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Research Article

PHYTOCHEMICAL CONSTITUENTS FROM LEAVES OF *ELAEIS GUINEENSIS* AND THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Objective: This study aims to evaluate the phytochemical constituents qualitatively and quantitatively, as well as the antioxidant and antibacterial activity of extracts from oil palm leaves.

Methods: In the present study, both qualitative and proximate (quantitative) phytochemical analysis were carried out using standard methods described previously. Meanwhile, antioxidant activity will be evaluated using DPPH scavenging assay while the antibacterial activity will be evaluated using disc diffusion assay.

Results: Crude extraction showed that methanolic extraction produced highest yield (8.28%) followed by hexane (3.10%) and chloroform (3.08%) extraction. Phytochemical screening of methanolic extract of oil palm leaves revealed the presence of phenolic compounds such as flavonoids, tannins, coumarins, alkaloids, saponins, terpenoids, steroids, and carbohydrates. Proximate analysis of phytochemicals in methanolic extracts showed that flavonoids are the main phytochemical constituents which found to be the highest (257.00 ± 3.055 mg QE/g DW) in oil palm leaves. Antioxidant assay exhibited great antioxidant activity from the methanolic extracts with IC_{50} value of 0.646 mg mL^{-1}. Meanwhile, the antibacterial activity revealed that methanolic extracts showed broad spectrum activity to all the tested bacteria with inhibition zone of 7.7 – 11.3 ± 0.0 – 1.0 mm.

Conclusion: The presence of many bioactive compounds such as alkaloid and flavonoid might responsible for the great antioxidant and antibacterial activities which worth to be further explore.

Keywords: Oil palm leaf, Phytochemicals, Antioxidant, Antibacterial.

INTRODUCTION

Intense development of oil palm industry in Malaysia contributes to the country's economy growth mainly through the trading of crude palm oil and its products [1]. However, there is an abundance of waste being generated from the industry which can be turned to value-added products. The palm biomass such as oil palm trunk, fronds, leaves, mesocarp fibres, shell and empty fruit bunches can be used as fuel to generate heat and electricity. In addition, recycling of cut fronds, empty fruit brunches and palm oil mill effluent (POME) contributes in replenishing of nutrients in oil palm plantations [2]. Besides its economic and environmental value, oil palm waste may also valuable for medicinal purposes. It is noteworthy that every parts of this plant can be used medicinally. The juice squeezed from oil palm leaves can be applied on wounds to enhance healing while the sap is used as laxative, and in fermented form is effective for improving lactation in nursing mothers [3]. The pulverized roots are added to drinks to cure gonorrhea, menorrhagia as well as bronchitis while the fruit mesocarp oil and palm kernel oil are administered as poison antidote and used externally as lotions with addition of other herbs for skin diseases [3, 4]. Ethnobotanical studies have revealed the folklore medicinal claim of *E.guineensis* for treatment of cancer, rheumatism, headaches and as an aphrodisiac, liniment and diuretic [5].

Ethnomedicinal use of plants is one of the most successful tools used in pharmaceutical industry searching new therapeutic agents for various fields of biomedicine [6]. Biologically active compounds like alkaloids, phenolic compounds, saponins, flavonoids, and many others, with known antioxidant and antibacterial properties, can be of great significance in therapeutic treatments [7, 8, 9]. These raise the interest of researchers to further investigate leaves of *E. guineensis* since it has been reported to possess some medicinal values [3, 10]. Meanwhile, not only the oil palm fruit containing many bioactive compounds such as fatty acid and essential oil, but the leaves also contain leveled amount of bioactive compounds, in fact in much diverse and higher in concentration [11]. Previous study also suggested that these compounds might probably exert medicinal effect against many ailments such as cancer and antiviral effect [3, 12]. Therefore, this paper aims to investigate the phytochemicals compounds from oil palm leaves along with its antioxidant and antibacterial activity against some common bacterial pathogens such as *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* which frequently reported responsible for nosocomial infection.

MATERIALS AND METHODS

Plant Collection

Fresh leaves of *E. guineensis* were collected from mini-nursery of School of Science and Technology, Universiti Malaysia Sabah and used as the sample for this study. The leaves were separated, cut into smaller pieces and washed thoroughly under running tap water. The leaves were dried in an oven at 40°C for 72 hours. The dried leaves were blended to fine powder using a mechanical blender.

Solvent Extraction

Organic solvents such as methanol, chloroform and hexane were used for extraction. Sample (50 g) was dissolved in 500 mL of respective solvents by sonication for 10 minutes. The extract was filtered through Whatman No .1 filter paper using vacuum pump filtration and the extraction process was repeated for two times The extract was concentrated under reduced pressure at 40°C, 90 rpm using a rotary evaporator. The yields obtained were kept at -20°C for further analysis. Extraction yields were weighted and calculated the formula as followed:

$$\text{Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant powder}} \times 100$$

Phytochemical Study (Qualitative analysis)

Phytochemical studies were conducted qualitatively and quantitatively to identify the presence of bioactive chemical constituents such as alkaloids, flavonoids, terpenoids and steroids, saponins, tannins, phenolic compounds, coumarins and carbohydrates. Qualitative phytochemical analysis was done according to the standard protocols as described previously [13, 14, 15, 16], while quantitative phytochemical analysis was done based on standard proximate measurement as described by previous studies [17, 18, 19, 20, 21, 22].

Antioxidant Assay using DPPH Method

Methanol solution of 1, 1-diphenył2-picrylhydrazyl (DPPH) radical (0.004 % w/v) was prepared and stored at10°C in the dark. Sample in methanol solution with different concentrations of 0.0625 to 4 mg mL^{-1}were prepared. Aliquot (0.05 mL) of the sample solution was then added to 5.0 mL of methanolic DPPH solution. The mixture was shaken vigorously and left to stand for 30 min in the dark. Absorbance measurements were recorded immediately at 517 nm. The absorbance of the DPPH radical without the crude extract samples (control) and the reference compound DL-α-tocopheroland gallic acid were measured. All the determinations were performed in three replicates and average was counted [23]. The percentage of inhibition (PI) of the DPPH radical was calculated according to the formula as followed:

$$PI, \% = = \frac{AC - AT}{AC} \times 100$$

Where AC = Absorbance of the control and AT = absorbance of the sample

Test Microorganisms

In this study, pure cultures of four different pathogens (*Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pneumonia*) were obtained from Queen Elizabeth Hospital, Kota Kinabalu, Malaysia. The microbial cultures were preserved in 30% glycerol stock solution at -85°C freezer. Prior to the antimicrobial activity study, the test microorganisms were subcultured on Nutrient Agar (NA) media, incubated at 37°C for 24 hours then inoculums of the test microorganisms were grown into Nutrient Broth (NB) and adjusted according to Mac Farlands Standard to achieve approximately $1x10^8$ CFU/ml before introduced into the test media.

Antibacterial Assay

The antibacterial activity was determined by the paper disc diffusion method. Sterilized discs of Whatman No.3 paper (6 mm) were used. All extracts concentration of methanol, chloroform and hexane (10 to 100 mg mL^{-1}) were used which 60 µL of the extracts were loaded onto the discs. Respective solvent (40 µL) was the negative control, whereas chloramphenicol antibiotic discs (10 mg mL^{-1}, 30µg/disc) were used as positive control. Muller-Hilton Agar (MHA) medium was prepared, sterilised and then transferred into petri dish. Approximately 0.1 mL culture of bacterial was placed on the MHA media and spread throughout the plate. The petri dishes that placed with discs (positive control, negative control, and different extract concentrations) were kept at room temperature for 30 min. The experiment was performed in triplicates and then incubated at 37°C for 24 hours before zones of inhibition to bacteria were measured and average diameter of zone of inhibition was obtained. Cultured bacteria with halo equal to or greater than 7 mm are considered susceptible to the tested extract [24].

RESULTS

Extraction Yield

Different solvents have different resolving strength towards the plant constituents which resulted in different yield as shown in Table 1. The extraction of methanol resulted with the highest amount of yield. Oil palm leaves (200 g) used in methanol-polar solvent extraction obtained the highest yield (8.28%). For both non-polar solvent extractions (chloroform and hexane), the yields obtained were 3.08% and 3.10% respectively.

Table 1: Yield of oil palm leaves extracted using different types of solvents.

Solvents	Weight of leaves used (g)	Yield (g)	Yield (%)
Methanol	200	16.56	8.28
Chloroform	50	1.54	3.08
Hexane	100	3.10	3.10

Phytochemical study (Qualitative analysis)

Based on the highest yield obtained from Table 1, methanol was chosen for further used for extraction and phytochemical screening. The qualitative analysis of the phytochemicals in oil palm leaves revealed the presence of phenolic compounds, flavonoids, tannins, coumarins, alkaloids, saponins, terpenoids and steroids, and carbohydrates as shown in Table 2.

Table 2: Qualitative analysis of the phytochemical constituents of oil palm leaves extracted with methanol.

Group of Phytochemicals	Test	Results
Alkaloids	Dragendorff's test	+++
	Mayer's test	+++
Coumarins	NaOH test	+++
Phenolic compounds	Gelatin test	+++
Saponins	Frothing test	+++
Tannins	$FeCl_3$ test	+++
Terpenoids and Steroids	Salkowski test	+++
Flavonoids	Shinoda test	+++
Carbohydrate	Benedict test	+++

- indicates absence, + indicates presence, number of (+) indicates no. of replication

Phytochemical Study (Proximate analysis)

The qualitative analysis results in Table 2 showed promising phytochemical properties presence in oil palm leaves. Therefore, a proximate analysis of phytochemicals using methanolic extract of oil palm leaves was performed to further quantify the amount of these properties and results are as shown in Table 3. The finding suggests oil palm leaves are rich in flavonoids, tannins, carbohydrate and phenolic compounds. The total alkaloid content was 5.00 ± 0.099 mg g^{-1} DW, contributed the least amount of phytochemicals in this study. The flavonoid content of the extract was 257.00 ± 3.055 mg QE g^{-1} DW, which is the highest amount among the phytochemical groups in this study. The flavonoid content was calculated in terms of quercetin equivalent ($y = 1.0354x + 0.001$, $R^2 = 0.9991$). The content of total phenolic was 70.07 ± 1.501 mg GAE g^{-1} DW, which was measured in terms of gallic acid equivalent ($y = 1.7333x - 0.0297$, $R^2 = 0.9993$). The content of tannin in extract was determined from the regression equation of tannic acid calibration curve ($y = 0.9514x - 0.0107$, $R^2 = 0.9997$), contained 165.00 ± 1.155 mg TAE g^{-1} DW; while the saponin content obtained was 24.00 ± 3.000 mg $g^{-1}$of DW. In addition, the amount of primary metabolites such as carbohydrates and protein content were also calculated. The carbohydrate content was determined from the glucose standard curve ($y = 34.075x + 0.0569$, $R^2 = 0.9996$), showed a total amount of 80.00 ± 1.756 mg glucose g^{-1} DW of oil palm leaves. In protein content determination, bovine serum albumin (BSA) was used as standard, showed regression equation of calibration curve ($y = 0.1691x - 0.01$, $R^2 = 0.9989$). The protein content obtained from oil palm leaves was 20.00 ± 1.365 mg BSA g^{-1} DW.

Table 3: Mean values of phytochemical content and relative percentage of mean in oil palm leaves.

Group of Phytochemical	Quantity (mg g^{-1} DW) ± S.D.	Relative % of mean
Alkaloids	5.00 ± 0.099^{f}	0.81
Saponin	24.00 ± 3.000^{e}	3.86
Flavonoid	257.00 ± 3.055^{a}	41.38
Phenolic	70.07 ± 1.501^{d}	11.28
Tannin	165.00 ± 1.155^{b}	26.57
Carbohydrate	80.00 ± 1.756^{c}	12.88
Protein	20.00 ± 1.365^{e}	3.22

a,b,c,d,e,f indicate groups of homogenous subset based on One-Way ANOVA analysis. DW denotes dried weight. S.D. denotes standard deviation.

Antioxidant Activity determined using DPPH radical scavenging activity

The dose-response curves of DPPH radical scavenging activities of the methanolic extract from oil palm leaves to reference antioxidants is shown in Figure 1. The scavenging effect of extract on DPPH radicals increased from 0.063 to 4 mg mL^{-1}. Gallic acid and α-tocopherol have the maximum DPPH free radical scavenging activity of 99.26% and 88.07%, respectively. Meanwhile, the scavenging activity for methanolic leaves extract was up to 98.26% at 4.0 mg mL^{-1}.

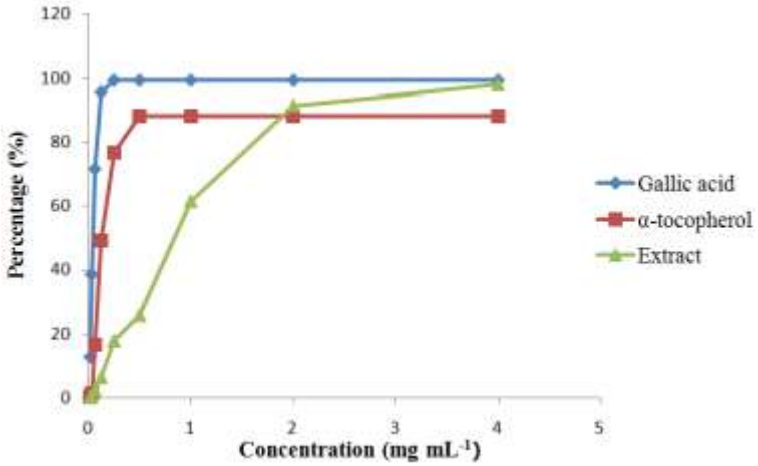

Fig. 1: DPPH radical scavenging activity of the methanolic extracts from oil palm leaves. Gallic acid and α-tocopherol were used as reference antioxidants.

Table 4 showed the amount of extract and reference antioxidants needed for 50% inhibition (IC_{50}). Lower IC_{50} value indicates higher antioxidant activity. IC_{50} value of the reference, gallic acid and α-tocopherol were 0.042 and 0.131 mg mL^{-1}, respectively. Meanwhile, the radical scavenging activity which showed by methanolic extract with IC_{50} was 0.646 mg mL^{-1}. This shows the gallic acid had the significant highest DPPH radical scavenging activity ($P < 0.05$), followed by α-tocopherol and lastly, the plant extract.

Table 4: IC_{50} values of reference antioxidants and methanolic oil palm leave extract for free radical scavenging activity.

Types	Gallic acid	α-tocopherol	Extract
IC_{50} value (mg mL^{-1})	0.042^{c}	0.131^{b}	0.646^{a}

a, b, c indicate groups of homogenous subset based on One-Way ANOVA analysis.

Antibacterial Activity

The antimrobial activity was assessed qualitatively and quantitatively with the presence or absence of inhibition zones, as summarized in Table 5. The methanolic extract from oil palm leaves showed antimicrobial activity against all tested microorganisms, with the exception to gram-negative bacteria *E. coli*. The methanolic extract showed almost similar intensity of antimicrobial activity against gram-positive bacteria, *Bacillus cereus* (11.0±0.0 mm) and *Streptococcus pneumonia* (11.3±0.6 mm); and gram-negative bacteria, *Pseudomonas aeruginosa* (11.0±1.0 mm) when the concentration set to 6mg per disc. However, chloroform and hexane extracts showed no activity to all tested microorganisms. There is no growth or activity observed on any negative disc loaded with respective solvents. Growth inhibition observed on standard disc (chloramphenicol) confirmed none of the microorganisms tested were chloramphenicol resistant.

Table 5: Antimicrobial activity of oil palm leaves extract with respective solvents against several types of bacteria.

Test Organism	Size of inhibition zone (mm) with respective plant extracts					
	MeOH				CHCl3	Hexane
	6mg/disc	3mg/disc	0.6mg/disc	CHL		
Bacillus cereus	11.0±0.0	9.7±0.6	8.0±0.0	16.0±1.2	n.d	n.d
Streptococcus pneumoniae	11.3±0.6	9.7±0.6	7.7±0.6	12.0±0.6	n.d	n.d
Escherichia coli	n.d	n.d	n.d	24.0±1.2	n.d	n.d
Pseudomonas aeruginosa	11.0±1.0	9.7±0.6	7.7±0.6	11.0±0.6	n.d	n.d

Values presented are means of three replicates ± standard deviations. Each disc loaded with approx. 60 µL or 6mg, 3mg and 0.6mg/disc of plant extract respectively. n.d= not detected. CHL= Chloramphenicol (10mg mL^{-1}, 30µg/disc). MeOH= Methanol; CHCl3=Chloroform

DISCUSSION

Extraction was done in order to separate the biologically active portions of plant using selective solvents through standard procedures. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. As shown in Table 1, the methanol extracts obtained the most yields. Previous study reported that methanol can extract most of the active components which are polar in nature such as anthocyanins, saponins, tannins, xanthoxyllines, totarol, quassinoids, flavones, polyphenols etc., whereas chloroform can extract terpenoids and flavonoids, and hexane can extract non-polar substances consists mainly of lipids [25]. Therefore, this finding suggests the choice of solvent plays an important role to obtain the highest extracts yield. Phytochemical study revealed all tested plant bioactive constituents were present in the oil palm leaves methanolic extracts. This is corresponding to a previous study which suggested that most of the polar solvents are able to resolve most of the plant bioactive constituents [24]. It is known that different phytochemicals have a broad range of pharmacological activities. For instance, saponins can be used as an anti-inflammatory agent and in treatment for tuberculosis; steroids were used as allergy, arthritis and coronary failure therapy, control in menstrual cycle and increasing women fertility; alkaloids can increase nutrient absorption and blood circulation, reduce pain and stimulate nerve system as it has narcotic effect; and tannins are reported to possess anti-irritant, anti-secretolytic, anti-phlogistic, antimicrobial and anti-parasitic effects [26]. Moreover, flavonoids are well documented to have important effects on various biological systems. Flavonoids have been referred as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activities [27].

The quantification of the amounts of the phytochemicals present in oil palm leaves confirmed flavonoid, tannin and phenolic are the main phytochemical constituents in oil palm leaves. Based on the study, the high content of phenolic, tannin and flavonoid compounds may contribute to its antioxidant activity. Previous study suggested if the IC_{50} value of an extract is less than 10 mg mL^{-1}, it indicates the extract is an effective antioxidant [28]. In this study, the IC_{50} value of

the methanol extract of *E. guineensis* leaves was 0.646 mg mL^{-1}, which means the extract potentially to be an effective antioxidant. Antioxidant capacity has been linked to many health-promoting properties; as they are able to scavenge free radicals which involved in many disorders such as neurodegenerative diseases, cardiovascular diseases and cancer [29]. The results from the antibacterial bioassay showed activity against *B. cereus*, *S. pneumonia* and *P.aeruginosa*, with the inhibition zone between 7.7 ± 0.6 mm and 11.3 ± 0.6 mm. This antibacterial effect might probably contributed by the presence of strong antibacterial compounds from the extract or due to the presence of combination of those bioactive compounds which show diverse action mechanisms. Biological actions are primarily due to these components in a very complicated concept of synergistic or antagonistic activities. Indeed, many antimicrobial activities in plants have been reported to be a result of the bioactive compounds present in plants, such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids, terpenoids and etc. [30, 31]. Saponins and tannins which are known antimicrobial agents have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them [32]. The potential as antimicrobial compounds may be further explored by identifying the bioactive constituents responsible for the action.

CONCLUSION

The phytochemical screening demonstrated the presence of different types of phytochemical constituents in oil palm leaves including phenolic compounds, flavonoids, tannins, coumarins, alkaloids, saponins, terpenoids and steroids which could be responsible for the biological activities. This study supports further research to isolate, purify, and characterize the active constituents from oil palm leaves; as it is needed to be elucidated for the drug discovery as well as development of new antibacterial agent

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