Antioxidant and Hepatoprotective Activity of Methanolic Extracts of *Elaeis Guineensis* Jacq Leaf

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Summary

In this study, the antioxidant properties of methanol extract of leaves *Elaeis guineensis* was evaluated through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity. Methanol extract of leaves *E. guineensis* exhibited good antioxidant activity (50.14 ± 1.711 at 1.0 mg/ml) with EC_{50} values of 810 µg/ml. In addition, the hepatoprotective activity of methanol extract of leaves *E. guineensis* was evaluated against carbon paracetamol induced hepatic damage in mice. The histopathology of rat liver was also done. The extracts at dose of 200 mg/kg were administered orally once daily. The results of this study strongly indicate that methanol extract of leaves *E. guineensis* has potent hepatoprotective action against paracetamol induced hepatic damage in mice. This study suggests that possible mechanism of this activity may be due to free radical-scavenging and antioxidant activities which may be due to the presence of polyphenols in the extracts.

Key words: Antioxidant activity, *Elaeis guineensis,* hepatoprotective activity

Introduction

There has been interest in the contribution of free radical reaction participating in reactive oxygen species to the overall metabolic perturbation that result in tissue injury and disease. Reactive oxygen such as superoxide anion, hydrogen peroxide, and hydroxyradical are generated in specific organelles of cells (Mitochondria and Microsomes) under normal physiological condition. These reactive oxygen species can damage DNA, so as to because mutation and chromosomal damage oxidize cellular thiols and abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (1, 2). Recently, various phytochemicals and their effect on health, especially the suppression of active oxygen species by natural antioxidant from tea, spices and herbs, have been intensively studied (3, 4).

Elaeis guineensis is a perennial monocot belonging to the family Palmae and tribe Cocoineae. It gives the highest oil yield per hectare of all the economic oil crops (5). It is an important crop for Malaysia and contributes significantly to the national economy (6). *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. Folk remedies of oil palm include treatment for cancer, headache and rheumatism and as an aphrodisiac, diuretic and liniment (7). Hence, the present study was carried out to determine the antioxidant and hepatoprotective activity of *E. guineensis* leaf extract.

Materials and methods

Standards and reagents: Standards; BHT (Butylated hydroxytoluene) and (+)-catechin, were purchased from Sigma (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH_) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol was obtained from Pronalab (Lisbon, Portugal).

Plant collection and extraction: Fresh samples of oil palm leaves were collected in February, 2008 near Semeling, Sungai Petani.. Plants were identified by a botanist of School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Leaves of selected plants were dried (room temperature) and powdered with a mortar.

Preparation of plant extracts: Some 100g of dried and powdered plant material were extracted at room temperature with 500 mL of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the antioxidant assays.

Total phenolic content: Contents of total phenolics in the extracts were estimated by a colorimetric assay based on procedures described by Singleton and Rossi (8), with some modifications. Basically, 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (0.250–2.500 mM; Y = 0.2903X; R2 = 0.999) and the results were expressed as mg of gallic acid equivalents/g of extract (GAEs).

DPPH radical-scavenging activity: Various concentrations of oil palm leaves methanolic extract (50.0 µl) was mixed with 5.0 ml of methanolic solution containing DPPH radicals (0.004% w/v). The mixture was shaken vigorously and left to stand for 30 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution (9).

The extract concentration providing 50% of radical scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration. BHT was used as standard.

Animals: Wister albino mice of either sex were used for the study of the crude extracts. Institution Animal Ethics Committee has approved the project. The animals were kept at 27±2 ◦C, relative humidity 44–56% and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and the food was withdrawn 18–24 h before the start of the experiment and water *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (10).

Paracetamol induced hepatotoxicity: Mices were divided into three groups (n=5). Group 1 (control) animals were administered a single dose of water (1ml/kg) daily for 7 days. Group II (Paracetamol) received water (1ml/kg body weight) once daily for 7 days and received Paracetamol (1ml/kg body weight) on day 5. Group III were administered orally a dose of 200 mg/kg of methanolic extract of *E. guineensis* in form of aqueous suspension once daily for seven days and paracetamol on day 5. Animals were sacrificed 24 hours after the last treatment. Liver was dissected out and used for histopathological studies (11).

Histopathological studies: The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration.

Statistical analysis: For all the experiments three samples were analysed and all the assays were carried out in triplicate. The results are expressed as mean values and standard error or standard deviation (SD). The differences between the oil palm leaves extract was analysed using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test with a = 0.05. This analysis was carried out using the SPSS v. 12.0.

Results and discussion

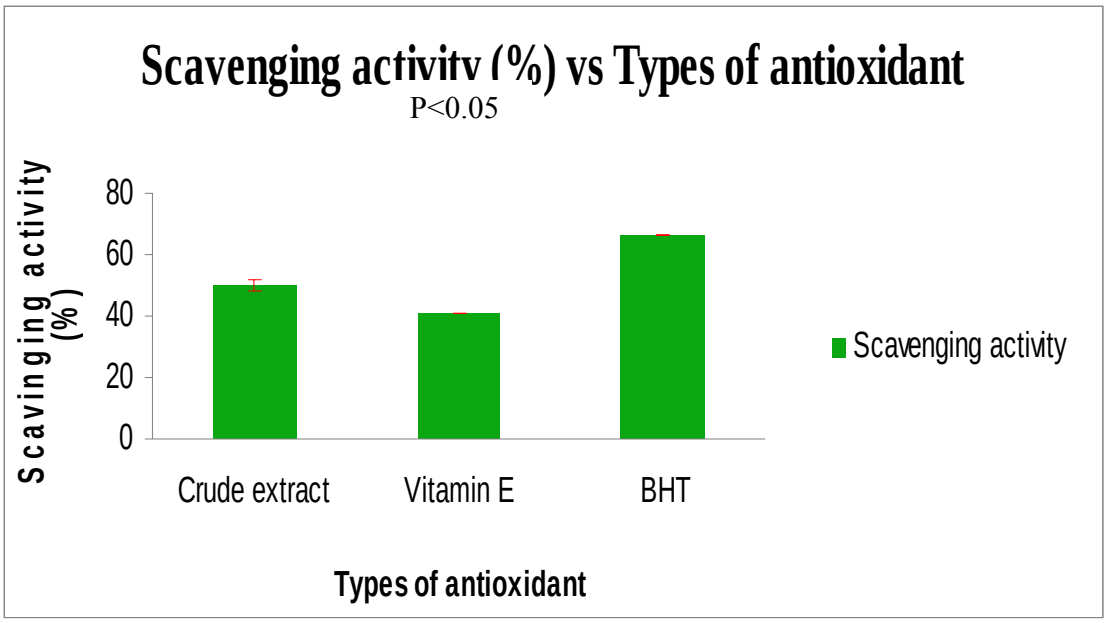
Table 1 present's polyphenols contents obtained for oil palm leaves extracts. The polyphenol content was 333.3 mM gallic acid equivalents (GAE) per liter of sample (mM l^{-1}). The antioxidant properties of the methanol extract of leaves *E. guineensis* was examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Figure 1 as comparable with known antioxidant BHT and vitamin E. The radical-scavenging activity (RSA) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH˙ solution in the absence of extract at 517 nm.

Table 1: The phenolic content of oil palm leaves.

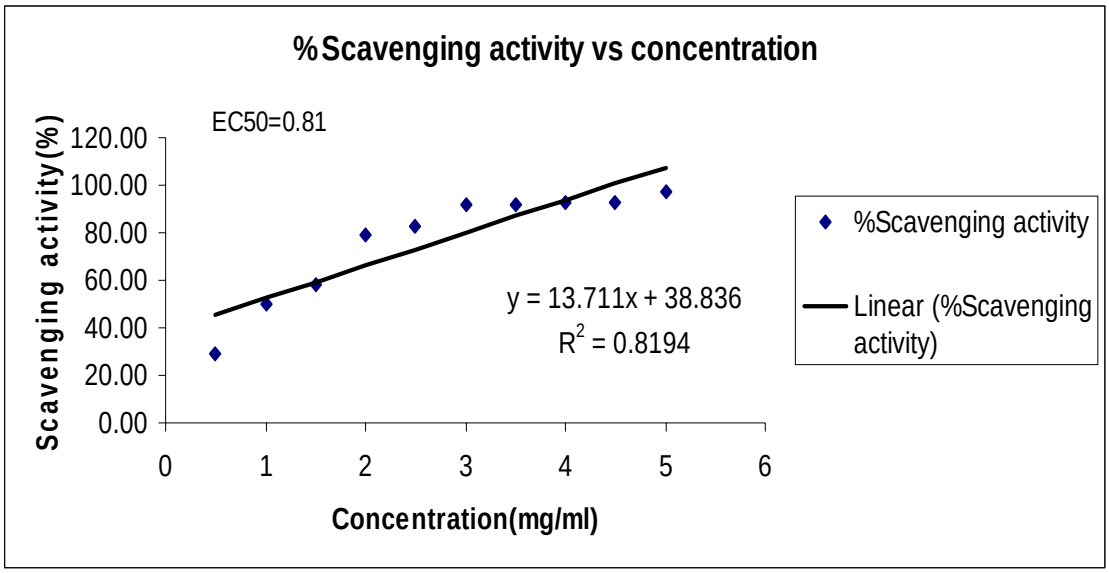
Sample	Mean ± Standard deviation
1	0.417 ± 0.031
2	0.436 ± 0.018
3	0.347 ± 0.008
Average	0.400 ± 0.047
Phenolic content	333.3 mM

From the analysis of Figure 1, we can conclude that the scavenging effects of leaves extracts on DPPH radicals was excellent (P<0.05), especially in the case of vitamin E but methanol extract of leaves *E. guineensis* revealed a low value of antioxidant activity compared with BHT. BHT has the highest percent scavenging activity that is 66.00 ± 0.010 compare to oil palm leaves that is 50.14 ± 1.711. The lowest is Vitamin E that is 41.00 ± 0.020. Figure 2 shows the EC_{50} value of methanol extract of leaves *E. guineensis* measured by DPPH radical-scavenging assays. Overall, methanol extract of leaves *E. guineensis* showed lower EC50 values of 810µg/ml. Lee et al. (12) reported that if the EC_{50} value of an extract is less than 10 mg/ml, it indicates that the extract is an effective antioxidant. In this study, the EC_{50} value of the methanol extract of leaves *E. guineensis* was less than 10 mg/ml, and this indicates that the extract was an effective antioxidant.

Figure 1: Free radicals scavenging activity of oil palm leave extract



. **Figure 2**: The EC_{50} values of crude extract of oil palm leaves.



Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well-preserved cytoplasm, nucleolus, prominent nucleus and visible central veins (Figure 3a). The liver sections of paracetamol-intoxicated mice showed massive severe necrosis, massive vacuolar degeneration and dilated blood sinusoids are filled with red blood cells (Figure 3b). The histological architecture of liver sections of the rats treated with methanol extract of leaves *E. guineensis* showed more or less normal lobular pattern hepatocytes with regenerating hepatocytes, diminution of fibrosis and reduction in fibrotic area (Figure 3c). Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic agent (13). At therapeutic doses, paracetamol is considered a safe drug. However, it can cause hepatic necrosis, nephrotoxicity, extra hepatic lesions, and even death in humans and experimental animals when taken in overdoses. Paracetamol hepatotoxicity is related to excessive oxidative stress mainly caused by the electrophile and highly reactive metabolite of PCM (NAPQI) (14). Paracetamol induced hepatic liver cell damage was studied in mice as its toxicity was maximum in mice. The animals became very weak and their body weight reduced considerably during one week experimental study. However, with the treatment of crude extract there was no reduction in the body weights and food consumption.

Conclusion

As far as we know, this is the first report concerning the antioxidant activity of methanol extract of leaves *E. guineensis*. The work herein indicates that the methanol extract of leaves *E. guineensis* present the good antioxidant activity values. The results obtained indicate a high potential of application for this methanol extract of leaves *E. guineensis* as an antioxidant. It can be included in foods with notable benefits for mankind or animal health.

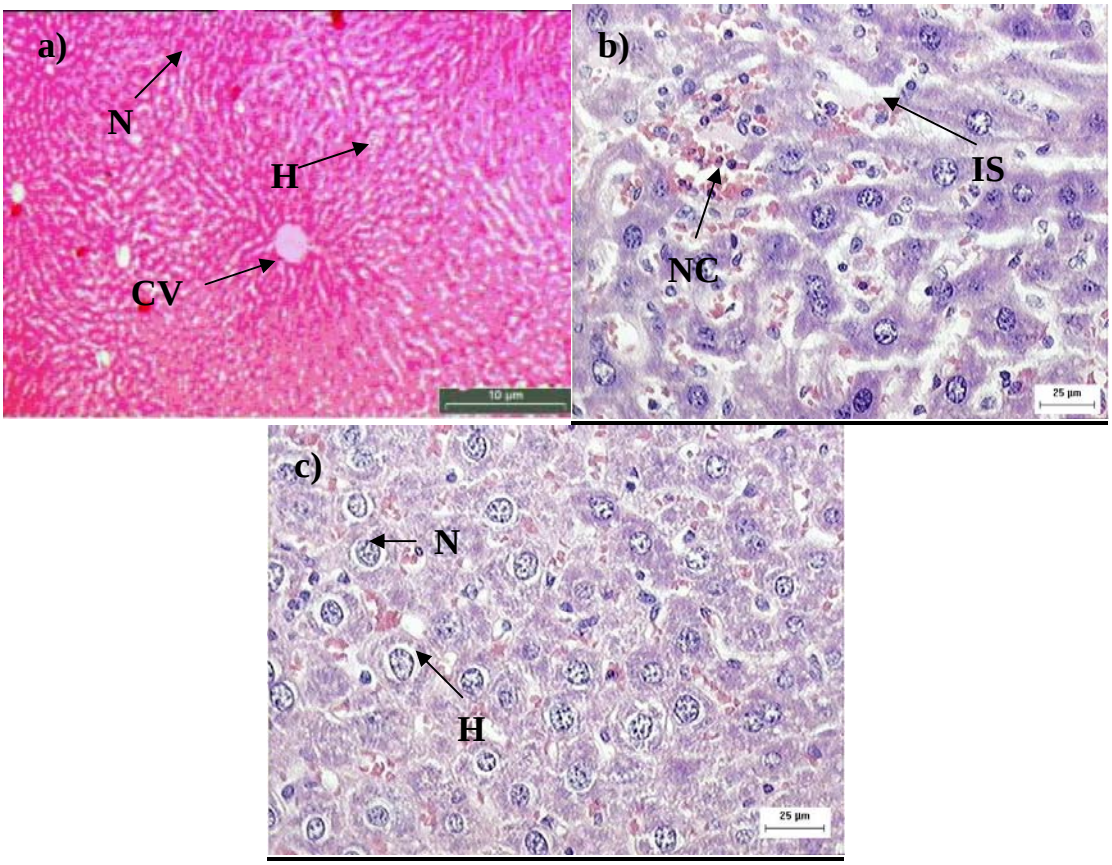


Figure 3: a) Photomicrograph of liver tissue of control mice showing normal hepatic cells with central vein. b) Photomicrograph of infected liver tissue of mice when given paracetamol to induce damage showing severe necrosis, massive vacuolar degeneration and dilated blood sinusoids are filled with red blood cells. c) Photomicrograph of treated liver tissue of mice with crude extract of oil palm leaves showing normal hepatocytes with regenerating hepatocytes, diminution of fibrosis and reduction in fibrotic area.(N: Nucleus, CV: Central vein, H: Hepatocytes, NC: Necrosis, IS: Intercellular space).

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