Original Article

Antioxidant properties of palm fruit extracts

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Phenolic compounds have been shown to exhibit bioactive properties, and in particular antioxidant effects. A phenolic-rich fraction has been isolated from the aqueous by-product obtained during the milling of oil palm fruits. The objectives of the study were to determine the phenolic content of the crude and ethanolic extracts of oil palm fruits and to evaluate the antioxidant properties of these extracts. The total phenolics content of the crude and ethanol extracts as determined by the Folin-Ciocalteu method were found to be 40.3 ± 0.5 and 49.6 ± 0.6 mg GAE/g extract (dry basis), respectively. The radical scavenging activity of the extracts determined using 2,2 diphenyl-1-picrylhydrazyl radical (DPPH·) indicated that both crude and ethanol extracts exhibit hydrogen-donating capacity, and have antiradical power (ARP) comparable to ascorbic acid. The DPPH radical scavenging activity of the extracts were less than that of gallic acid, but the time-course variations of the scavenging curves suggest that the extracts acted by a mechanism similar to that of gallic acid. The electron-donating potentials of the extracts were inferred from the hydrogen peroxide scavenging and reducing power assays. The reducing power of crude and ethanol extracts at 1 mM GAE were found to be comparable to that of 0.3 mM gallic acid. The extracts indicated complete scavenging of hydrogen peroxide at concentrations above 0.4 mM GAE. These findings suggest that the crude and ethanol extracts are able to scavenge free radicals, by either hydrogen or electron donating mechanisms, and can therefore act as primary antioxidants.

Key Words: palm fruit, antioxidants, free radicals, phenolics

Introduction

The oil palm (*Elaeis guineensis)*, is the source of two important edible oils, i.e palm oil from the mesocarp, and palm kernel oil from the kernel. The extraction of palm oil involves a milling process, where substantial amounts of water are introduced into the system. Water-soluble phytochemicals from the palm fruit mesocarp partition into the aqueous phase, and can be recovered through a combination of centrifugation and membrane filtration technologies.[1] The product obtained from this process[2] contains several phenolic compounds, including gallic, chlorogenic, protocatechuic, gentisic, coumaric, ferulic and caffeic acids, as well as catechins, hesperidin, narirutin and 4-hydroxybenzoate.[1]

Phenolic compounds exhibit a wide range of biological and physiological properties due to their ability to act as antioxidants,[3,4] free radical scavengers[5,6] and chelators of divalent cations.[7] The antioxidant activity of phenolic compounds is influenced by several factors, i.e. position and degree of hydroxylation, polarity, solubility, reducing potential, and stability of the phenoxy radical.[8] The structure of phenolic compounds, in particular the position and degree of hydroxylation, is of primary importance in determining their antioxidant activity.[9] However, phenolic compounds, which account for one of the largest and most widely distributed group of phytochemicals[10], vary considerably in structure with over 8000 naturally-occurring compounds having been identified.[11] In this work, the antioxidant properties of the palm fruit extracts were studied by their ability to scavenge free radicals using the 2,2 diphenyl-1-picrylhydrazyl radical (DPPH·) reducing power and hydrogen peroxide scavenging assays.

Materials and methods

Chemicals and instruments

Methanol was of analytical grade. DPPH, gallic acid, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride and phosphate buffered saline were purchased from Sigma-Aldrich Co. Ltd (St. Louis, MO, USA), hydrogen peroxide from Sigma-Aldrich Laborchemikalien GmbH (Seelze), Folin-Ciocalteu reagent from Sigma-Aldrich Chemie GmbH (Riedstr), and sodium carbonate from Asia-Pacific Specialty Chemicals (NSW, Australia). Reverse osmosis (RO) water was used for preparation of aqueous solutions. Palm extracts had been prepared at the laboratories of the Malaysian Palm Oil Board (MPOB; Bandar Baru Bangi, Malaysia).[2]

Absorbance measurements were recorded by a Shimadzu UV-160A UV-Visible Reading Spectrophotometer (Shimadzu Corporation, Japan) using disposable cuvettes (Sarstedt, Nümbrecht, Germany) for visible range, and quartz cuvettes for measurements in the ultraviolet (UV) range.

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Determination of total phenolics content

Total phenolics content was determined by the Folin-Ciocalteu method as modified by Gao *et al.*[12] Gallic acid was used as a standard, and the total phenolics content was expressed as mg gallic acid equivalents (GAE)/g dry matter.

Radical scavenging activity

To 3 mL of 0.2 mM DPPH· solution in methanol in aluminum foil-wrapped test tube, 100 µL of sample was added. Test tubes were vortexed and incubated for 30 min at room temperature, and the absorbance read at 515 nm. Extracts were tested at concentrations of 0.2-1.0 mM GAE. Gallic and ascorbic acids were also tested at similar concentrations. The DPPH scavenging activity was

$$\%\ \text{DPPH}\cdot\ \text{Scavenging} = 100 \times \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right]$$

calculated as follows:[13,14]

where *Absorbance of Control is the absorbance in absence of standards or extracts*
Absorbance of Sample is the absorbance in presence of standards or extracts

Reaction kinetics

The method used was based on that described by Da Porto *et al.*[15] with slight modification. The procedure involved reaction of a 0.2 mM methanolic solution of DPPH· with antioxidant compounds at several molar ratios. Standards (gallic and ascorbic acids) were prepared as aqueous solutions of 0.1-1.0 mM, and 0.4-4.0 mM respectively. Crude and ethanol extracts were dissolved in water to produce solutions of 0.2-2 mM GAE. The reaction was started by addition of 100 µL of extract or standard antioxidant solution to 3 mL DPPH· solution in a test tube. Absorbance of the reaction mixture at 515 nm was then measured at 5 s intervals over varying periods of time, between 5 to 10 minutes.

Reducing power

The reducing power of extracts was determined by the method of Oyaizu[16] as modified by Chang et *al.*[17] using extract solutions of concentration 0.1-1.0 mM GAE.

Scavenging of hydrogen peroxide

A modified method based on that of Ruch *et al.,* [18] was used to determine the ability of the extracts to scavenge hydrogen peroxide. Hydrogen peroxide (43 mM) was prepared in phosphate buffered saline (pH 7.4). Standards (gallic and ascorbic acids) and extract solutions were prepared at concentrations of 0.1 to 1.0 mM. Aliquots of standard or extract solutions (3.4 mL) were added to 0.6 mL of hydrogen peroxide solution. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm. The percentage of scavenging was calculated as follows:[19]

$$\%\ H_2O_2\ \text{Scavenging} = 100 \times \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right]$$

where *Absorbance of Control is the absorbance in absence of standards or extracts*
Absorbance of Sample is the absorbance in presence of standards or extracts

Results and Discussions

Total phenolics contents of extracts

The total phenolics contents of the crude and ethanol extracts were found to be 40.33 ± 0.48 and 49.58 ± 0.63mg GAE/g extract (dry basis), respectively. It is postulated that the treatment with ethanol may have resulted in the precipitation of non-phenolic compounds, and thus contributing to the higher phenolics content in the ethanol extract.

DPPH radical scavenging activity

The scavenging of the DPPH radical by hydrogen-donating antioxidants (AH, Equation 1) is characterized by a rapid decline in the absorbance at 515 nm, followed by a slow step where the absorbance depreciates more gradually. The rapid reaction between antioxidants and DPPH· occurs with the transfer of the most labile H atoms to the radical, while the subsequent slow step depends on the residual H-donating capacity of antioxidant degradation products.[20, 21]

$$\text{DPPH}\cdot + \text{AH} \longrightarrow \text{DPPH-H} + \text{A}\cdot \qquad \textit{(Equation 1)}$$

The scavenging behaviour of both crude and ethanol extracts are similar to that of gallic acid, as seen from the disappearance of DPPH· over time curves (Fig. 1a-c). Ascorbic acid, on the other hand exhibits a very rapid initial step (Fig. 2), and the disappearance of the purple colour of DPPH· occurs almost immediately upon contact between reactants. These observations on the scavenging rates of gallic and ascorbic acids are consistent with the observations reported by Sanchez-Moreno *et al.,*[22] who classified these compounds as displaying, intermediate and rapid kinetic behaviour, respectively.

The DPPH radical scavenging activity of gallic acid, ascorbic acid, crude and ethanol extracts increases in a dose-dependent manner. Gallic acid exhibits higher DPPH radical scavenging activity than either ascorbic acid or palm extracts when tested at concentrations of 0.2-1.0 mM GAE. For example, at a concentration of 1.0 mM, the DPPH radical scavenging activity of gallic acid (94.5 ± 0.3%) was more than 6 times that of ascorbic acid (14.6 ± 1.1%), crude extract (15.4 ± 0.4%), and ethanol extract (14.2 ± 0.7%). On the other hand, the radical scavenging activities of the extracts are comparable to that of ascorbic acid.

The molar ratio of antioxidant/DPPH required to decrease the concentration of DPPH to 50% of its initial value, denoted as EC_{50} or Efficient Concentration, is an indicator of antiradical activity, i.e the lower the EC_{50}, the more potent the scavenging activity.[23] The EC_{50} can be obtained from plots of percentage (%) remaining DPPH· as a function of molar ratio (MR) of antioxidant/DPPH (Fig. 3). It has been suggested that the reciprocal of EC_{50}, called the antiradical power (ARP) provides a clearer and more direct indicator of the radical scavenging potency of a compound.[23] It is observed that the ARP of gallic acid

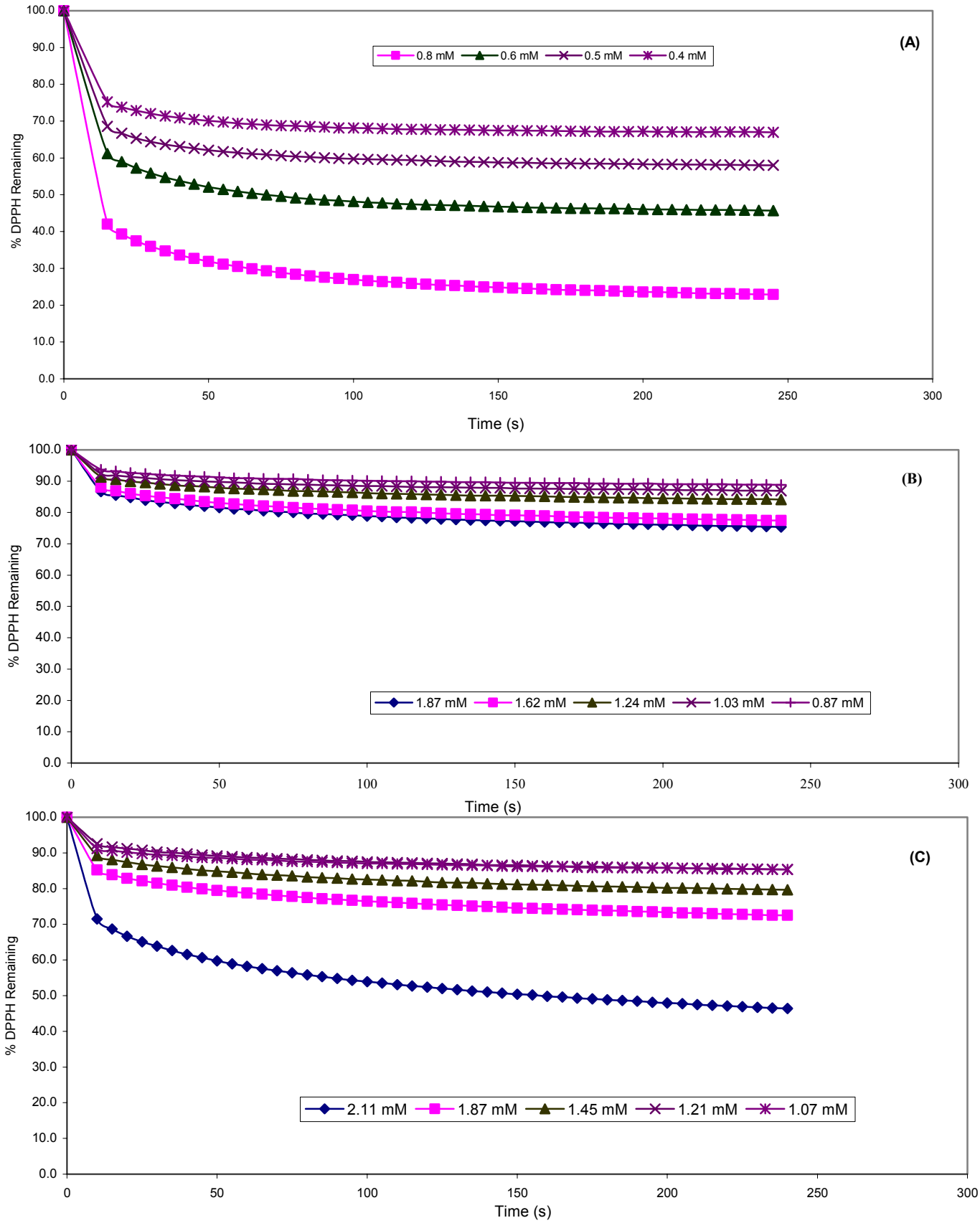

Figure 1 : Scavenging of DPPH radical by varying concentrations of (A) gallic acid, (B) crude extracts, and (C) ethanol extract

(13.05) is higher than that of ascorbic acid (3.78), crude extract (3.14) or ethanol extract (3.31). These ARP values for gallic and ascorbic acids agree with the values reported previously.[23]

This high ARP for gallic acid is attributed to the number of hydroxyl groups that are available to donate hydrogen atoms to the DPPH radical. The ARP of the crude and ethanol extracts are respectively, 3.14 and 3.31, which are comparable to those of eugenol (3.7) and zingerone (3.7), and higher than that of ferulic acid (2.33).[23] While the ARP of the ethanol and crude extracts are comparable to that of ascorbic acid, the radical scavenging curve discussed earlier suggests that the mechanism of DPPH radical scavenging by the extracts could be similar

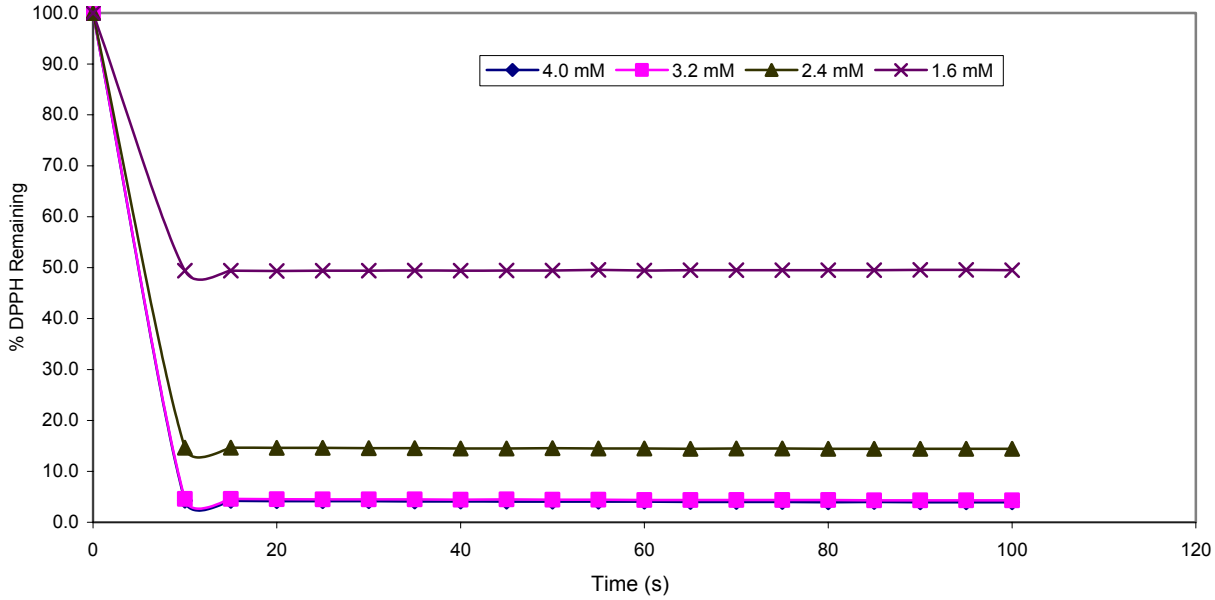

Figure 2. Scavenging of DPPH radical by ascorbic acid

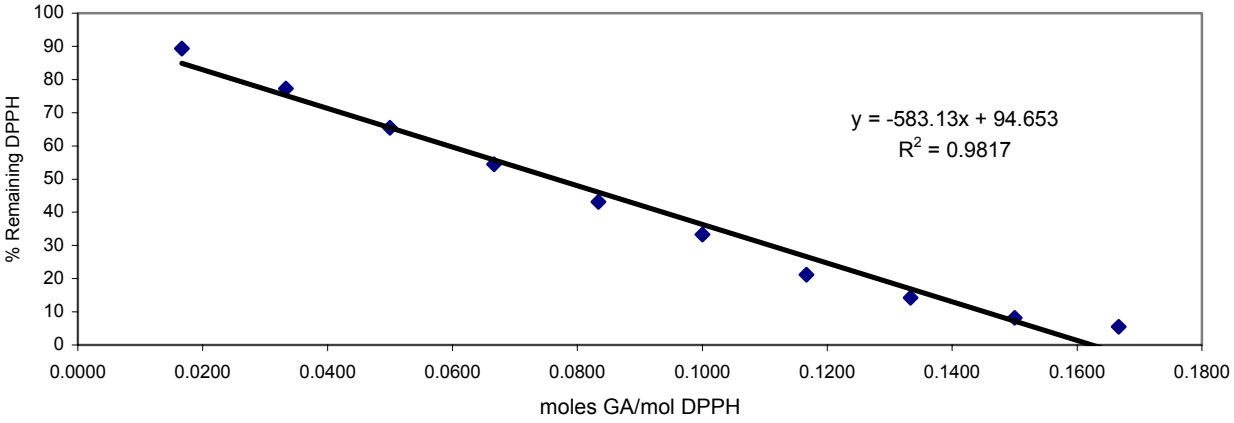

Figure 3. Plot of % remaining DPPH against Molar Ratio (gallic acid/DPPH)

to that of gallic acid. Though the exact composition of the phenolic compounds present in the crude extracts have yet to be fully elucidated, the presently available data identifies some of these compounds as gallic, chlorogenic, protocatechuic, gentisic, coumaric, ferulic, and caffeic acids, as well as catechins, hesperidin, narirutin, and 4-hydroxybenzoate.[1] As reported by Brand-Williams *et al.,*[23] gallic, gentisic, caffeic, and proto-catechuic acids exhibit potent antiradical activity, ferulic acid exhibits a moderate activity, but coumaric and vanillic acids exhibit poor activity. It could therefore be expected that the palm extracts could exhibit moderate anti-radical activity due to the combined antiradical activity of the different phenolic compounds present.

Reducing power

Reducing power assay measures the electron-donating capacity of an antioxidant.[24] The reduction of the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm, and an increased absorbance is indicative of higher reducing power. The reducing power of the extracts increased progressively over the concentration range studied (0.1 to 1.0 mM GAE). Extract solutions at 1mM GAE had comparable reducing power to gallic acid at 0.3 mM. These findings suggests that the palm extracts are capable of donating electrons, and could therefore react with free radicals or terminate chain reactions. The reducing power of ascorbic acid was also studied over the 0.05-0.6 mM concentration range and it was found that the reducing power increased in a dose dependent manner up to a concentration of 0.3 mM, but did not show any clear trend beyond that range.

Hydrogen peroxide scavenging

The scavenging of hydrogen peroxide by the standards (gallic and ascorbic acid) and extracts after incubation for 10 minutes increased with increased concentration. Both extracts exhibited higher hydrogen peroxide scavenging activity than either gallic or ascorbic acids at similar concentrations. While hydrogen peroxide itself is not very reactive[25], it can generate the highly reactive hydroxyl radical (OH) through the Fenton reaction[26,27] *(Equation 2)*. Thus, the scavenging of hydrogen peroxide is an important antioxidant defense mechanism.[28]

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^- \quad \textit{(Equation 2)}$$

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in Equation 3.[29]

$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O \quad \textit{(Equation 3)}$$

The scavenging of hydrogen peroxide by phenolic compounds has been attributed to their electron-donating ability.[30] The crude and ethanol extracts have high electron-donating abilities, and 100% scavenging was achieved with concentrations of crude and ethanol extracts at 0.4 mM GAE. In comparison, the hydrogen peroxide scavenging activity of gallic acid and ascorbic acid at 1.0 mM were found to be 93.2 ± 0.6% and 70.4 ± 2.5%, respectively.

Conclusion

The phenolics profile of crude and ethanol extracts are similar, though the latter has higher total phenolics content. The ability of palm fruit extracts to act as hydrogen donors is indicated by their ability to scavenge DPPH free radicals, and in this respect the DPPH radical scavenging activity of both crude and ethanol extracts are comparable to that of ascorbic acid. The radical scavenging kinetics, on the other hand suggests that the scavenging mechanism of the extracts is similar to that exhibited by gallic acid. The results of the reducing power and the hydrogen peroxide scavenging suggests that the extracts can also act as electron-donors, and as such can terminate radical chain reactions.

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Antioxidant properties of palm fruit extracts
椰子提取物的抗氧化性研究

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摘要: 很多研究表明酚类化合物具有生物活性,特别时在抗氧化性方面.油椰子粉末经水提取，分离出富含酚类的成分．本研究的目的是为了测定油椰子的粗提取物和醇提取物中的酚类的含量，并对这些提取物的抗氧化性进行研究．用 Folin-Ciocalteu 法测定粗提取物和醇提取物的总的酚类含量，其结果分别为 40.3 ± 0.5 and 49.6 ± 0.6 mg GAE/g(干重)．利用 DPPH.测定提取物清除自由基的能力，结果表明粗提取物和醇提取物都具有较强的贡献氢原子的能力，并且具有与维生素 C 相似的清除自由基的能力.这两种提取物清除 DPPH 自由基的能力虽然不如没食子酸强，但是它们作用机制与没食子酸相似．这两种提取物能清除过氧化氢，并具有还原性，所以我们推断出其具有贡献电子的能力．1 mM GAE 的粗提取物和醇提取物的还原能力与 0.3 mM 的没食子酸的还原能力相当．浓度高于 0.4 mM GAE 的提取物能完全清除过氧化氢．这些结果表明油椰子的粗提取物和醇提取物能通过贡献出氢离子或电子来清除自由基，因此可以作为一种抗氧化剂.

关键词: 椰子，抗氧化剂，自由基，酚类化合物