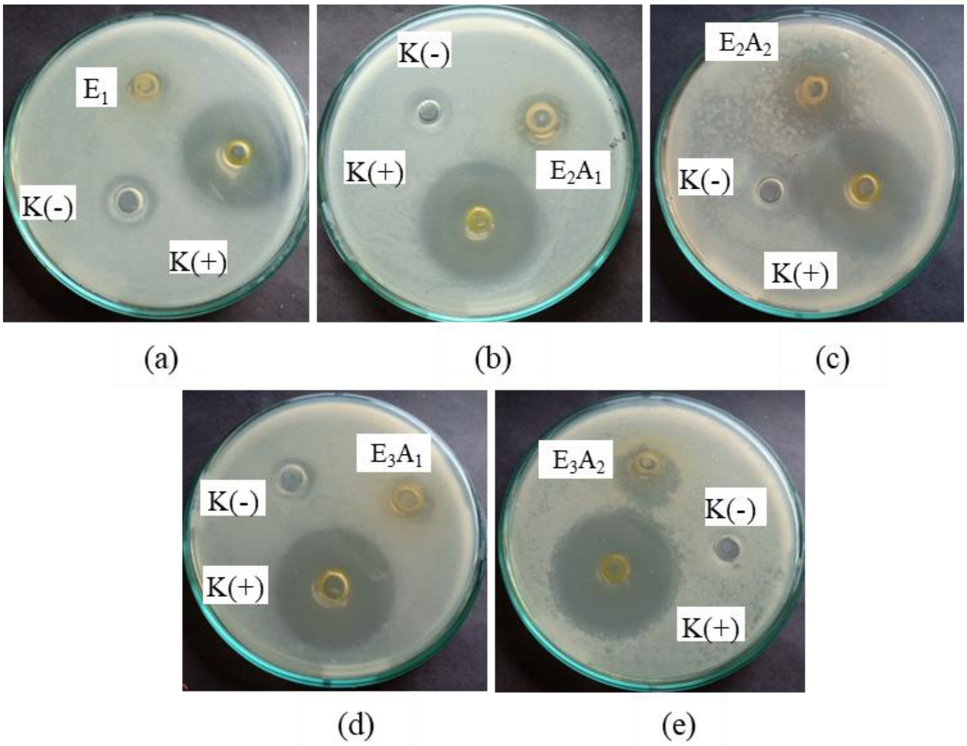

Figure 3. Antibacterial activity of *Escherichia coli*

Table 2. Pineapple peel extract has antibacterial action against *Staphylococcus aureus* and *Eschericia coli* bacteria.

Extract	Average diameter of inhibition zone (mm)		Inhibition zone response
	Staphylococcus aureus	*Eschericia coli*	
E_1	8.36	8.67	Moderate
E_2A_1	10.43	12.60	Strong
E_2A_2	19.00	19.31	Strong
E_3A_1	9.10	9.35	Moderate
E_3A_2	11.20	16.80	Strong
Positive control	32.96 ± 0.7	34.31 ± 0.6	Very strong
Negative control	5 ± 0.1	5 ± 0.1	Weak

Analysis of the antibacterial activity test results for pineapple peel extract reveals varying responses across different solvent types and bacteria. Notably, extracts using ethanol, hydrochloric acid, ethanol, and sodium hydroxide exhibit strong inhibitory effects, while the ethanol extract shows a comparatively weaker response. This discrepancy can be attributed to variations in the thickness and composition of bacterial cell walls, influencing their resistance to antibacterial activity. Phenol derivatives, an extract component, engage with bacterial cells through an adsorption process involving hydrogen bonds [16–18]. At lower concentrations, weakly bonded phenol-protein complexes form, promptly decomposing and allowing phenol penetration into cells, leading to precipitation and denaturation of proteins. At higher concentrations, phenol induces protein coagulation and lysis of cell membranes [19–20]. The findings of this study underscore the positive impact of phenolic compounds from pineapple peel on the activity of *Staphylococcus aureus* and *Escherichia coli* bacteria. These outcomes lay the groundwork for further exploration of the potential of natural compounds as effective and sustainable antimicrobial agents.

Conclusions

MAE presents an eco-friendly option for extracting phenolic compounds from pineapple peel. By employing a microwave heating power of 200 watts, an extraction time of 20 minutes, a 20 g sample, and a solvent mixture of 90 ml ethanol and 10 ml hydrochloric acid, it was possible to obtain an extract with elevated concentrations of phenolic compounds and potent antibacterial activity. The microwave extraction procedure must be optimized in order to successfully recover phenolic chemicals from pineapple waste. This optimization is a crucial foundation for enhancing the value of by-products by applying environmentally friendly technologies.

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Conflicts of Interest

The authors declare no conflict of interest.

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