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Antibacterial activity of *Saccharum officinarum* leaves extract against food-borne disease

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Abstract. Food-borne diseases are mainly caused by germs contaminated food which deliver serious illnesses such as intoxication, infection or combination of both. The germs could be *Escherichia coli* and *Staphylococcus aureus*. Previous study revealed *Saccharum officinarum* baggase competence in depressing these bacteria growth. Leaf should afford a better role for having no carbohydrate deposition. For that reason, its petroleum ether extract was prepared in a dilution series of 100 %, 75 %, 50 % or 25 % and used for antibacteria evaluation by paper disc method. The result indicated diminished growth of both bacteria by 100 % and 75 % extract, though not as effective as the positive control containing ampicillin. Furthermore, the Gram-positive bacteria was deprived more than the negative one. GCMS chromatogram depicted some fatty acid appearances that might contribute to the antimicrobe activity. A future study of antibacterial effect of each isolated compound should be managed. However, this study has already verified that the petroleum ether extract of *Saccharum officinarum* leaves were found to be an antibacterial agent against *Escherichia coli* and *Staphylococcus aureus*.

1. Introduction

Saccharum officinarum is known as sugarcane or cane, a crop for sugar production. This is a plant with an alteration on the photosynthesis efficiency so the products can be raised up over years [1]. Cane utilization has generated some wastes, such as baggase, rind and leaves. Baggase and rind are cane residues during sugar or ethanol manufacture. Baggase is the matter that remains after stem was milled, while rind is the detached outmost layer of the stem as the cane was refined. If both waste are formed because of the sugar industry, then the leaf waste is engaged since the first cultivation of the plant. The waste is occurred accidentally during fall leaves or in purpose which is commenced prior to stem grinding. Green harvesting in order to reduce air pollution for burning cane leaves has induce an abundant number of this kind of waste [2].

The leaves waste is barely occupied for human wealth. In spite of this, some medicine men in some countries take advantages of this enduring parts of the plant. The one suggested the implication for diuretic improvement, while the other try to handle kidney function in improving the flow of dejecta within urinary tract [3]. Secretion is in sequence to the purpose of digestion. In view of that, both recommendation can be infered to the function of the plants leaves in handling food-borne diseases.

Food-borne diseases are mainly caused by pathogen contamination in food which can give a serious challenge and a result of severe illnesses such as food intoxication, infection or combination of both.



People had once been accustomed by synthetic chemical pharmacies to cure diseases. This habit could be dangerous if continuously done for ages. The drugs may effect the kidney instead of making it better. A pattern in taking prescribes was subsequently improved. Presently, people change this habit by taking pharmaceuticals of plants which contain some metabolites such as phenolics which have significant antibacterial, antiviral, and antiseptic activities [4, 5]. The phenolic compounds give an effect to the bacteria defense mechanism in accordance to the genetic inheritance of the bacteria, so the process seems to be adequate and balance [6].

Particular studies have shown remarkable action of cane extract in handling food-borne diseases as caused by *Escherichia coli* and *Staphylococcus aureus*. The outcome mentioned this valuable activity was given by the ethanol solution of baggase [7]. Another one has detailed the phytochemical of the cane leaves [8]. But, non of the experiment has already explained substances within leaves petroleum ether extract together with the antibacteria activity, especially for food-borne disease. Therefore, the current investigation becomes promising.

2. Methods

2.1. Cane leaves

The cane leaves were obtained from farmers at Rendeng, Kudus, Indonesia. The farmers are typical cane supplier for nearby sugar industry. The leaves of 12 months old plant were freshly grounded and dissolved in petroleum ether in the comparison of 1 : 3 (w/v).

2.2. Maceration

The leaves was macerated in petroleum ether solution o/n. The maceration solution was then filtered through a millipore. The residue was continuously macerated in identical solution 1 : 3 (w/v) for 2 more times. Filtrate of each maceration was combined and dried under vacuum at 50⁰C. The paste extract was weighted and stored at 4⁰C.

2.3. Microbial strain

Staphylococcus aureus ATCC 25926 and *Escherichia coli* ATCC 25922 were obtained from the Microbiology Laboratory of Universitas Diponegoro Medical Faculty. All successions were inoculated in nutrient broth (NB) media and incubated at 37⁰C for 16 – 18 hs. In the next day, the cultures were diluted in sterile 0.8% saline solution as in Mc Farland standard for antibacterial activity study.

2.4. Antibacterial activity by Oxford cup method

Antibacterial activity was examined by using Oxford cup method by some modifications. Each strain culture was uniformly spread onto individual NA plates by sterile cotton swabs and sterile cups (6 mm in diameter) were 1 hr soaked in a series of extract solution of 25 %, 50 %, 75 % or 100% with a clear cup of H₂O as the negative control and antibiotic contained cup as the positive control. Each series of cup was put on separated agar plate. The plates were incubated o/n at 37⁰C. The antibacterial effect was evaluated by measuring the diameters of inhibition zones. All tests were repeated in triplicate.

2.5. Substances identification by using GCMS

The substances contained in cane leaves were evaluated by GCMS. The paste extract was liquified in petroleum ether and filtered by microfilter to remove sediments. 2 μ l of the solution was employed for GC-MS analysis by using Clarus 680 Perkin Elmer analyzer. Helium (99.9%) was used as carrier gas in 1ml/min flow rate. Initial and detector temperature was 110 and 280⁰C respectively. Total running time was 30 minutes.

3. Result and Discussion

3.1. Antibacterial activity

The data of antibacterial activity which was evaluated by measuring inhibition zone diameters were compiled in Table 1. *S. aureus* is the representative of Gram-positif bacterium, while *E. coli* is the

negative one. Cane bagasse extract showed antibacterial activity opposed to both microbes [6]. The present experiment of leaf extract also exhibit similar obstruction. The inhibition zone of petroleum extract of the leaves for *S. aureus* was better than the ethanol extract of cane bagasse since the last one used 20 times higher concentration. In the contrary, *E. coli* unveil less depression. The average of inhibition zone diameters for Gram-positive bacteria were significantly larger than those of Gram-negative. This result suggests that cane leaf petroleum ether extract give a better recovery for Gram-positive caused disease.

Table 1. Average width of inhibition zones of cane leaves petroleum ether extract against pathogens.

Strain	Diameter of inhibition zones (mm)			
	25% extract	50% extract	75% extract	100% extract
Ampicillin	N/A	N/A	N/A	19.3
<i>S. aureus</i>	10.4	11.9	12.1	13.8
<i>E. coli</i>	0	0	8.2	8.4

The depiction of inhibition zones were given in figure 1. The positive controls of ampicillin (F of a and b) have notable compression to the bacteria. Thus, it ascertains that the bacteria are not the resistance ones. Moreover, the presentation confirm capability of cane leaves petroleum ether extract in lessening *E. coli* and *S. aureus* growth. *S. aureus* seems to be more susceptible to the extract. In the gram negative bacteria, the situation is a little bit different because the substances have to pass two layers of membrane. The outer membran and the cytoplasmic membrane. Especially at the former one, there is an interior coating of phospholipids and an exterior coating of lipopolysaccharide [9]. The substance become a barrier for *E. coli* and also for other Gram-negative bacteria. The barrier is in the outer membrane in the form of thick layer which is covering the cell wall. This cover is hardly accessed by liquid substances. In the other hand, *S. aureus* is a kind of Gram-positive which has single layer of peptidoglycan, so substances can easily diffuse through [10]. The diffused substances could disturb the cytoplasmic membrane and destroy the cell [11].

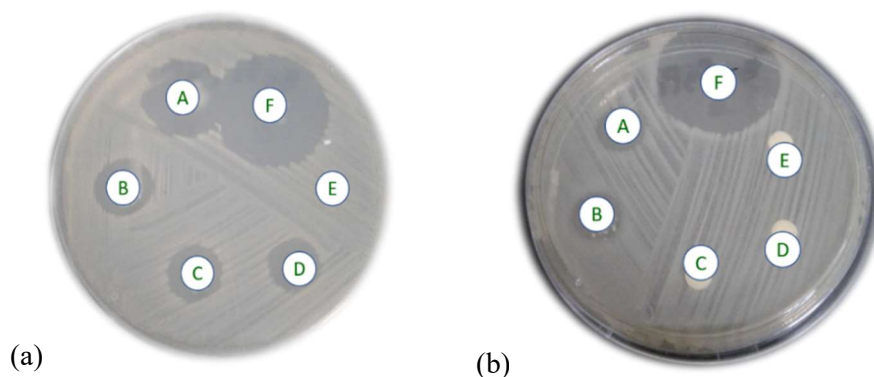


Figure 1. Inhibitory activity of petroleum ether extract of *Saccharum officinarum* leaves against (a) *S. aureus* and (b) *E. coli* with A is for 100% extract concentration, while B, C, and D are for 75%, 50% and 25% extract, respectively. E is the negative control with H₂O only and F is the positive control containing 100% ampicillin

SEM micrographs of comparable experiments of plant extracts controlled microbes exhibited cell wall disruption at low concentration of submission. In the next level, the membrane will detached and lysis. The cell permeability and integrity will transform and the cell will expand anonymously with many of membrane blebs. Finally, there are breakdown of plasm and organelles and leakage of cell contents. Thus, the cell is terminated [12].

3.2. Substances identification

The GCMS profile of the petroleum ether extract of the cane leaves was shown as Figure 2. There are a few number of detected matters since not every metabolites are volatile and dissolved in petroleum ether. There are considering 4 distinct peaks were occurred. The retention times of the peaks are 12.93 min, 13.70 min, 16.08 min and 16.34 min as detailed in Table 2.

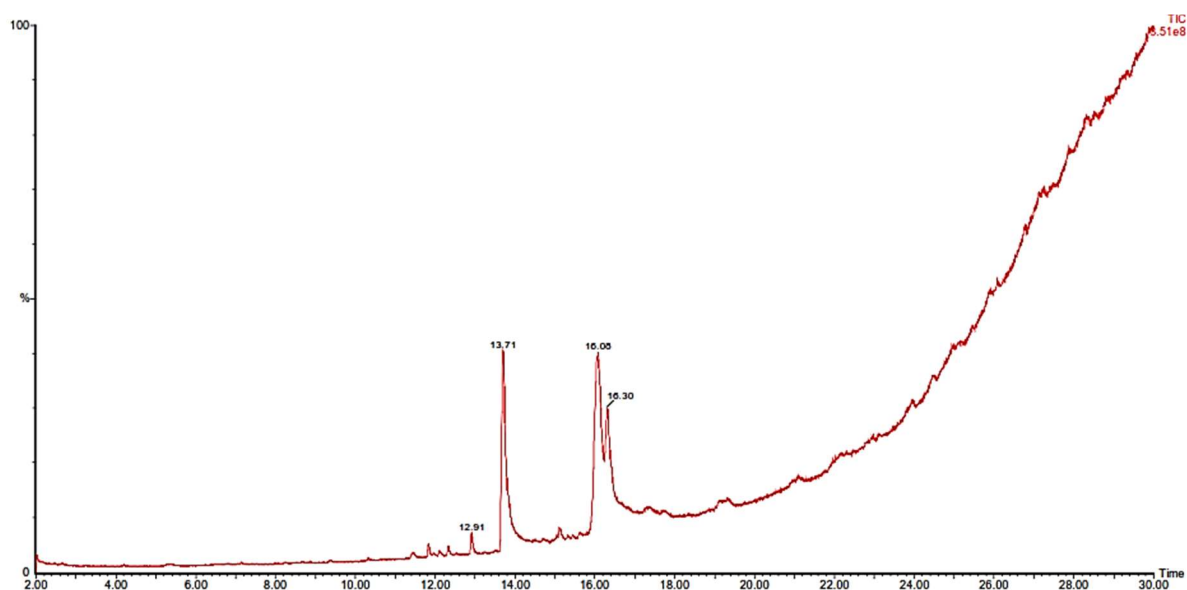


Figure 2. The GCMS profile of petroleum ether extract of cane leaves

The main peak is corresponded to the third detected peak. Chromatographic analysis of this peak revealed a molecular ion at m/z 282 (M^+) that can be proposed for a molecular formula of $C_{18}H_{34}O_2$. A kind of substance having this characteristics of molecular weight is cis-vaccenic acid. This fatty acid is an omega-7 one which is usually found in seed. The melting point of the omega-7 fatty acid is lower than that of omega-9 fatty acid, therefore it can be applied better in a low temperature environment, especially as feedstock. Vegetable oil containing omega-7 fatty acid was used to provide renewable sources of important elements and also advantageous health aspects such as cardiovascular fitness and cholesterol level reduction [13]. However, recent investigation show the capability of an extract containing vaccenic acid in reducing microbes growth such as for *Proteus mirabilis*, *Pseudomonas aerogenosa*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* [14]. This unsaturated fatty acid also inhibited methicillin-resistant *Staphylococcus aureus* RN4220/pUL5054 growth [15]. Henceforward, the present experiment confirm an accordance research of antimicrobes activities.

Table 2. The composition of the petroleum ether extract of cane leaves

RT	Area (%)	Component
12.928	1.666-	Hexadecanoic acid
13.703	31.229-	n-Hexadecanoic acid
16.084	37.197-	cis-vaccenic acid
16.339	29.908-	Oleic Acid

The dual initial substances of the chromatography represent hexadecanoic acid in the appearance of molecular ion at m/z 270 and 256 (M^+) in the formula of $C_{17}H_{34}O_2$ and $C_{16}H_{32}O_2$. It could be palmitic acid and the methyl ester. Palmitic acid is saturated fatty acid which is largely found in palm oil. The

administration of the oil has a significance role toward obesity and T2 diabetes mellitus while a sole palmitic acid noticeably ruins phosphorylation and activates insulin receptor in several cell types, contributing to the onset of insulin-resistance [16]. Crystallization of palmitic acid film of cicada and dragonfly wings confirms the bactericidal activity against Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* cells [17].

The RT of 16.34 min recommended a molecular ion at m/z 282 (M^+) with a suggested formula of $C_{18}H_{34}O_2$ and should be oleic acid. Oleic acid is monosaturated fatty acid with higher omega than vaccenic acid which is 9. This fatty acid exhibited an extraordinary influence in antimicrobes and antifungal. Furthermore, the capability of oleic acid and the other components of cane leaf petroleum ether extract in suppressing microbes growth has delivered an immense effect of the antimicrobe itself.

4. Conclusion

Petroleum ether extract of *Saccharum officinarum* leaves can be exploited as antimicrobes resources against food-borne bacteria, in this case were Gram-positive of *Staphylococcus aureus* and Gram-negative of *Escherichia coli*. This competence was supported by the properties of some fatty acids which are vaccenic acid, palmitic acid and oleic acid.

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