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The effect of extraction temperature on total phenols and antioxidant activity of *Gynura procumbens* leaf

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ABSTRACT
The effect of extraction temperature on total phenolic contents and free radical scavenging activity of *Gynura procumbens* leaf extract was investigated. The content of total phenolic were not significantly different (P > 0.5) at the extraction temperature of 40 °C and 50 °C. However, decrease in total phenolic content was observed from extraction temperature of 60 °C and above. The extracts obtained at lower temperature exhibited significant free radical-scavenging activity compared to extraction at higher temperatures.
KEYWORDS: Antioxidant, Extraction temperature, *Gynura procumbens*, Total phenolics.

INTRODUCTION

Polyphenols are bioactive constituents present in food plants which are very important in the control and prevention of tissue damage by activated oxygen species due to their antioxidant effects (1). The antioxidant properties of polyphenols are generally accepted as the basis of their therapeutic effect. Polyphenols reduce oxidative stress; possibly by inhibiting the formation of lipid peroxidation products in biological systems (2). The stability of polyphenols in plant extracts depends on many factors including the drying and extraction method. Stability studies of phenolic constituents in the different stages of processing of raw materials are needed to ensure efficacy of the finished product.

Gynura procumbens (*Merr.*, Compositae) is a medicinal plant native to South East Asia, where it has been used to treat urinary lithiasis, edema, eruptive fever, influenza, rheumatism, colon cancer, haemorroids and diabetes (3). The methanolic extract of *G. procumbens* was reported to demonstrate antioxidant activity using *in vitro* models (4). The active chemical constituents of *G. procumbens* leave include, flavonoids, sterols and their glycosides (5 - 6). The development of simple and rapid extraction methods for analysis of polyphenols in plant extracts is of great significance in the quality control of herbal medicine and botanical supplements. Therefore, the aim of present study was to evaluate the effect of heating on total phenolic contents and free radical scavenging activity (FRSA) of the leaf extracts of *G. procumbens* obtained at different extraction temperatures.

MATERIALS AND METHODS

Chemical and reagents

Gallic acid, Butylated hydroxytoluene (BHT), quercetin (QUE), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company (St.Louis MO, USA). Methanol was obtained from Merck All other solvents were analytical grade or HPLC grade.

Plant material

The *Gynura procumbens* was obtained from Penang Island, Malaysia. A voucher specimen (10117) was deposited at the herbarium of the School of Biological Sciences, Universiti Sains Malaysia. The leaves of *G. procumbens* were dried at 35 °C in an air oven and finely powdered.

Extraction

Samples of the powdered leaf (0.5 g) was weighed and transferred into conical flasks. The samples were extracted with 100 ml of 80% aqueous methanol. The suspension of the leaf samples were incubated for four hours at different temperatures (40 °C, 50 °C, 60 °C, 80°C, and 100°C) with intermediate shaking. The extracts were vacuum filtered (Whatman No. 1) and

each filtrate was adjusted to final volume of 100 ml with methanol.

Contents of total phenolics and total soluble solids

The total phenolic content in extracts was determined by using Folin-Ciocalteu reagent and external calibration with gallic acid (GA). Briefly, 0.2 ml of extract solution in a test tube and 0.2 ml of Folin-Ciocalteu reagent were added and the contents mixed thoroughly. After 4 min, 1 ml of 15% Na_2CO_3 was added, and then the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 760 nm by using Lamda 45 UV-Vis spectrphotometer (Perkin-Elmer, USA). The content of total phenolics was calculated by using gallic acid (GA) calibration curve. The results were expressed as mg GA / g dry material. The content of total soluble solids of an aliquot (20 ml) of extract was determined in triplicate (7).

Free radical scavenging activity

The free radical scavenging activity of the extracts was measured by 1,1,-diphenyl-2-picryl-hydrazil (DPPH) using the method of Hatano et al. (8) with some modification. Briefly, DPPH solution (0.1 mM) was prepared in methanol and to 2 ml of this solution was mixed with 0.2 ml of samples of extracts. The volume of the solution was adjusted with methanol to a final volume of 3 ml. After incubation at room temperature for 30 min, the absorbance of the mixture was measured at 517 nm against methanol as blank using Lamda 45 UV-Vis spectrphotometer (Perkin-Elmer, USA). BHT (0.01 mg/ml) and QUE (0.01mg/ml) were used as positive control. The activities of the samples were evaluated by comparison with a control (containing 2 ml of DPPH solution and 1 ml of methanol). Each sample was measured in triplicate and averaged. The activity was calculated according to the formula:

% Inhibition = $[(A_C - A_S) / A_C] \times 100$

where A_C is the absorbance value of the control and A_S is the absorbance value of the test solution.

Statistical analysis

Results were given as mean ± standard deviation of three replicates. Experimental results were analysed by SPSS 10 (SPSS Inc. Chicago, IL). Differences between means were determined using Turkey multiple comparisons. P values < 0.05 were regarded as significant.

RESULTS

The effect of extraction temperature on total soluble solids and total phenolic contents - Table 1 shows the effect of different extraction temperature on total soluble solids and total phenolic contents of the extracts obtained at different temperatures. Increasing temperature of extraction increased the total soluble solids. However, the increase was observed only up to extraction temperature of 60 ^oC. Though the highest total soluble solids were at 60 ^oC, the value was not significantly different (P > 0.5) from that of the extract obtained at 50 ^oC. For the temperatures studied, extraction above 60 ^oC showed a significantly lower total phenolic content compare to extractions at 40 ^oC and 50 ^oC. There was no significant difference (P>0.5) between the total phenolic content of the extracts obtained at 40 ^oC and 50 ^oC. The value of the extract obtained at 60 ^oC was significantly lower than that of the extractions at 40 ^oC and 50 ^oC. The content of total phenolic of all the extracts at the temperatures studied decreased in the order 40 ^{o} C > 50 ^oC > 60 ^oC > 80 ^oC and100 ^oC.

The influence of heating on free radical scavenging activity of extracts

The result of free radical-scavenging activity of the extracts obtained at different temperatures by DPPH method is shown in Table 1. The spectral change of DPPH solution after mixing it with the solution of the extracts and reference standards (BHT and QUE) is shown in Figure 1. All the extracts demonstrated inhibitory activity against the DPPH radical. For the temperatures studied, extractions at 40 ^oC showed the highest free radical-scavenging activity however, the value was not significantly different (P>0.05) from that of extraction at 50 ^oC. The extracts obtained at extraction temperature above 60 ^oC showed significantly lower FRSA compared to extracts at 40 ^oC and 50 ^oC. The order of FRSA of extracts and reference standards was: QUE > BHT > 40 ^oC > 50 ^oC > 60 ^oC > 80 ^oC > 100 ^oC.

DISCUSSION

Processing techniques involving extraction solvent, pH, light and heat can markedly influence the levels and efficacy of bioactive compounds of dietary supplements such as polyphenolic compounds. Polyphenolic constituents of botanicals are unstable compounds and their degradative reactions occurred throughout the stages of formulation process of a dietary supplement. Botanical extracts and beverages involve heating, which may have an impact on bioactivity. Beverages in general require pasteurization known to affect the activity of some polyphenols. The temperature during extraction affects the stability of

Table 1 : Effect of different extraction temperature on total soluble solids, total phenolic contents, and free-radical scavenging activity of Gynura procumbens leaf.

Extraction Temperature (^{o}C)	Total soluble solids (%,w/w)	Total phenolics (mg GA/ g dry material)	DPPH activity (%)
40	8.28± 0.09a	21.74 ± 0.25a	58.21 ± 1.37a
50	10.54± 0.10b	21.46 ± 0.28a	58.08 ± 1.22a
60	10.65± 0.12b	20.15 ± 0.19b	47.94 ± 1.15b
80	9.41± 0.08c	12.22 ± 0.12c	34.17 ± 1.13c
100	9.36± 0.06c	8.54 ± 0.08d	26.68 ± 0.76d
BHT	NA	NA	69.46 ± 1.51e
Quercetin	NA	NA	70.22 ± 1.48e

Values are expressed as means ± standard deviation (n = 3). NA, not applicable. ; Means with different letters in columns indicate significantly different at p<0.05

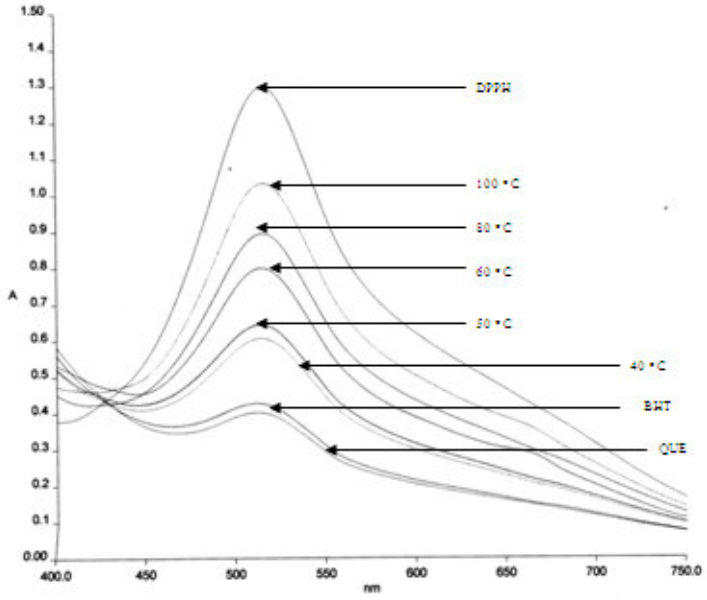
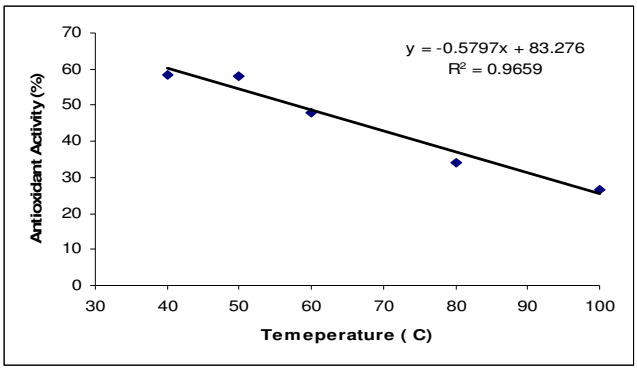


Figure 1 : UV spectral change of DPPH solution (0.1 mM) after mixing with, the extracts at different temperature (40 ^{o}C, 50 ^{o}C, 60 ^{o}C , 80 ^{o}C , 100 ^{o}C), butylated hydroxytoluene (BHT, 0.01 mg/ml), and quercetin (QUE, 0.01 mg/ml).



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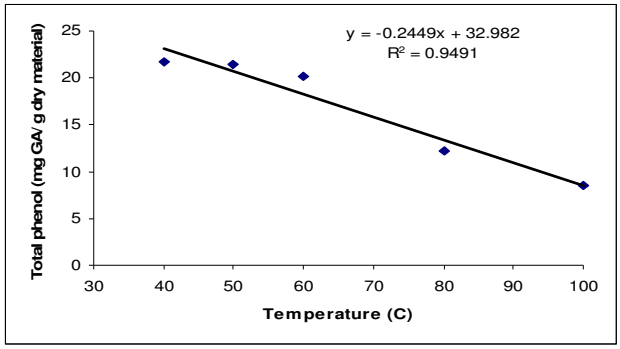

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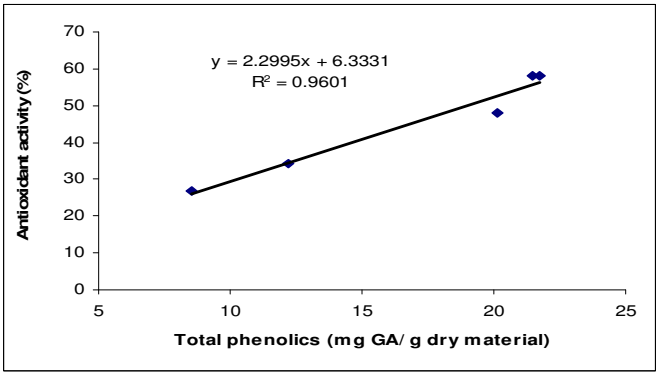

c

Figure 2 : Relationship between (a) extraction temperature and total phenolic contents (b) extraction temperature and free radical-scavenging activity (c) total phenolic contents and free radical-scavenging activity of Gynura procumbens leaf extracts.

polyphenols due to chemical and enzymatic degradation which cause reduction in total phenolic contents and loss of antioxidative activity. Moreover, antioxidants properties of phenolics make them sensitive to oxidation. The present study was focused on total phenolic contents and antioxidative effect of *G. procumbens* leaf extracts obtained at different temperatures. The effect of extraction temperature on total phenolic contents and FRSA activity of the 80% methanol was investigated. For all the temperatures investigated, the highest content of total phenols were observed in the extracts obtained at 40 °C followed by extraction at 50 °C. Significant lower levels of total phenols were demonstrated by extractions at temperatures above 60 °C which may be due to degradation of the phenolic compounds at elevated temperatures. The results of the present study also indicate a significant reduction in FRSA of the samples extracted at temperatures above 60 °C compared with the values of extraction at 40 °C. This was expected due to reduction in total phenolic contents which occur at temperature above 60 °C. Larrauri et al. (9) reported that phenolic antioxidants exhibit significant decomposition at temperatures above 60 °C. Additionally, the presence of polyphenol oxidases may decrease the amount of antioxidants present in an extract. At the extraction temperature above 60 °C, the polyphenol oxidase may have been activated quick enough to instigate the degradation of the markers. Metabolites of polyphenols have considerable antioxidant properties but at elevated temperature they can be degraded into another compound with insignificant antioxidant potential.

The relationship between extraction temperatures with total phenolic contents and FRSA is given in Figure 2. As expected, strong negative correlation was observed between the extraction temperature with total phenolics (r_{xy} = -0.9491, Figure 2A) and FRSA (r_{xy} = -0.9659, Figure 2b). The strong negative correlation between extraction temperatures with total phenolics and FRSA suggests that extraction at elevated temperature is partly responsible for significant degradation of phenolic antioxidants. There was a strong positive correlation between the total phenolics and FRSA (r_{xy} = 0.9601, Figure 2c). This indicates that the antioxidant activity of *G. procumbent* leaf is due

to its phenolic constituents. This result is in agreement with other reports in literature which showed positive strong correlation between antioxidant activities and total phenolic contents (10 - 12). Our previous investigations have shown that polyphenols and their glycosides (rutin and kaempferol-3-O-rutinoside) are the major bioactive components in *G. procumbens* leaves (5). These phytochemicals have been reported to possess high antioxidant activities (13 - 14).

In summary, extraction of *G. procumbens* leaf with 80% methanol at temperature below 60 ^{0}C, gave a greater retention of polyphenolic compounds and greater expression of free-radical scavenging activity. On the other hand, lesser expression of antioxidant activity occurred at elevated temperatures (above 60 ^{o}C) due to the degradation of bioactive polyphenolic antioxidants in the extracts.

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