The antioxidant effect of *Carissa carandas* unripe fruit extracts and fractions

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ABSTRACTS

The fresh unripe and ripe fruits of *Carissa carandas* fruits were extracted using non-polar solvent *i.e* hexane, followed by chloroform and methanol. The extracts were then tested for their antioxidant activities using FTC and TBA methods. The chloroform and methanol extracts of unripe fruits showed better activities than BHT. The chloroform and methanolic extracts were fractionated using paper chromatography into eight fractions i.e. Five fractions from the chloroform extracts and three fractions from the methanolic extracts. The fractions were labeled as MF1, MF2, MF3, CF1, CF2, CF3, CF4 and CF5. The fractions were then tested for their antioxidant activities. As expected, all fractions showed very strong antioxidant activity and the strongest antioxidant activities were indicated by fraction MF1 and CF1. However, the optical density values of those fractions are higher then the chloroform and methanolic extracts. The major components of MF1 and CF1 are found as two unidentified monoterpenoids. The results revealed that the mixture of compounds in the chloroform and methanolic extracts exhibited better antioxidant activity than the isolated fractions. Based on this finding, it is concluded that the synergistic effects of the constituents in the chloroform extracts of the *Carissa carandas* unripe fruit performed the best antioxidant property.

INTRODUCTION

Carissa carandas, a shrub of the family Apocynaceae, possibly a native to Southeast Asia is selected in this study. This plant is widely cultivated throughout the tropical and subtropical regions for its fruit and as an ornamental plant because of its stiff, zig-zag branches, opposite green leaves, white and fragrant flowers in terminal cymes, ovoid to globose reddish small berry fruits. This plant also can be made as an excellent hedge (Hsuan Keng, 1990).

The root juice of this plant has been used in traditional medicine to treat various microbial diseases such as diarrhoea, dysentery and skin diseases (Taylor, 1996). The Malays conserve the fruits as pickle by boiling them in vinegar or salt, making cordial and jams (Burkill, 1966).

Natural antioxidants from plant extracts provide a measure of production that slows the process of oxidative damage (Bergman *et al.*,2001). Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extracts. In the present study, we have investigated the *in-vitro* antioxidant effect of the extracts and fractions from Carissa carandas unripe fruits using FTC dan TBA methods.

MATERIAL AND METHODS

Plant extraction

A kilogram of the fresh unripe and ripe fruits of *Carissa carandas* were dried in the drying cabinet (Protech, Malaysia). The materials were then successively extracted using non-polar solvent *i.e* hexane, followed by chloroform and methanol in a Soxhlet apparatus for 24 hours. The I extracts were evaporated under reduced pressure. The final residues were used for the bioassays.

Ferric thiocyanate (FTC) method

The method of Kikuzaki and Nakatani (1993) was slightly modified. A screw-cap vial containing a mixture of 4mg (4ml) of sample (final concentration 0.02%) in 99.5% ethanol. 4.1 ml of 2.5% linoleic acid (Sigma, USA) in 99.5% ethanol. 8.0 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in an incubator (WTB Binder, Germany) at 40°C in the dark. To 0.1 ml of this mixture, 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate (Sigma, USA) were added. Three minutes after addition of 0.1 ml of 2X10-2 M ferrous chloride (Sigma, USA) in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. This step was repeated every 24hours until the control reached its maximum absorbance value.

Thiobarbituric (TBA) method

Two ml of 20% trichloroacetic acid (Fisher, UK) and 2 ml of 0.67% TBA (Fisher, UK) solution were added to 2.0 ml from the mixture (containing sample) prepared in the FTC method (Ottolenghi, 1959). This mixture was kept in a water bath (100°C) for 10 min and after cooling to room temperature, it was centrifuged at 3000 rpm for 20 min. Antioxidant activity was based on the absorbance of the supernatant at 532 nm at one day after the final day of the FTC assay.

Fractionation of the best extracts

The chloroform and methanolic extracts of unripe fruits that showed the best antioxidant activities were fractionated using paper chromatography. The extracts were applied as a streak on 12-15 sheets of Whatman no 3 paper (46 x 57 cm) and run in solvent BAW overnight. The dried papers were viewed under UV and were cut into fractions based on the band and eluted in 80% methanol overnight. The fractions were concentrated and were used for the antioxidants assay. The compounds in the best fractions were identified.

Statistical analysis

The results are the means ± SEM of triplicates from 2-4 independent experiments. Statistical values ($p<0.05$) were determined by one-way ANOVA using Graphpad Prism version 3.0

RESULTS AND DISCUSSION

The antioxidant activity was evaluated using inhibition of linoleic acid autooxidation in a water alcohol system. This assay system represents inhibition of lipid peroxidation. Both of FTC and TBA methods were used. FTC method measures the amount of peroxide produced during the initial stage of lipid oxidation whereas TBA method measured the later stage of lipid oxidation. The chloroform and methanolic extracts of the unripe fruits showed very strong antioxidants activities as compared to BHT (a commercial antioxidant). The pattern of activity was very similar for both methods. Those two extracts were fractionated using paper chromatography with BAW as the solvent system. The fractions were then tested for antioxidant activity. All fractions showed very low antioxidant activity and the strongest antioxidant activities were indicated by fraction CF1 and MF1. However, their optical density values are higher then their original extracts. Their major components were identified as monoterpenoids. The results revealed that the mixture of compounds in the extracts exhibited better antioxidant activity than the isolated fractions. Based on this finding, it is concluded that the synergistic effects of the constituents in the chloroform extract of the unripe fruit performed the best antioxidant property.

Figure 1 Antioxidant activity of various extracts of the unripe and ripe fruits of *Carissa carandas* using FTC method

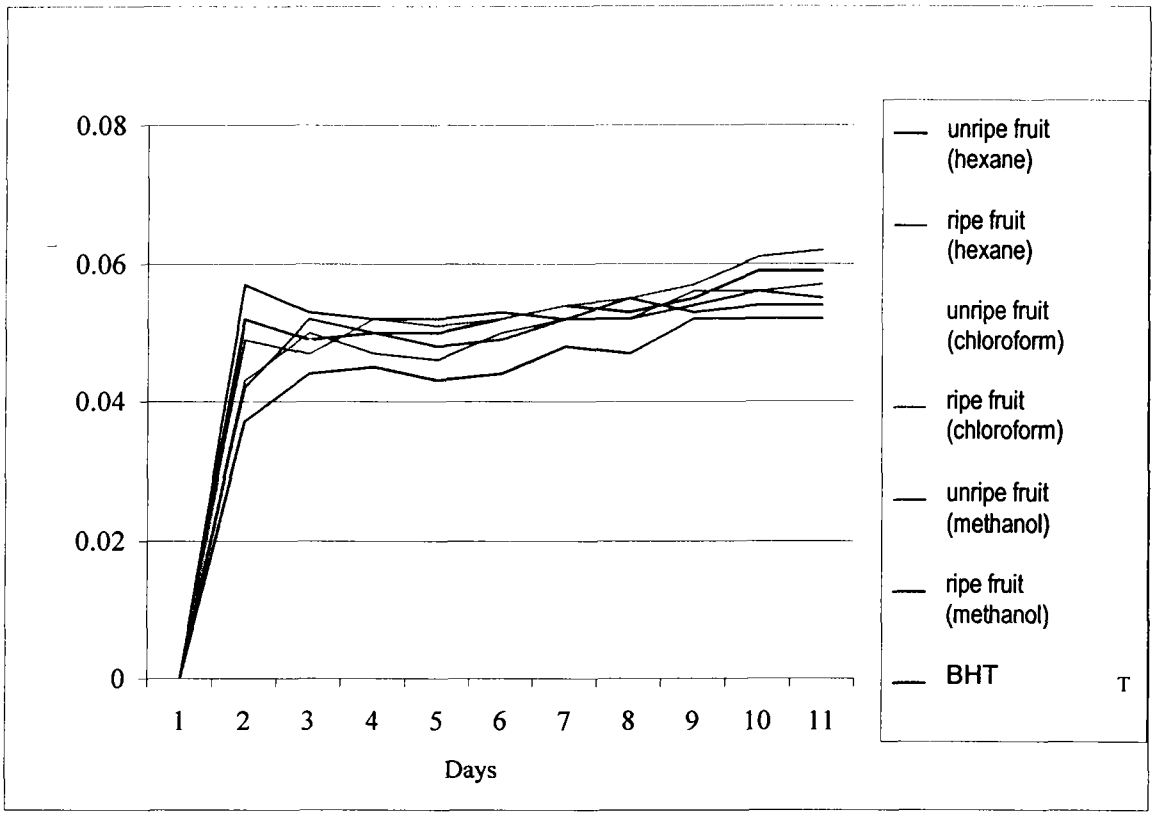


Figure 2 Antioxidant activity of various fractions from *Carissa carandas* unripe fruits using FTC method

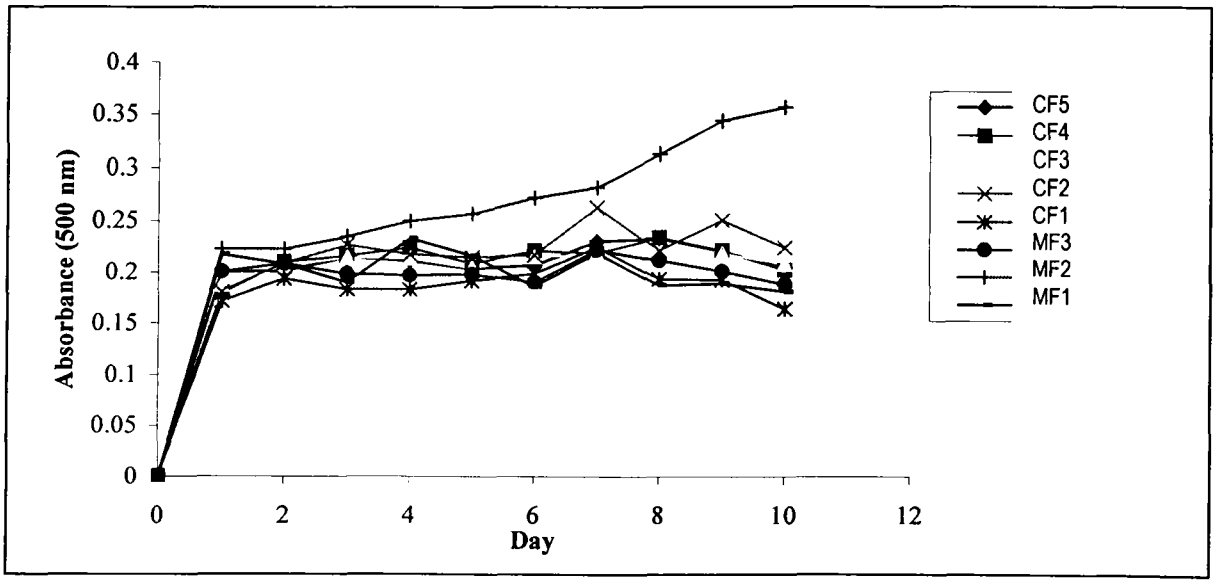


Figure 3 Antioxidant activity of various extracts of the unripe and ripe fruits of *Carissa carandas* using TBA method

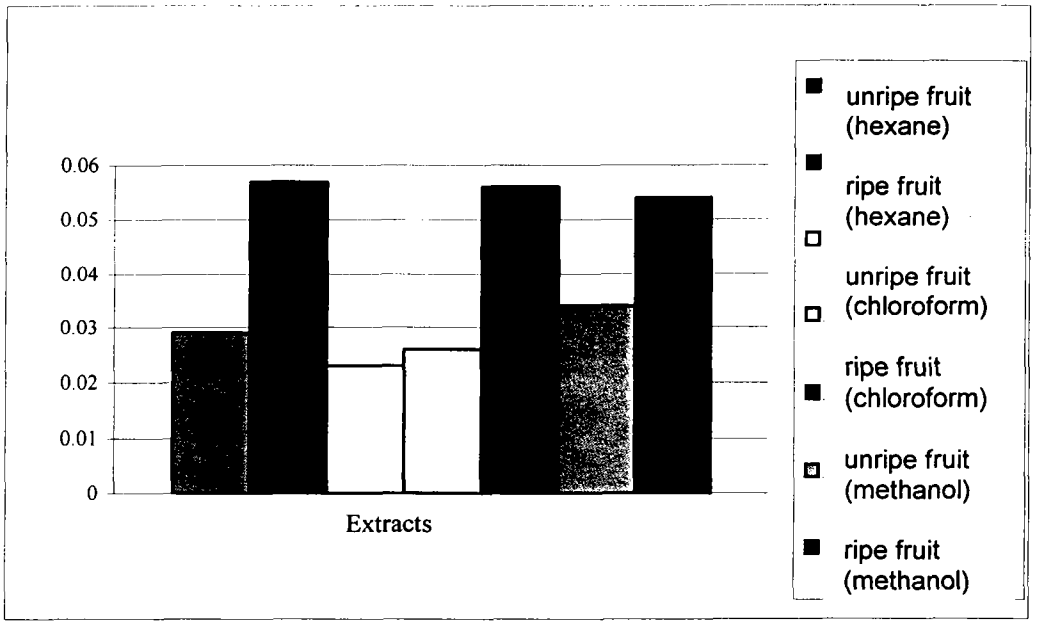


Figure 4 Antioxidant activity of various fractions various fractions from *Carissa carandas* unripe fruits using TBA method

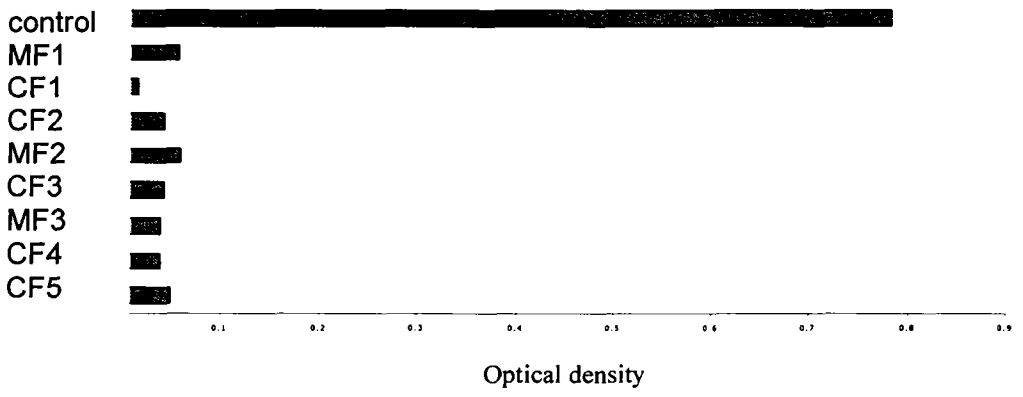
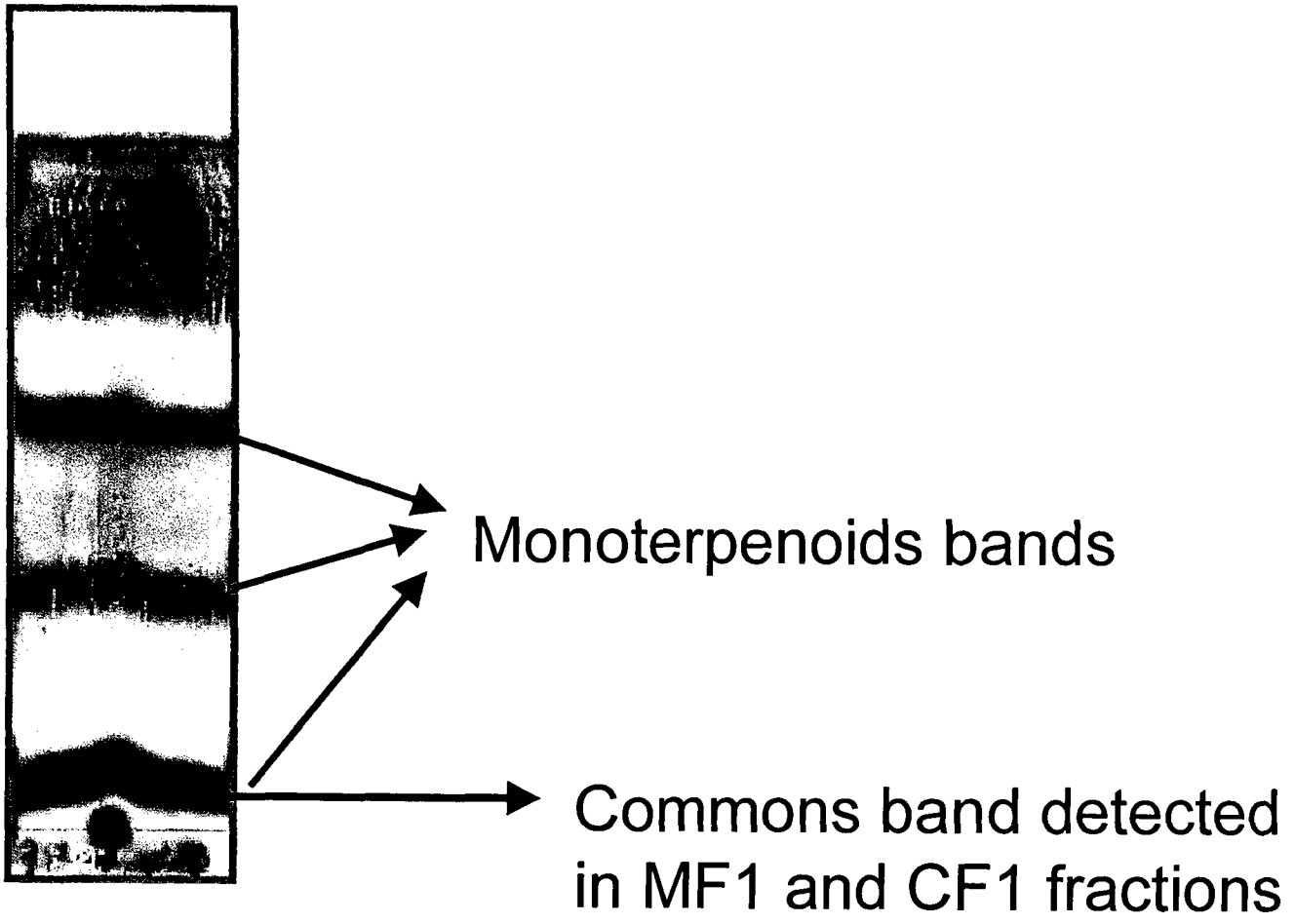


Figure 5 Monoterpenoid detection in the chloroform unripe fruit of *Carissa carandas*



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