

Antimicrobial Activity of *Elaeis Guineensis Leaf*

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Summary

This research was conducted to study the antimicrobial activity of the oil palm (*Elaeis guineensis*) leaf. The dried leaves material was extracted with methanol. Screening of the methanol extract of *E. guineensis* for antimicrobial activity showed that the extracts possesses significant antimicrobial activity against Gram positive, Gram negative bacteria, yeast and the fungi tested. The minimum inhibitory concentration (MIC) values for the methanol extract of *E. guineensis* had been determined and were found in the range of 6.25-12.500 mg/ml. The methanol extract of *E. guineensis* at the MIC, half MIC and two time MIC concentration were found to inhibit the early growth phase of the yeast *Candida albicans*. Microscopic studies showed that the methanol extract of *E. guineensis* caused some physiological and morphological changes in the treated cells of *C. albicans*.

KEY WORDS: antimicrobial activity, *Elaeis guineensis*, *C. albicans*, Scannin Electron Microscopy

Introduction

According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries. Malaysia is gifted with a very rich plant life and the use of some of the traditional medicines and food preparations need to be well documented (1). Among the many plants with healing properties in Malaysia is *Elaeis guineensis* Jacq. Much still remains unknown about the *E. guineensis* leaf, and research will be required on many levels as data are deficient on status, extent and utilization leaf extract.

E. guineensis is a perennial monocot belonging to the family Palmae and tribe Coccoineae. It gives the highest oil yield per hectare of all the economic oil crops (2). It is an important crop for Malaysia and contributes significantly to the national economy (3). *E. guineensis* originated from West Africa where it was growing wild and later developed into an agricultural crop. It was first introduced to Malaya in early 1870's as an ornamental plant. In 1917 the first commercial planting took place in Tennamaran Estate in Selangor, laying the foundations for the vast oil palm plantations and palm oil industry in Malaysia.

All parts of the plants are useful. The wood is used as frames for buildings and the sap is fermented into palm wine. The oil from the fruit mesocarp and the seeds are used for cooking and for making soaps, creams and other cosmetics. The fresh sap is used as a laxative and the partially fermented palm wine is administered to nursing mothers to improve lactation. Soap prepared with ash from fruit-husk is used for the preparation of a soap used for skin infections. A root decoction is used in Nigeria for headache. The pulverized roots are added to drinks for gonorrhea, menorrhagia and as a cure for bronchitis (4). The leaf extract and juice from young petioles are used as application to fresh wounds. The fruit mesocarp oil and palm kernel oil are administered as poison antidote and used externally with several other herbs as lotion for skin diseases. Palm kernel oil is applied to convulsant children to regulate their body temperature. Folk remedies of oil palm include treatment for cancer, headache and rheumatism and as an aphrodisiac, diuretic and liniment. Hence, the present study was carried out to determine the antimicrobial activity of *E. guineensis* leaf extract.

Materials and methods

Plant material

The *E. guineensis* leaf was collected from various areas in Peninsular Malaysia, in December 2007. The plant material was dried in an oven at 60 °C for 7 days.

Extraction procedure

Approximately 100 g of dried sample was added to 300 mL of methanol and soaked for 4 days. Removal of the sample from solvents was done by filtration through cheesecloth and the filtrate was concentrated using a rotary evaporator in vacuo to one-fifth volume in a centrifugal evaporator at 60 °C and then sterilized by filtration using a 0.22-mm membrane for antimicrobial assay. The leaf extract was further dried in an oven at 60 °C.

The dried extract was then redissolved in 10% DMSO (v/v) to yield solution containing 100.0 mg of extract per mL.

Test microorganisms and growth media

The following Gram-positive and Gram-negative bacteria, yeasts, and molds were used for antimicrobial activities studies: bacteria included *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*; yeasts included *Candida albicans*, and *Saccharomyces cerevisiae*; molds included *Aspergillus niger* (CGMCC 3.316). The bacterial strains were grown in Mueller–Hinton agar (MHA) plates at 37 °C, whereas the yeasts and molds were grown in sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28 °C. The stock culture was maintained at 4 °C.

Antimicrobial activity

Disk diffusion assay

Antibacterial and antifungal activities of 4 plant parts extracts were investigated by the disk diffusion method (5, 6). The MHA plates, containing an inoculum size of 10^6 colony-forming units (CFU)/mL of bacteria or 2×10^5 CFU/mL yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L-shaped glass rod. Then disks (6.0-mm dia) impregnated with 25 µL of each extracts at concentration of 100.0 mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent alone in the center to serve as a control, and antibiotic disks (6.0-mm dia) of 30 µg/mL chloramphenicol (for bacteria), and 30 µg/mL of Miconazole nitrate (for fungi) were also used as positive controls. All of the plates were incubated at 37 °C for 18 to 24 h for bacteria and at 28 °C for 48 to 96 h for fungi. The zones of growth inhibition around the disks were measured after 18 to 24 h of incubation at 37 °C for bacteria and 48 to 96 h for fungi at 28 °C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms. All of the experiments were performed in triplicate. The results are reported as the average of 3 experiments.

Determination of Minimum Inhibitory Concentrations (MIC)

A 16 h culture was diluted with a sterile physiological saline solution [PS; 0.85% (w/v) sodium chloride] with reference to the 0.5 McFarland standards to achieve an inoculum size of approximately 10^6 colony forming unit mL⁻¹. A serial dilution was carried out to give final concentrations between 1.563-200.00 mg crude extract per mL. The tubes were inoculated with 20 µL of the bacterial suspension per mL nutrient broth, homogenized and incubated at 37 °C. The MIC value was determined as the lowest concentration of the crude extract in the broth medium that inhibited the visible growth of the test microorganism.

The MICs of the leaf extracts of *E. guineensis* determined by twofold dilution method against *A. niger*. A test sample was dissolved in the 10% DMSO (v/v). These

solutions were serially diluted and were added to PDB (potato dextrose broth) to final concentrations of 1.563 to 200.00 mg crude extract per mL. The spore suspensions of *A. niger* was obtained from their respective 10 days old culture, mixed with sterile distilled water to obtain a homogenous spore suspension of 1×10^8 spore/ml. A 10 µl spore suspension of each test strains was inoculated in the test tubes in PDB medium and incubated for 2–7 days at 28 °C. The control tubes containing PDB medium were inoculated only with fungal spore suspension. The minimum concentrations at which no visible growth was observed were defined as the MICs, which were expressed in mg/ml (7).

Effect of *E. guineensis* leaf extract on *C. albicans* cells by scanning electron microscopy study

One ml of the *C. albicans* cell suspension of the concentration of 1×10^6 cells per mL was inoculated on a Sabouraud dextrose agar plate and then incubated at 30°C for 12 hours. Two ml of the extract at the concentration of 100 mg per mL was dropped onto the inoculated agar and further incubated for another 36 hours at the same incubation temperature. A 10% DMSO treated culture was used as a control. A small block of yeast containing agar was withdrawn from the inoculated plate at 0 and 36 hours fixed for scanning (8).

Growth profile of *C. albicans* in the presence of *E. guineensis* leaf extract

In order to assess the fungicidal effect with $\frac{1}{2}$, 1 or 2 fold MIC concentration over time, growth profile curves were plotted (9). A 16 h culture was harvested by centrifugation, washed twice with phosphate saline and resuspended in phosphate saline. The suspension was adjusted using the McFarland standard and was then further diluted in phosphate saline to achieve approximately 10^7 colony forming units mL⁻¹. Leaf extract was added to aliquots of 25 mL Mueller-Hinton broth (MHB) in a 50 mL Erlenmeyer flask in a water bath at 37°C, in an amount which would achieve concentration of $\frac{1}{2}$, 1 and 2 fold MIC (3.13 mg/mL) after addition of the inocula. Extract free medium was used as control. Thereafter 1 mL of inoculum was added to each Erlenmeyer flask. After addition of the inoculum a 1 mL portion was removed the flask and the growth of *C. albicans* was monitored using this portion by measuring the optical density at 540 nm (UV-9100, Ruili Co., China). The growth of *C. albicans* was measured every 4 h for 48 h by the above method.

Results and discussion

The disk diffusion assay and Broth dilution technique results of *E. guineensis* leaf extract showed in Table 1. As shown in Table 1, the extracts from the *E. guineensis* displayed antibacterial and antifungal activity against all/or some of the tested gram positive and gram negative bacterial and yeasts and mold, with the diameters of zone inhibition ranging between 12 and 14 mm. The extract was inhibited the growth of five of the bacterial strains tested, namely *S. typhi* (12mm), *S. aureus* (13mm), *B. subtilis* (12mm), *E. coli* (12mm) and *P. aeruginosa* (14mm).

Table 1. Antimicrobial activity (zone of inhibition and MIC_a) of *Elaeis guineensis* leaf extract compared with commercial antibiotic.

Microorganism	Zone of inhibition (mm) ^a			MIC (mg/mL) ^b Of the extract
	Extract	Chloramphenicol	Miconazole nitrate	
Bacteria				
<i>Salmonella typhi</i>	12	22	ND	6.25
<i>Klebsiella pneumoniae</i>	-	24	ND	-
<i>Proteus mirabilis</i>	-	22	ND	-
<i>Bacillus subtilis</i>	12	23	ND	12.5
<i>Staphylococcus aureus</i>	13	22	ND	6.25
<i>Escherichia coli</i>	12	23	ND	12.5
<i>Pseudomonas aeruginosa</i>	14	24	ND	6.25
Yeast				
<i>Candida albicans</i>	14	ND	23	6.25
<i>Saccharomyces cerevisiae</i>	-	ND	24	-
Fungi				
<i>Aspergillus niger</i>	13	ND	22	12.5

^aThe values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL extract and 30 µg/mL chloramphenicol and miconazole nitrate

^bBroth dilution method, mean value n = 3.

Furthermore, the fungi studied, *A. niger* (13mm) and *C. albicans* (14mm) were also susceptible to the extracts of *E. guineensis*. However, the *S. cerevisiae* was resistant against the extracts tested. In contrast, the inhibition zone of solvent control methanol was zero, so that it was not active against all of the tested microorganisms. However, 2 antibiotics, 30 µg/mL of chloramphenicol and 30 µg/mL of Miconazole nitrate were more effective than any of the extracts from of *Cassia spectabilis*, with the diameters of zone inhibition ranging between 22 and 24 mm except the leaf extract of *E. guineensis*.

Antimicrobial potency of the leaf extract of *E. guineensis* against the tested bacteria and fungal strains were expressed in MIC as presented in Table 1. The MIC values against these bacteria, and fungal strains ranged from 6.25 to 12.5 mg/ml (Table 1). The MIC results also indicated the leaf extract is effective against gram-positive bacteria, Gram negative bacteria and fungal strains.

Further study particularly we concentrate on dermatophytic fungi *C. albicans* because the increasing prevalence of drug resistant *C. albicans* recovered from hospitalized patients is a major concern worldwide (10). Also, *C. albicans* is the most

abundant and significant species in human beings and known to be responsible for infections in people, and can cause vulvovaginitis, oral thrush, nosocomial infection, and candidiasis (11, 12).

Further evidence of antiyeast activity against *C. albicans* of the extract was obtained from SEM study (Figure 1). Figure 1 shows the SEM photomicrographs of the untreated and extract treated cells of *C. albicans*. Cells treated with Minimal Inhibitory Concentration of the crude extract underwent considerable morphological changes in comparison with the control when observed under scanning electron microscope (Figure 1). Untreated cells (Figure 1.A) showed many oval and smooth cells in appearance and some at a budding stage. After 36 hours of exposure (Figure 1.B), cells completely collapsed and cavitated. It appeared that, the cells had lost their metabolic functions completely at this stage because of effect from the extract.

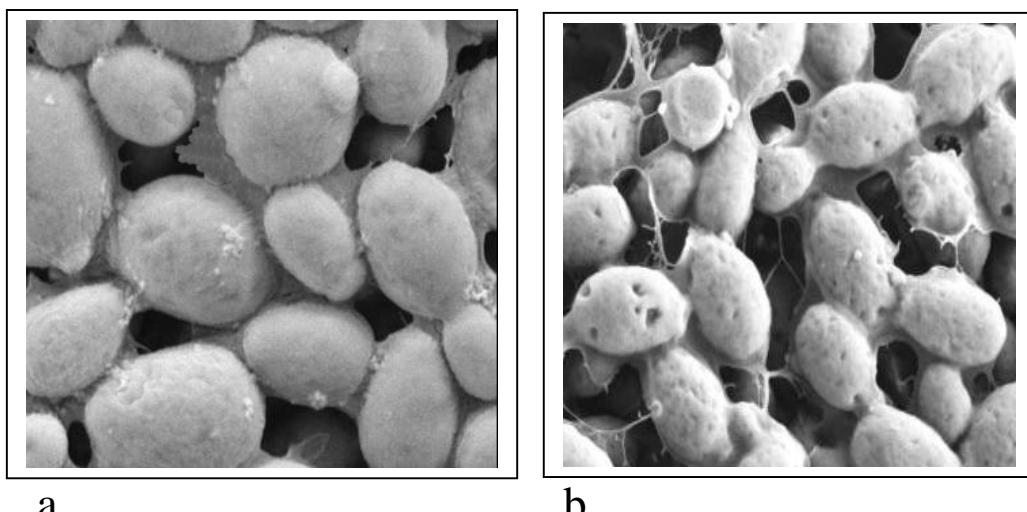


Figure 1. SEM micrographs of the (A) untreated and (B) extract-treated cells of *Candida albicans*.

The growth profile of *C. albicans* in the presence of *E. guineensis* leaf extract was utilized in this study to confirm MIC findings and to evaluate the ability of *E. guineensis* leaf extract to eliminate *C. albicans* growth *in vitro*. In the case of 1 and 2 fold MIC concentrations the extract inhibited the yeast growth within 4 h and subsequent regrowth was not seen (Figure 2). The growth profile suggested that the extract significantly inhibited *C. albicans* growth and also prolonged antimicrobial activity against the organism as determined by growth curves.

In our present study, a wide range of human pathogenic microorganisms were examined, including not only Gram-positive and Gram-negative bacteria, but also yeasts and molds. Significant antifungal activities also found against the *C. albicans* in this study. This may indicate that the *E. guineensis* extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial and antifungal agents from natural plant sources.

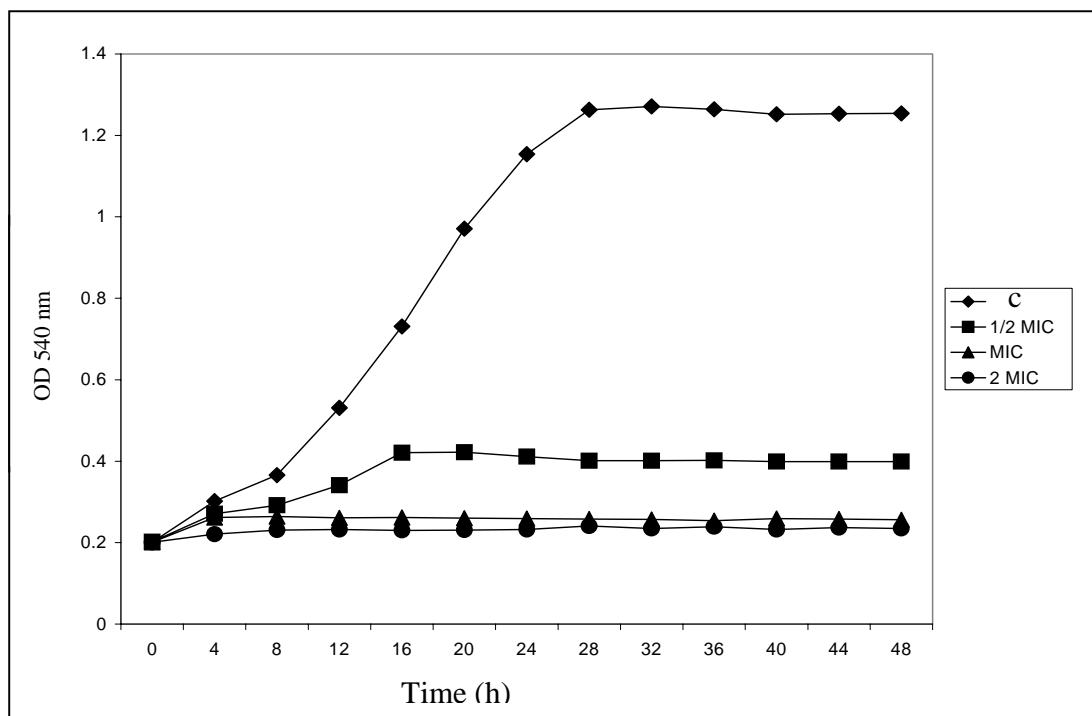


Figure 2: Growth profile for *Candida albicans* in Mueller-Hinton broth with 0 (control, c) and 3.13 mg/ml (1/2 MIC), 6.25 mg/ml (MIC) dan 12.5 mg/ml (2MIC) concentration of *Elaeis guineensis* leaf extract.

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