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Research Article
Antibacterial Activity Testing and Ethanol Extract Characterization of Oil Palm Fronds (*Elaeis guineensis* Jacq)

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Abstract

Background and Objective: Oil palm fronds are palm oil plantation waste material that can be utilized as feed and have antioxidant and antibacterial activity. This study was conducted to examine the antibacterial activity and identify the components of the ethanol extract of oil palm fronds. **Materials and Methods:** Oil palm frond extraction was performed using the maceration method in 96% ethanol. Identification of the extract was performed by phytochemical screening and antibacterial activity tests involved the paper disc method with a 5% extract concentration. The test bacteria were *Escherichia coli* and *Staphylococcus aureus*. Measurements were made through observation and the results were then compared with those in the literature. Antibacterial activity was measured according to the zone of inhibition based on the diameter of the clear zone formed around the well. **Results:** The maceration process using 96% ethanol resulted in 30.65 g of extract. Oil palm fronds contained tannins and steroids according to phytochemical screening. Oil palm ethanol extract has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with low activity (2 mm zone of inhibition). **Conclusion:** The results of this study indicate that oil palm frond extract can be utilized as a natural antibacterial source.

Key words: Oil palm frond, extract ethanol, antibacterial, *Escherichia coli* and *Staphylococcus aureus*, Tannin, Steroid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobials are compounds that can inhibit the growth of pathogenic microorganisms (bacteriostatic effects) or kill bacteria (bactericidal effects) that come in contact with the antibiotic. Synthetic antimicrobial compounds have a negative effect on animals, including cattle and the humans who consume them, such as leading to increased drug dosages and other side effects. Animals given antibiotics cannot be slaughtered within a certain time of treatment and antibiotics increase microbial resistance to certain drugs. The increase in cases of pathogenic bacterial resistance has hastened the search for new sources of antimicrobial compounds through the development of ways to utilize natural medicinal substances that have antibacterial activity. Antibiotics obtained naturally from microorganisms are called natural antibiotics. Some types of plants that have natural antimicrobial activity include onions[1], garlic (*Allium sativum* L.) and leeks (*Allium porrum* L.)[2], ginger and honey[3] and *Capsicum annuum* L.[4]. The use of natural antimicrobials is expected to minimize negative effects on livestock animals and the humans who consume them.

Oil palm fronds are palm oil plantation waste material that can be utilized as feed[5] and have antioxidant activity[6]. Oil palm fronds are useful as an antibacterial[7] because they contain chemical compounds such as flavonoids (chrysoeriol and luteolin)[8], alkaloids, phenolics, steroids and tannins[9]. The fronds also show antimicrobial activity and can improve healing of infected wounds[10]. The potential antimicrobial activity of ethanol extracts of oil palm fronds has not been widely reported. The objective of this study was to extract and identify antimicrobial compounds in ethanol extracts of oil palm fronds that are potentially useful as natural antibiotics and that may be used as a substitute for synthetic antibacterials.

MATERIALS AND METHODS

The present study was conducted at the Faculty of Agriculture and Animal Sciences, State Islamic University of Sultan Syarif Kasim, Riau, Indonesia, the Biotechnology Research Center of the Indonesian Sciences Institute, Indonesia and the Laboratory of Biota Sumatera Andalas University during the period from June-October, 2016.

The materials used in the study were oil palm fronds obtained from oil palm plantations in the Riau province of Indonesia. All reagents were purchased from commercial sources and used as supplied, including ammonia, 96% ethanol (Merck, Germany), NaOH, HCl, Mayer reagent, Dragendorff reagent, Liebermann-Burchard reagent, amyl alcohol, magnesium powder, iron (III) chloride, nutrient agar, DMSO (Merck, Germany) and tetracycline (Kimia Farma, Indonesia).

The equipment used in this study included bottles and jars (Pyrex), a vacuum evaporator (Buchi, Germany), micropipettes (Eppendorf, US), droppers, dropper plates, a water bath, reaction tubes (Pyrex), petri dishes (Pyrex), cotton buds (Kimia Farma, Indonesia), a bunsen burner, agar plate, paper discs (Sigma-Aldri, Indonesia) and calipers (Krisbrow, Indonesia).

Research implementation: Oil palm frond sections used included the front two-thirds (2/3) of the frond (consisting of 150-200 pieces of leaves), which were then chopped using a leaf chopper and dried in the sun. A total of 540 g of dry oil palm frond was extracted using a maceration method (without heat) with a 96% ethanol solvent for 24 h, which was repeated 8 times (8×24 h). Every 24 h, the ethanol filtrate is separated and then concentrated by a vacuum evaporator. The concentrated ethanol extract was then weighed. Afterward, phytochemical screening was performed according to Farnsworth[11] and antibacterial activity testing of the oil palm extract was performed according to a method described by Handayani *et al.*[12].

Statistical analysis: This study used an experimental method to test antibacterial activity and to identify antibacterial compounds in ethanol extracts of oil palm fronds. Identification of antibacterial activity was performed through observation and then compared with the results in the literature. Antibacterial activity was measured based on the zone of inhibition, which was measured as the diameter of the clear zone formed around the well. Davis and Stout[13] have previously suggested assessment of bacterial inhibition activity based on the diameter of this clear zone. If the inhibition zone diameter is $\geq$20 mm, then inhibition is considered to be very strong, a diameter of 10-20 mm indicates strong inhibition, a diameter of 5-10 mm indicates medium inhibition and a diameter $\leq$5 mm represents weak inhibition. Mudi and Ibrahim[14] stated that if the inhibition zone diameter is smaller than 6 mm, this indicates that the extract is inactive, but if the diameter is greater than 6 mm, then the extract is classified as having antimicrobial activity.

RESULTS AND DISCUSSION

Oil palm frond extraction was performed with the maceration method, which is a simple yet widely used method that is suitable for heat-resistant samples that are thermostable and damage resistant. Maceration methods have been reported by Maiti *et al.*[15], Varghese *et al.*[16], Adewale[17], Irshad *et al.*[18], Pathan *et al.*[19] and Amanpour *et al.*[20]. Before the maceration process was performed, the palm oil fronds were dried and smoothly ground to facilitate their dissolution so that the maceration process occurs more quickly. Macerating powders can expand the overall surface area, accelerate system equilibrium and increase the effectiveness of extraction [21].

The solvent used in this extraction process was 96% ethanol because ethanol is a universal and economical polar solvent, is readily available, is the best solvent for low molecular weight compounds and is often used to extract antimicrobial compounds from plants. Ethanol is a universal solvent that can extract polar, non-polar and semi-polar compounds[22]. The use of ethanol to extract bioactive compounds from plants was reported by Wendakoon *et al.*[23] for boldo, hops, licorice and yerba mansa and by Sen and Batra[24] for *Melia azedarach* L. The use of ethanol in the extraction process yields maximum amounts of total phenolics and total flavonoids[25].

A comparison was performed for the yield of extracts produced from the initial sample. A total of 540 g of oil palm frond was extracted by using 96% ethanol, yielding 30.65 g of crude extract, for a 5.68% yield (Fig. 1). Similar results have been reported by Korompis *et al.*[26] for the skin of langsat (*Lansium domesticum Correa*) and by Quan *et al.*[27] for *Angelica sinensis*, but the result was lower than that reported by Paibon *et al.*[28] and by Enabulele and Ehiagbonare[29].

Factor affecting the yield are extraction time, temperature, particle size of the material, solvent extraction method used and solid/solvent ratio[30]. The process of milling oil palm fronds into the smaller particle results in a finer particle size, greater surface area, faster reactions and enhanced absorption, therefore, it is expected that yield will be higher. The content of the compounds in the sample also affects the yield. The samples used here included all parts of the palm oil plant (leaves, sticks and midribs) from the front two-thirds of the frond (150-200 leaves) and had been aged (approximately 14 years), resulting in low levels of polar compounds. This low polarity causes a low yield. Castello *et al.*[31] reported that seperate extractions from leaves, roots and hypocotyls of *Bixa orellana* with ethanol produces extracts with high antimicrobial activity. The low water

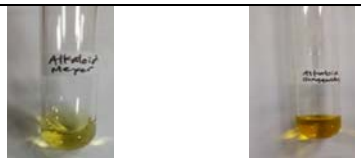
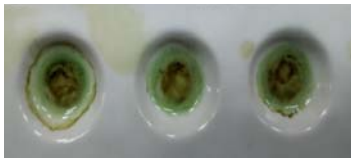
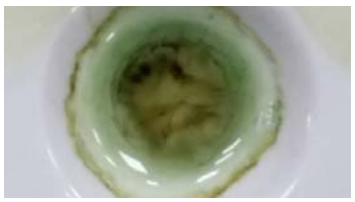
Fig. 1: Extract from oil palm fronds using ethanol as a solvent

content of 96% ethanol leads to a low solubility of the water-soluble compounds present in oil palm fronds. Extraction of waxy corn starch with 50, 70 or 90% ethanol all yielded greater starch extraction than was achieved with 96% ethanol[32].

The identification of chemical compounds in oil palm ethanol extract showed that oil palm fronds contains steroid and tannin compounds, characterized by the blue and green colors in Table 1. Tannins and steroids are secondary metabolites in plants of the phenolic and triterpenoid class of potential antimicrobials[33]. The discovery of tannin and steroid compounds from oil palm ethanol extracts indicates that oil palm fronds have potential as an antimicrobial. Some studies have reported similar findings, such as those by Sasidharan *et al.*[9], Chong *et al.*[7] and Masola *et al.*[34], who demonstrated that the oil palm fronds contain chemical compounds, including flavonoids, alkaloids, phenolics, steroids and tannins, that have potential as antimicrobials.

The mechanism by which tannins act as antimicrobials is related to protein and cellular activity. This is because tannins can damage polypeptide compounds in cell walls, activate microbial cell adhesion and disrupt transport proteins so that microbial growth is inhibited. The ethanol extract of *Jatropha curcas* L. and herbal meniran (*Phyllanthus niruri* L.) contains tannins, flavonoids and saponins and has antibacterial activity against coliform bacteria and *Staphylococcus aureus*[35,36].

Table 1: Chemical characterization of ethanol extract of oil palm fronds (*Elaeis guineensis* Jacq)

Compound groups	Results	Documentation	Characteristics
Alkaloid	-		
Steroid	+		The formation of a blue or green color
Terpenoid	-		
Flavonoid	-		The compound is indicated by the dark blue or dark green color
Saponin	-		
Tanin	+		
Kuinon	-		

Lipid membranes and liposomes are the main targets of steroids in their role as antimicrobial compounds. This is through interactions of steroids with lipophilic compounds in phospholipid membranes that decrease membrane integrity such that the cells become brittle and lyse[36,37].

The discovery of tannin and steroid compounds in palm oil ethanol extracts shows that oil palm fronds have antimicrobial activity. Sasidharan *et al*.[9] reported that tannins, saponins, alkaloids, flavonoids, steroids and terpenoids in oil palm leaves have antimicrobial activity that can decrease the number of microbes and accelerate wound healing in mice.

Antimicrobial activity was measured as the inhibitory ability of the extract against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. Lovmar[38] reported that the inhibition of cell wall synthesis, alteration of cell membrane permeability or active transport through cell membranes, inhibition of protein synthesis and inhibition of nucleic acid synthesis will inhibit microbial growth.

The antimicrobial activity test results for the effects of palm oil ethanol extract on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria are characterized by the inhibition zone around the well. The formation of an inhibitory zone suggests that the extract has antibacterial activity[39]. The inhibitory zones formed in the

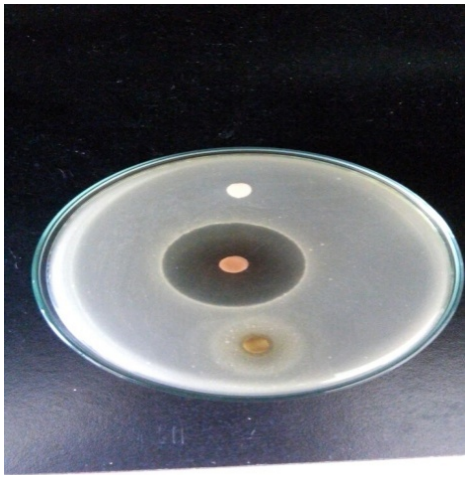


Fig. 2: Antimicrobial activity of oil palm (top: Negative control, middle: Positive control, bottom: Palm oil extract)

antimicrobial activity tests of palm oil ethanol extract for both gram-positive and gram-negative bacteria at a concentration of 5% were 2 mm in diameter in each case. This shows that the 96% ethanol extract had nearly the same antimicrobial activity against both gram-positive bacteria and gram-negative bacteria (Fig. 2). Increased concentrations would increase the diameter of the clear zone and therefore, the antibacterial

activity, as reported by Lodhia *et al.*[40] for palmarosa, evening primrose, lavender and tuberose and by Masola *et al.*[34] for *Adansonia digitata* (Bombacaceae, African baobab).

The bacterial inhibition activity demonstrated in this study is classified as weak because the inhibition zone was 2 mm in diameter. Davis and Stout[13] stated that a bacterial inhibitory zone $\leq$5 mm represents weak inhibition. The low antimicrobial activity demonstrated in the study was due to the low concentration of extract used, i.e., 5%. This concentration is the minimum concentration for antibacterial activity tests of a crude extract[12]. Based on this finding, the extract of palm oil has potential for use as an antibacterial. A higher concentration of the extract would be expected to increase the antimicrobial activity as increasing the concentration of an extract increases the diameter of the inhibitory zone formed due to the greater abundance of active compounds in the extract[40-42]. An increased concentration of *Bauhinia variegata* leaf extract (50-200 μg mL^{-1}) led to increase in antioxidant activity from 41-56%[43].

The low antimicrobial activity produced in the present study is also associated with the type of solvent used. Ethanol is a universal polar solvent that only dissolves polar compounds. The use of a polar solvent causes many polar compounds to come out of solution in the extract because they are mixed with many other polar compounds. This causes the activity of the resulting antimicrobial compound to be low. The low antimicrobial activity demonstrated in this study, as shown by the 2 mm diameter inhibitory zone, was also because of the low concentration of extract used (5%). A higher concentration of extract is expected to increase antimicrobial activity. The higher the concentration of the extract, the greater the diameter of the clear zone and therefore, the antimicrobial activity is greater[40,44,45].

CONCLUSION

Oil palm ethanol extract contains tannins and steroids and has a low antibacterial activity (2 mm inhibition zone) against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 5%. The ethanol extract of oil palm fronds can be developed as a natural antibacterial source that can be used as a substitute for synthetic antibacterials.

SIGNIFICANCE STATEMENT

The study found that oil palm fronds contains tannin and steroid compounds and their extract has antibacterial activity. This study indicated that oil palm fronds can be utilized as a natural antibacterial source. Therefore, this research could lead to a new view that oil palm fronds can be used as a natural antibacterial and used as a substitute for synthetic antibacterials.

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