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PHYTOCHEMICAL SCREENING AND IN VITRO ANTIBACTERIAL ACTIVITY OF *Elaeis guineensis* LEAVES EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

(Penyaringan Fitokimia dan Aktiviti Antibakteria Daun Sawit (*Elaeis Guineensis)* In Vitro Terhadap Bakteria Patogen Manusia)

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Abstract

Chloroform and methanol extracts of *Elaeis guineensis* leaves were investigated for *in vitro* antibacterial activity against the human pathogenic bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Four different concentrations of both extracts consists of 50, 100, 200 and 300 mg/ml were prepared for antibacterial activity using disc diffusion method. The results revealed that chloroform and methonal extract showed high toxicity against all bacterial strain tested. However, both extracts is more effective and exhibit better inhibiting activity against gram positive bacteria, *S. aureus* compared to gram negative bacteria (*E. coli* and *P. aeruginosa*). Methanol extract of *Elaeis guineensis* leaves shows greater inhibition zone compared to chloroform extract as phytochemical screening revealed that this extracts contain terpenoids, tannins and saponin. The highest antibacterial activity was exhibited by 300mg/ml methanolic extracts against *S. aureus* which inhibited 10.67 ± 0.33 mm of the diameter zone. Followed by 200mg/ml methanolic extracts and 300mg/ml chlorofom extracts against *S. aureus* which inhibited 9.17 ± 0.17 mm and 8.33 ± 1.67 mm respectively. This result revealed the potentials of *Elaeis guineensis* as antibacterial agent in combating infections from human pathogenic bacteria. However, further studies, including identification and purification of the active compounds, will need to be pursued.

Keywords: antibacterial activity, *Elaeis guineensis* extracts, human pathogenic bacteria, inhibition zone

Abstrak

Aktiviti antibakteria ekstrak klorofom dan metanol daun sawit terhadap *Escherichia coli, Pseudomonas aureginosa* dan *Staphylococcus aureus* (bakteria penyebab penyakit manusia) telah dikaji secara *in vitro*. Ekstrak daun sawit disediakan dalam kepekatan yang berbeza (50, 100, 200 and 300 mg/ml) untuk kajian menggunakan kaedah resapan cakera. Hasil kajian mendapati ekstrak klorofom dan metanol daun sawit menunjukkan kadar toksik yang tinggi terhadapt semua strain bakteria yang diuji. Walaubagaimanapun, kedua-dua ekstrak lebih efektif dan menunjukkan kadar perencatan yang lebih tinggi terhadap bakteria gram positif iaitu *S. aureus* berbanding bakteria gram negatif (*E. coli* and *P. aeruginosa*). Kandungan terpenoid, tanins and saponin yang dikenalpasti semasa saringan fitokimia penyumbang kepada ekstrak methanol daun sawit yang menunjukkan kadar perencatan yang lebih tinggi berbanding ekstrak klorofom. Kadar perencatan yang paling tinggi adalah 10.67± 0.33 mm dari 300 mg/ml ekstrak metanol terhadap *S. aureus.* Diikuti oleh 200 mg/ml ekstark metanol dan 300 mg/ml ekstrak klorofom yang masing-masing merencatkan 9.17 ± 0.17 mm dan 8.33 ± 1.67 mm pertumbuhan *S. aureus.* Hasil kajian menunjukkan bahawa daun kelapa sawit berpotensi untuk digunakan sebagai agen antibakteria. Walaubagaimanapun, kajian lanjut, termasuklah penulenan dan pengenalpastian sebatian aktif perlu dijalankan.

Kata kunci: aktiviti antibakteria, ekstrak *elaeis guineensis*, bakteria penyebab penyakit manusia, kadar perencatan

Introduction

Bacteria can be pathogenic to human as they can cause bacterial infection and serious health complications. Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* are pathogens in wound infections. These pathogenic bacteria also can cause various diseases. *S. aureus* is considered to be major pathogen and responsibles for a wide range of human diseases, including septicaemia, endocarditis, pneumonia includes wound, bone and joint infections [1]. These bacteria produce toxins that can destroy membranes and directly damage tissue and leads to transformation of scar tissue [2,3]. In addition, *S. aureus* also produced toxin that responsible for causing toxic shock syndrome [4] and sometimes involved death [5]. Certain *E. coli* strains are causing a variety of diseases [6] and cause serious infections in the urinary system [3]. *E. coli* strains are the major cause of diarrhea in both humans and animals. It is estimated to cause 600 million diarrhea cases and 800,000 deaths worldwide principally in children under the age of five years old. Such disease cases become worst as antibiotic resistance rates in *E. coli* are rapidly rising [7]. *P. aeruginosa* is a type of parasite and can invade the urinary and cause meningitis [5]. *P. aeruginosa* also found to be causative agent of blue-green purulence in the wounds of patients [8].

Bacterial infections can be treated using antibiotics and antibacterial agents, which interfere with the growth and reproduction of the bacteria. Antibiotics are probably the most successful family of drugs so far developed for improving human health. Both synthetic and natural antibiotics and antibacterial agents can help us combat bacteria which may harm us. Synthetic antibacterial which commonly use in daily routine give negative effect in human health. Triclosan, a synthetic antibacterial found in the personal care product like soap, deodorants, and toothpaste is highly toxic and causes cancer [9]. They will promote leukemia, nervous system disorders and liver problem. Natural antibiotics differ from synthetic antibiotics in terms of safety profiles which safer than synthetic antibiotics. The long term side effects of using natural antibiotics are much better compared to synthetic antibiotics. Because of the safety issues, researcher tends to find new antibacterial agent from natural sources.

Plants were believed to have a good antibacterial activity as a lot of research which tested plants for antibacterial activity showed positive result. In 2012, the extract of *Ocimum santum, Ocimum gratissimum, Aegle marmelos* and *Adhatoda vasica* showed a good potential as antibacterial agents against human pathogen [10]. While, Mahesh & Satesh [11], had proven that leaf, root and bark methanol extracts of *Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritian* showed the antibacterial activity against *B. subtilis, E. coli, P. fluorescens, S. aureus, X. axonopodis pv. malvacearum, A. flavus, D. turcica* and *F. verticillioides.*

Elaeis guineensis leaves extracts were chosen for the antibacterial assay as they contain the needed phytochemical compound for health [12]. Originated from Family of *Arecaceae*, *Elaeis guineensis* can be used as a dietary supplement to provide a natural source of mixed vitamin E, mixed carotenoids, vitamin K, squalene, sterols, and other nutrients. Besides, oil palm waste also can save cost in pharmacy field and the utilization of waste material can promote clean environment. Additionally, phytochemical screening also had been done for analyzed metabolites, which were responsible for curing ailment. Plants contained various bioactive compounds that had been recognized to possess many properties, including antioxidant, anti-allergic, anti-inflammatory, and antiviral and so forth [13].

Materials and Methods

Plant Extraction

Elaies guineensis fresh leaves samples were cut into small pieces and were air-dried for one week. Dried *E. guineesis* leaves samples were macerated with chloroform and methanol (successive extraction) for 24 hours. The samples were extracted three times at room temperature, filter and evaporated. Four different concentrations were prepared (50, 100, 200 and 300 mg/ml) for each extracts.

Bacterial Strains

The human pathogenic bacteria tested in this assay were obtained from Biological Laboratory 3, Universiti Teknologi MARA (Pahang). Antibacterial assay were performed against three bacteria strains, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

Antibacterial Assay

Antibacterial assay were carried out by *in vitro* disc diffusion method [14]. Autoclaved Mueller-Hinton agar was poured into sterile petri dishes and was left to solidify. Bacteria inoculant was uniformly spread over the agar plates. Sterile 6 mm AA discs were saturated with 1.5ul of *E. guineensis* leaves chloroform and methanol extracts. The disc were then placed on the top of the agar plate and incubated at 37°C for 24 hours. Antibacterial activities were evaluated by measuring the diameter of the inhibition zone. Standard antibiotic, gentamicin was used as a positive control while discs containing DMSO were used as negative control.

Phytochemical Screening

Qualitative phytochemical screenings of *E. guineensis* leaves of chloroform and methanol extracts were performed for detection of tannins, saponin, flavonoids, terpenoids, alkaloids, and glycosides. The phytochemical were conducted using techniques by Ayoola et al. and Okoli et al. [15,16] with slightly modification. Test for tannins: Dried powder samples were boil in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration. Test for saponin: About 2 g of powder samples were boiling in distilled water and shake vigorously for a stable persistent froth. The frothing was then added with 3 drops of olive oil and observed for the formation of emulsion.

Test for flavonoids: A portion of the powdered plant sample heated with 10 ml of ethyl acetate over a steamed bath for 3 min. The mixture was filtered and 4 ml of the filtrated shake with 1 ml of dilute ammonia solution. Yellow colour appearance indicates a positive test for flavonoids. Test for terpenoids: 5ml extracts were mixed with 2ml chloroform ($CHCl_3$). The solution was then added with 3ml H_2SO_4 to form layer. Reddish brown colours indicate the present of terpenoids. Test for alkaloids: 0.2g of extract was added with 2% H_2SO_4. The solution was then filtered and added with Mayer's reagent. Orange red colour indicates the presence of alkaloids. Test for glycosides: The extract was hydrolyzed with HCl solution and neutralized with NaOH and added with Fehling's A and B. The red colour indicates the presence of glycoside compound.

Results and Discussion

Table 1 shows the evaluation of antibacterial activities of *E. guineensis* chloroform leave extracts against *S. aureus, E. coli* and *P. aeruginosa.* Different concentration on *E. guineensis* chloroform extracts gives different result.

Table 1. Antibacterial activities of *E. guineensis* chloroform leaves extracts against human pathogenic bacteria

Concentration (mg/ml)	**Inhibition zone diameter (mm) ± Standard deviation**		
	S. aureus	***E. coli***	***P. aeruginosa***
50	6.00 ± 0.00	4.00 ± 2.00	6.17 ± 0.17
100	7.17 ± 1.67	4.17 ± 2.09	6.83 ± 0.17
200	7.67 ± 1.67	4.33 ± 2.17	7.33 ± 0.17
300	8.33 ± 1.67	4.50 ± 2.25	0.00 ± 0.00
Positive control	12.50 ± 2.00	11.80 ± 2.00	11.80 ± 2.00
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Evaluation of antibacterial activities against *S. aureus* showed the highest inhibition zone at 300 mg/ml concentration. For antibacterial activities against *E. coli*, the lowest concentration (50 mg/ml) gives the lowest inhibition zone, 4 mm and was increased proportionately with the increasing of the concentration. While, there was no inhibition zone observed for 300 mg/ml concentration of chloroform extract against *P. aeruginosa*. Result revealed that *E. guineensis* chloroform leave extracts showed good antibacterial activities against *S. aureus*.

Table 2 shows the evaluation of antibacterial activities of *E. guineensis* methanol leave extracts against *S. aureus*, *E. coli* and *P. aeruginosa.*

Table 2. Antibacterial activity of *E. guineensis* methanol leaves extracts against human pathogenic bacteria

Concentration (mg/ml)	**Inhibition zone diameter (mm) ± Standard deviation**		
	S. aureus	***E. coli***	***P. aeruginosa***
50	6.17 ± 0.17	4.17 ± 2.09	6.67 ± 0.17
100	7.50 ± 0.29	4.67 ± 2.35	7.17 ± 0.17
200	9.17 ± 0.17	6.50 ± 0.29	7.67 ± 0.33
300	10.67± 0.33	4.83 ± 2.42	2.17 ± 2.17
Positive control	12.50 ± 2.00	11.80 ± 2.00	11.80 ± 2.00
Negative control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

The lowest inhibition zone of antibacterial activities showed by *E. guineensis* methanol leaves extracts against *S. aureus* was 6.17mm (50 mg/ml) and the highest was 10.67mm (300 mg/ml). Methanol extracts were more effective for antibacterial activity against *S. aureus* rather than chloroform solvent. As for antibacterial activities against *E. coli*, the widest inhibition zone was shown at concentration 200 mg/ml (6.5 mm). Evaluation of antibacterial activities against *P. aeruginosa* revealed that highest concentration had the lowest inhibition zone.

E. guineensis methanol leaves extracts were most effective against gram positive bacteria, *S. aureus* than gram negative bacteria, *E. coli* and *P. aeruginosa*. Kensa and Yasmin [13] found that different cell wall membrane structure attribute to this result. Gram positive bacteria have only single layer, lacking outer layer membrane and the negative bacteria have multi-layered structure and outer membrane. According to Nastrol et al. [17] gram positive bacteria more susceptible by having only an outer layer peptidoglycan layer which not effective permeability barrier.

Result also revealed that *E. guineensis* methanol leaves extract has potential act as antibacterial agent as majority of active compound being dissolved in methanol solvent [18] and methanol solvent also proven to be more effective solvent for plant extraction than ethanol, n-hexane and water [19].

Higher concentration of *E. guineensis* leaves extracts does not guarantee greater inhibition zone. Mesaros et al. stated that *P. aeruginosa* can produce antibiotics-inactivating enzymes [20]. As a result, antibiotics resistance will be build. Bacterial inhibition will not occur at this stage [21].

Table 3. Phytochemical screening test of *E. guineensis* leaves extracts

Compound	**Chloroform Leaves Extracts**	**Methanol Leaves Extracts**
Flavanoids	+	-
Terpenoids	-	+
Alkaloids	-	-
Gylcosides	-	-
Tannins	-	+
Saponin	-	+

Plant contains various biological active compounds which have potential for the development as medical agent. Phytochemical screening revealed the present of saponin, terpenoid and tannis in the *E. guineensis* methanol leaves extracts and the present of flavonoid in *E. guineensis* methanol extracts (Table 3). These compounds can serve as protection agent against microorganism. Tannins and flavonoid is the main phenolic compound in most plants which can serve as supplements in fight against various diseases [12]. While terpenoid participate in the plant defence which important in controlling pest, pathogen and weed [22].

Conclusion

The finding of the study revealed that phytochemicals from *E. guineensis* leaves extracts able to treat bacterial infections. It is demonstrated the potential of phytochemicals from *E. guineensis* leaves in developing a new and novel antibacterial agents. Showing promising result for all the bacteria tested, the phytochemicals present in *E. guineensis* leaves may serve as an affordable and new source for the treatment of bacterial infection.

Further toxicological and pharmacological studies will be useful to confirm the effectiveness of using *E. guineensis* leaves as alternative source in combating infections in human being which may also be lower cost and less toxicity. Phytochemical constituents in oil palm leaves seemed to have the potential to act as a source of useful drugs and also to improve the health status as the presence of various compounds that are vital for health.

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