Total phenolics content and antioxidant activity of hot water extracts from dried *Ficus deltoidea* leaves
(Kandungan jumlah fenolik dan aktiviti antioksidan ekstrak air panas daripada daun kering *Ficus deltoidea*)

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Keywords: *Ficus deltoidea*, total phenolics, antioxidant activity, hot water extracts, mas cotek

Abstract
Total phenolics content and antioxidant activity of hot water extracts at temperatures ranging from 55 °C to 107 °C and several sample-to-water ratios (ranging from 1:45 to 1:120, g:ml) were determined for the dried leaves of two accessions of *Ficus deltoidea*. Total phenolics content and antioxidant activity had higher values in accession MFD 6. A significant difference (*p* <0.05) in the total phenolics content between the ratios of 1:45 and 1:120 was observed in both accessions at all temperatures tested. However only the temperature of 107 °C caused a significant difference (*p* <0.01) in antioxidant activity between the ratios of 1:45 and 1:120 in both accessions. A positive linear correlation (*R*² = 0.65–0.76) which was statistically significant (*p* <0.01) was demonstrated between radical scavenging activity and total phenolics content in both accessions. The data indicate that *F. deltoidea* leaf has potential as a good source of phenolic antioxidants.

Introduction
*Ficus deltoidea*, or locally known as ‘mas cotek’, is a medicinal plant that is gaining popularity among herbal practitioners. Traditionally, the leaves of *F. deltoidea* are boiled and the decoction taken for general health (Musa 2005). The decoctions are used for post-natal treatment and to treat leucorrhoea disease. In a few studies, this *Ficus* species showed other medicinal properties such as being anti-inflammatory, anti-diabetic, anti-bacterial, anti-diarrhoea and anti-ulcer (Mohd. Lip et al. 2005).

Hadijah et al. (2004) concluded that *F. deltoidea* tea showed no signs of toxicity in rats based on a sub-acute toxicity study. *Ficus deltoidea* tea has high potential in reducing total cholesterol, LDL-cholesterol and the risk of cardiovascular disease by decreasing the antherogenic index (LDL/HDL ratio) and increasing the percentage of HDL/total cholesterol ratio (Hadijah et al. 2007).

To date, 31 different accessions have been successfully collected. These accessions are characterized based on their leaf, flower morphology (size, shape and colour) and growth habit. Some plants have smaller and more elongated leaves with parallel veins, whereas others have larger, thicker, more rounded leaves and reticulate veins.

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Arif Zaidi et al. (2006) found that the total polyphenol contents of leaf and fruit of *F. deltoidea* had no significant difference at 5% probability level. However, the total polyphenol contents of the leaves harvested from the top portion of the plants were significantly ($p < 0.05$) higher than the rest, i.e. the middle and bottom portions. Typical phenolics that possess antioxidant activities are mainly phenolic acids and flavonoids. Phenolic acids are a major class of phenolic compounds widely occurring in the plant kingdom (Wojdylo et al. 2007).

Extraction efficiency is a function of process conditions. Previous findings reported the influence of some variables (e.g. temperature, time contact, solvent-to-solid ratio) on the phenolic yields that are capable of being extracted from diverse natural products such as almond hulls, pine sawdust or apple byproducts (Pinelo et al. 2005).

Natarajan et al. (1962) found that the rate of extraction of various constituents (e.g. caffeine and tannins) increased greatly as the temperature rose to 85 °C. Some non-catechin substances are increasingly extracted into the tea infusion with a rise in brewing temperatures (Liang et al. 1999). Liang and Xu (2003) observed a sharp increase in tea cream when the extraction temperature rose from 50 to 60 °C. Hinneburg and Neubert (2005) found that at a lower temperature (25 °C), the use of a lower concentration of ethanol leads to decreased antioxidant activities as well as longer extraction time (24 h).

The extraction procedure is important because its conditions will determine the quality and yield of the individual constituents. Temperature, solvent concentration and extraction time are the most important factors that influence the extraction efficacy in terms of quality and yield (Hinneburg and Neubert 2005). The amount of polyphenols extracted is also dependent on the extraction method (Wojdylo et al. 2007). Proestos and Kokaitis (2006) found the amount of extractable phenolic substances decreased with decreasing polarity of the solvent.

Mixtures of alcoholic solvents and water are commonly employed by researchers to extract phenolics from natural sources (Velioglu et al. 1998; Kim et al. 2003; Pinelo et al. 2005). Generally, polyphenols are extracted with methanol/water or acetone/water (Santos-Buelga and Williamson 2003). Mixtures of ethanol and water are used as non-toxic and environmentally friendly solvents, and have been shown to be effective in the extraction of quercetin glycosides (Keinanen 1993).

Extraction by water at 125 °C was used to extract a flavour compound from rosemary by Basile et al. (1998). Water offers a better choice over methanol due to its merit of being non-toxic compared to methanol and yet having superior extraction efficiency. It also offers a better choice in obtaining antioxidant-rich extracts (Wong et al. 2006). Hence, the aim of this study was to investigate the influence of extraction temperature and sample-to-water ratio on polyphenolic content and antioxidant activity during extraction from *F. deltoidea*.

**Materials and methods**

**Plant materials**

*Ficus deltoidea* accessions of MFD 4 and MFD 6 (*Plate 1*) were collected from the cultivated plants for the study. Samples from both were intact leaves from branches cut at the age of 6 months. These two accessions have different characteristics in terms of leaf morphology and agronomic characters, i.e. plant height (MFD 6 is taller than MFD 4), yield (MFD 4 is greater than MFD 6) and canopy size (MFD 6 is bigger than MFD 4) and both have potential for commercial planting (Musa et al. 2006).

The leaves were washed, drained and dried in a convection-type laboratory oven (Memmert, model 800) at a pre-set temperature of 40 °C for 35 h to reach a final moisture content of 9.12 ± 0.96% (wet basis). The dried leaves were then ground using a dry mill blender, and uniformity of
particle size was obtained by sieving with a 710 µm mesh sieve (Retsch, Test Sieve A200, Germany) for further analysis.

**Extraction process**

Ground sample of 1 g was used for extraction with distilled water. Sample-to-water ratios of 1:45, 1:70, 1:95 and 1:120 (g:ml) were used in this study. The lowest ratio is based on a cup of water containing about 240 ml of water for 2 g tea sachet. The mixtures were heated on a hotplate (Favorit) for 60 min and stirred at 10-min intervals. The temperatures recorded were 55.4 ± 0.5 °C, 75.1 ± 0.3 °C and 107.4 ± 0.9 °C.

The mean (± standard deviation) temperature was obtained and recorded when the temperature was constant. The temperatures of mixtures were recorded using a type K thermocouple (Hanna 9043) with the tip of the thermocouple submerged into the mixtures in the middle of the hotplate. The beaker (150 ml) containing the mixtures was covered with aluminium foil to minimize evaporation in order to maintain the sample-to-water ratio.

The extracted product was cooled at room temperature prior to being filtered through filter paper (Whatman No. 2). The volume of the infusion was set at 110 ml for all the temperature regimes. This was to eliminate the influence of volume differences when assaying the antioxidant activities and total phenolics content.

**Determination of total phenolics content**

Total phenolics content was determined using the Folin-Ciocalteu reagent (according to Ragazzi and Veronese 1973). An extract (0.5 ml) was introduced into a test tube, added with 0.5 ml Folin-Ciocalteu’s reagent and 8 ml deionized water. Sodium carbonate (20%), 1 ml was added and the mixture was vortexed for 10–15 s. The absorbance of the sample was measured at 725 nm by a UV-Visible spectrophotometer (Shimadzu UV 1601). This method is based on the formation of a blue-coloured product by redox reaction with the Folin-Ciocalteu reagent. The absorbance of coloured solutions is proportional to the polyphenol concentration. A standard curve was plotted using 20, 40, 60, 80, 100 and 120 µg/ml of gallic acid (Sigma). The total phenolics content was calculated for every sample in µg gallic acid equivalent (GAE) per ml.

**Free radical scavenging assay (DPPH)**

The DPPH method has been widely used to measure the antioxidant capacities of different residual and natural products. It is a rapid, simple, sensitive and practical assay (Moure et al. 2000; Peng et al. 2000; Siriwardhana and Shahidi 2002). The free radical 2,2-diphenyl-1-picrylhydrazyl DPPH scavenging assay was done using the procedure described by Cervato et al. (2000), with slight modification. A reaction mixture consisting of 2.9 ml of 60 µM (0.024 g/litre) DPPH in absolute ethanol and 100 µl of sample was left in the dark at room temperature for 30 min. The mixture was then measured for absorbance.
Total phenolics content of Ficus deltoidea

by a spectrophotometer at 517 nm. The radical scavenging activity of each sample was calculated according to the following formula for inhibition percentage of DPPH:

\[
I_p^{\text{DPPH}}(\%) = \frac{(A_B - A_A)}{A_B} \times 100
\]

where,

- \( A_A \) = the absorbance value of the test
- \( A_B \) = the absorbance value of the blank

**Statistical analysis**

All analyses were done in triplicate and the data were presented as means ± standard deviation (SD) using Microsoft Excel version 2003. The data were statistically analysed by t-test in which two samples were assumed to have equal variances. Only variables with a confidence level superior to 95% (\( p < 0.05 \)) were considered to be significant.

**Results and discussion**

**Extraction process**

The mixture temperatures at the middle of the hotplate during the extraction process are shown in Figure 1. It was noticed that the mixture temperature was higher particularly for a higher sample-to-water ratio and started to increase beyond the preset temperature after 30 min of heating. The aluminium foil cover of the beakers seemed to have caused heat build-up thus affecting the mixture temperature. It was important to cover the beakers in order to prevent the beneficial compounds from escaping in the steam or reacting with oxygen, as well as to maintain sample-to-water ratio.

**Total phenolics content**

The amount of total phenolics (Figure 2), measured by the Folin-Ciocalteu method, varied widely in the extracts, and ranged from 134.29 ± 30.13 to 239.57 ± 32.55 µg GAE/ml and from 214.83 ± 32.41 to 401.73 ± 47.35 µg/ml for MFD 4 and MFD 6 respectively. Extraction at a temperature of 75 °C and a sample-to-water-ratio of 1:120 contained the highest total phenolics for both MFD 4 and MFD 6. The sample-to-water ratio of 1:45 at 107 °C led to a lower level of phenolics (\( p < 0.01 \)) in MFD 4 as well as in MFD 6 (\( p < 0.05 \)) compared to 1:120. In most cases, total phenolics was higher at lowest sample-to-water ratio (i.e. 1:120) for each temperature used but it seemed to decrease when the temperature was increased, particularly in MFD 6. In other words, some of the sample-to-water ratios contributed significantly to total phenolics, whereas there was no significant difference among the temperatures.

![Figure 1. Temperature of mixtures throughout the extraction process for 60 min with heating at 55, 75 and 107 °C and at various sample-to-water ratios](image-url)
From the results, it was concluded that the amount of phenolic phytochemicals are affected by cultivar and processing procedure in agreement with the findings of de Freitas and Glories (1999) and Kalt et al. (2001). The best results for extractable substances were in general obtained for the lowest solid-liquid ratio. Pinelo et al. (2005) found that the highest value of total phenolics was reached when extraction was carried out under the conditions of higher temperature, longer time of contact and higher solvent-to-solid ratio.

**Antioxidant activity**

Antioxidant activity of the samples extracted using the various sample-to-water ratios and temperatures were assayed for MFD 4 and MFD 6 ranged from 42.92 ± 8.34% to 74.40 ± 7.01% and from 71.57 ± 4.35% to 87.69 ± 3.51% respectively (Figure 3). Antioxidant activities increased with increasing temperature in both MFD 4 and MFD 6. At each temperature, antioxidant activity was highest at the sample-to-water ratio of 1:120 which was significantly different \( (p < 0.05) \) compared to sample-to-water ratio of 1:45.

From this study, the free radical scavenging activity of butylated hydroxyanisol (BHA), butylated hydroxyl toluene (BHT), catechin and quercetin was 93.64, 88.99, 95.46 and 96.13% respectively. It was suggested that *F. deltoidea* extracts exhibited moderate antioxidant activity when compared to the standard antioxidants.

**Relationship between phenolic contents and antioxidant activity**

Overall, the total phenolics content and antioxidant activity varied from 101.26 to 277.16 µg GAE/ml (mean: 202.83 ± 38.31 µg GAE/ml) and from 33.58 to 79.90% (mean: 59.75 ± 11.43%) respectively, in MFD 4. In MFD 6, the total phenolics content and antioxidant activity varied from 181.49 to 437.52 µg GAE/ml (mean: 309.40 µg GAE/ml) respectively.

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Total phenolics content of *Ficus deltoidea* ± 55.00 µg GAE/ml) and from 67.03 to 91.22% (mean: 81.86 ± 6.96%) respectively. The relationship between total phenolics content and antioxidant activity of the plant extracts is shown in Figure 4. There was a significant (p < 0.01) positive relationship between antioxidant activity and total phenolics in both the accessions, MFD 4 and MFD 6, implying that the antioxidant activity of these extracts is largely due to the presence of phenolic compounds. This is in agreement with previous findings regarding the relationships of phenolic content and antioxidant activity of extracts from natural products (Ninfali et al. 2002; Katsube et al. 2004; Mello et al. 2005; Djeridane et al. 2006; Katalinic et al. 2006).

**Conclusion**

Both total phenolics content and antioxidant activity were higher in MFD 6. As the temperature increased from 55 °C to 107 °C for a 60 min extraction, total phenolics content appeared to decrease but antioxidant activity increased. The increase of antioxidant activity was probably contributed by the flavonoid contents, hence there is a need to do further studies. It was observed that the lowest sample-to-water ratio (i.e. 1:120) had the highest value of total phenolics as well as antioxidant activity. The different temperatures used for the extraction did not significantly affect total phenolics and antioxidant activity for both MFD 4 and MFD 6. Apparently, all temperatures used affected the total phenolics as well as the antioxidant activity, particularly at the highest sample-to-water ratio (i.e. 1:45) compared to 1:120 (p <0.05) for both accessions of MFD 4 and MFD 6.

**References**


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**Abstrak**

Kandungan jumlah fenolik dan aktiviti antioksidan di dalam ekstrak air panas pada jualat suhu daripada 55 °C hingga 107 °C dan pelbagai nisbah sampel-kepada-air (jualat daripada 1:45 hingga 1:120, g:ml) ditentukan pada daun kering daripada dua aksesi mas cotek. Kandungan jumlah fenolik dan aktiviti antioksidan didapati tinggi dalam aksesi MFD 6. Perbezaan yang nyata (*p* <0.05) dikesan pada kandungan jumlah fenolik antara nisbah 1:45 dan 1:120 dalam kedua-dua aksesi pada semua suhu yang digunakan. Walau bagaimanapun, perbezaan yang ketara (*p* <0.01) pada aktiviti antioksidan antara nisbah 1:45 dan 1:120 bagi kedua-dua aksesi hanya berlaku pada suhu 107 °C. Korelasi lelurus positif (*R*² = 0.65–0.76) dengan perbezaan signifikan (*p* <0.01) telah ditunjukkan antara ‘radical scavenging activity’ dengan jumlah kandungan fenolik dalam kedua-dua aksesi. Data yang diperoleh menunjukkan daun mas cotek berpotensi sebagai sumber yang baik untuk bahan antioksidan fenolik.