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Full Length Research Paper

Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*

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The aim of this study was to determine and compare the antioxidant activity of methanolic and chloroformic extracts of *Momordica charantia* (MC) fruit. In this study, the total antioxidant and free radical scavenging activities in methanolic and chloroformic were measured by ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) methods. Total phenol and flavonoid contents of the MC extracts were also evaluated. The total antioxidant activity results indicated that, the inhibition percent of methanolic extract was significantly higher than the inhibition percent of chloroformic extract in the FTC and TBA methods. A higher IC_{50} value for free radical scavenging was found for methanolic extract when compared with chloroformic extract. Methanolic extract contained a significantly higher concentration of total phenols and flavonoids when compared with chloroformic extract. Methanolic extract contained more potent antioxidant and high polyphenol compounds when compared with chloroformic extract. The present study, confirmed that, the type of solvent has an important role in detecting plant compounds. The natural plant antioxidants and phenolics compounds in MC have the capability of being used in food systems to preserve food quality.

Key words: *Momordica charantia*, antioxidant activity, polyphenol compounds, phenolics, flavonoids.

INTRODUCTION

Oxidative stress has been recognized to have a pathological role in many types of chronic diseases such as diabetes, heart disease and cancer. Oxidative stress occurs when the formation of free radicals increases (Elmastas et al., 2006). In oxidative stress, the balance between the formation of reactive oxygen species and amount of antioxidants is destroyed. Oxidative stress causes damage to cell components, such as proteins, lipids and nucleic acids (Rahimi et al., 2005; Wright et al., 2006; Gladine et al., 2007) and eventually leads to cell death (NazIroglu et al., 2004; Emekli-Alturfan et al., 2009).

Antioxidant effects of various medicinal plants used in traditional therapeutics are associated with their antioxidant properties (Sathishsekar and Subramanian, 2005; Aiyegoro and Okoh, 2009). Moreover, the use of fruits and vegetables containing antioxidant agents diminish the possibility of chronic diseases such as diabetes (Myojin et al., 2008), cancer and cardiovascular diseases (Saha et al., 2004; Horax et al., 2005; Semiz and Sen, 2007). In addition, consumption of plants and/or food containing antioxidant agents may help the body to

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Abbreviations: MC, *Momordica charantia;* **MDA,** malondialdehyde; **FTC,** ferric thiocyanate; **TBA,** thiobarbituric acid; **DPPH,** 1,1-diphenyl-2-picryl-hydrazyl; **TBARS,** thiobarbituric acid reactive substances; **BHT,** butylated hydroxytoluene; **BHA,** butylate hydroxyanisole; **TBHQ,** teri-butylhydroquinone; **PG,** propyl gallate.

decrease oxidative injury (Elmastas et al., 2006).

Current scientific articles have focused on plant natural antioxidants (Emekli-Alturfan et al., 2009). Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins and phenolic acids (Lee et al., 2004; Horax et al., 2005). Polyphenols, especially flavonoids are generally known as the antioxidant agent in plant extracts (Bernardi et al., 2008). Multiple actions of phenolic components remove free radicals in the polar and lipid phase and inhibit different types of oxidizing enzymes.

The plant of *Momordica charantia* (MC), also known as bitter melon, belongs to the cucurbitaceous family and is cultivated as an indigenous vegetable in tropical regions (Virdi et al., 2003; Pitipanapong et al., 2007; Budrat and Shotipruk, 2009). The biological components of MC include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Grover and Yadav, 2004). The phenolic compounds of MC have been reported to exhibit antioxidant activity (Horax et al., 2005; Budrat and Shotipruk, 2009). Antioxidant, anti-diabetes, anti-inflammatory, anti-bacterial and anti-cancer effects of MC have also reported (Grover and Yadav, 2004; Budrat and Shotipruk, 2009). Synthetic antioxidants, such as butylate hydroxyanisole (BHA), teri-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and propyl gallate (PG) are carcinogenic (Singh et al., 2002; Gülçin et al., 2004).

There are various methods to estimate the antioxidant activity of compounds in plant extracts. However, one method alone is unable to recognize all possible mechanisms characterising an antioxidant (Dorman et al., 2003; Erkan et al., 2008). Until now, there has not been a report on the chloroformic extract of MC fruit and the methanolic (polar) and chloroformic (non-polar) extracts of MC using methods with ferric thiocyanate (FTC) for the initial stage of lipid peroxidation and thiobarbituric acid (TBA) for the secondary stage of lipid peroxidation. Thus, the current study was carried out to characterize the antioxidant activity of these extracts by various methods. Furthermore, this study also measured the phenolic and flavonoid contents in these extracts.

MATERIALS AND METHODS

Plant materials

Fresh whole MC fruit were collected from local markets in Malaysia and prepared for extraction using two different procedures.

Preparation of the extracts

Methanolic extract

The methanolic extract was prepared based on a method previously described by Virdi et al. (2003). A total of 500 g of dried whole fruits of MC (with seeds) was weighed and mixed with 5 L (1:10) of methanol. The mixture was then, left for 1 h in the dark at 50°C. The mixture was then, filtered and evaporated to dryness under reduced pressure using a rotary evaporator (BUCHI Rotavapor R-220) to produce the yield. The yield was 31 g of powder per kg of dried whole fruit (3.1%). The extract was then kept at -80°C until use.

Chloroformic extract

The chloroformic extract preparation was also done according to the method previously described by Virdi et al. (2003). A total of 500 g of dried whole fruits of MC (with seeds) was weighed and mixed with 5 L of chloroform. The extraction was executed as described earlier. The yield was 20 g of powder per kg of dried whole fruit (2%). The extract was then kept at -80°C until use.

Antioxidant activity

Ferric thiocyanate (FTC) method

Different extracts (4 mg) and standards (4 mg; BHT, vitamin C and vitamin E) were mixed with 4 ml of absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. The mixture was placed at 40°C (0.1 ml) and was then mixed with 9.7 ml of 75% (v/v) ethanol and 0.1 ml 30% ammonium thiocyanate. Three minutes after adding ferrous chloride (0.1 ml of 2×10^{-2} M ferrous chloride), the absorbance was measured at 500 nm in a spectrophotometer (Secomam, Domont, France). This step was repeated every 24 h until the control reached its maximal absorbance value. The mixture without added sample was used as a control (Saha et al., 2004; Gülçin et al., 2007, 2009). The inhibition of lipid peroxidation (%) was estimated by the following formula:

$$\% \text{ Inhibition} = 100 - ((A_1 - A_0) \times 100),$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts (Elmastas et al., 2007).

Thiobarbituric acid (TBA) method

Extracts (2 ml) and standard solutions (2 ml) on the final day (day 8) of the FTC assay were added to 1 ml of 20% aqueous trichloroacetic acid and 2 ml of 0.67% aqueous thiobarbituric acid. After boiling for 10 min, the samples were cooled. The tubes were centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was evaluated at 532 nm in a spectrophotometer (Saha et al., 2004). The antioxidant activity was calculated by percentage of inhibition in this method as follows:

$$\% \text{ Inhibition} = 100 - [(A_1 - A_0) \times 100]$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts (Elmastas et al., 2007).

Free radical scavenging activity

The ability of methanolic and chloroformic extracts of MC to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Jain et al. (2008). MC extracts (3 ml) with six different concentrations (15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were mixed with 1 ml of a 0.1 mM ethanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 min intervals against a blank (pure ethanol) (Singh et al., 2002). The percentage of radical scavenging activity was

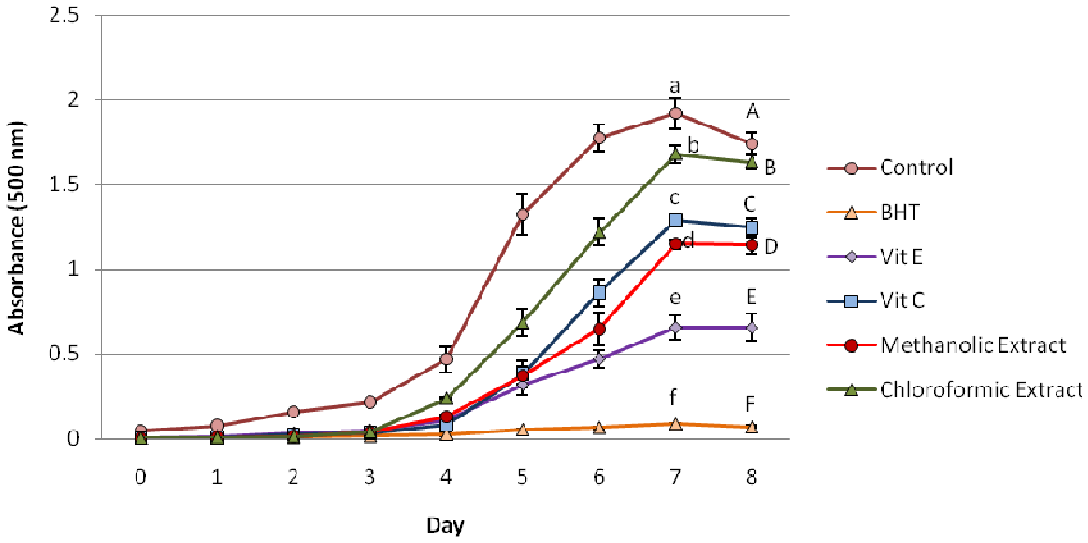

Figure 1. Antioxidant activity of the standards and different type of MC extract as measured by FTC method (mean ± SD). Each experiment was executed in triplicate and repeated three times. Different lowercase letters at day 7 and uppercase letter at day 8 indicate significant different (P ≤ 0.05).

calculated using the following formula:

Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts (Elmastas et al., 2007).

Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentrations (1.56, 3.12, 6.25, 12.5, 25 and 50 µg/ml). The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals (Jain et al., 2008).

Total phenol assay

The total content of different MC extract types was evaluated using a method described by Kim et al. (2003). The extract solution (0.5 ml) with a concentration of 1000 µg/ml was added to 4.5 ml of deionized distilled water and 0.5 ml of Folin Ciocalteu's reagent which was then added to the solution. After mixing the tubes, they were maintained at room temperature for 5 min followed by the addition of 5 ml of 7% sodium carbonate and 2 ml of deionized distilled water. After mixing the samples, the samples were incubated for 90 min at 23°C. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. The standard curve was prepared by gallic acid in five different concentrations (50, 100, 150, 250 and 500 mg/L).

Total flavonoid assay

The flavonoid content of each extract was measured based on methods described by Ebrahimzadeh et al. (2008). Briefly, 0.5 ml of sample (5 g/L) was mixed with 1.5 ml of methanol and then, 0.1 ml of 10% aluminium chloride was added, followed by 0.1 ml of potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in different concentrations (12.5, 25, 50, 80 and 100 mg/L).

Statistical analysis

Results were expressed as means ± standard deviations (SD). Statistical comparisons were made using the student t-test, one-way analysis of variance (ANOVA) and Tukey's post-hoc test. Correlations between the IC_{50} and total phenolic and flavonoid contents were established using regression analysis at a 95% significance level. P ≤ 0.05 was identified as a significant difference.

RESULTS

Ferric thiocyanate (FTC) method

As shown in Figure 1, the absorbance of methanolic extract at 7 day and 8 day (1.14 ± 0.027 and 1.14 ± 0.053, respectively) was significantly (P < 0.05) decreased when compared with the absorbance of chloroformic extract at 7 and 8 day (1.68 ± 0.052 and 1.63 ± 0.044, respectively). The absorbance of methanolic extract at 7 and 8 day was markedly (P < 0.05) lower than the absorbance of vitamin C (1.28 ± 0.034 and 1.24 ± 0.056, respectively) and higher than the absorbance of vitamin E (0.65 ± 0.072 and 0.65 ± 0.082, respectively). In contrast, chloroformic extract had a significantly (P < 0.05) higher absorbance at 7 and 8 day when compared with vitamins C and E. The absorbance of methanolic extract and chloroformic extract were markedly (P < 0.05) higher than the absorbance of BHT at 7 and 8 day (0.08 ± 0.006 and 0.06 ± 0.006,

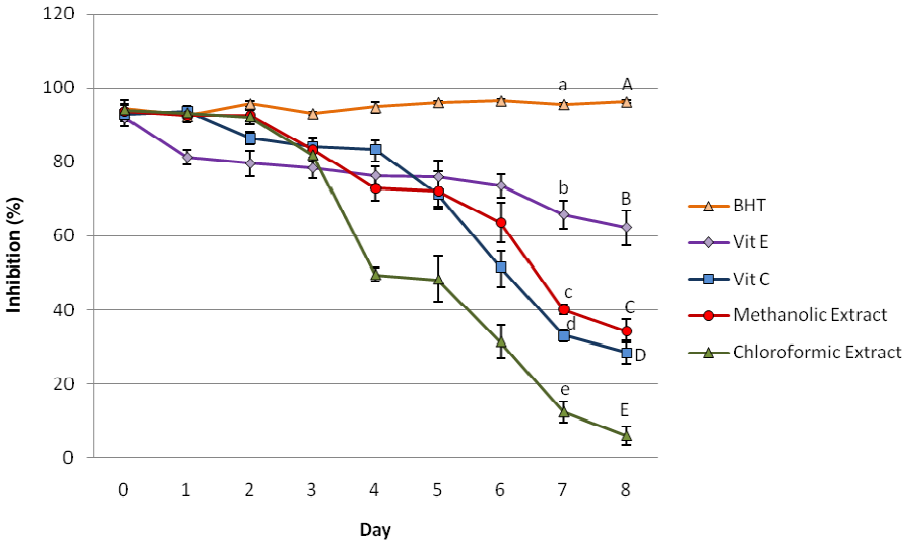

Figure 2. Percentage of inhibition of linoleic acid peroxidation as measured by FTC method (mean ± SD). Each experiment was executed in triplicate and repeated three times. Different lowercase letters at day 7 and uppercase letter at day 8 indicate significantly different P ≤ 0.05).

Table 1. Total antioxidant activity of samples by TBA method.

Sample	Absorbance (nm)	Percentage of inhibition
Control	1.23 ± 0.067^{a}	0^{A}
BHT	0.04 ± 0.005^{b}	96.22 ± 0.435^{B}
Vitamin E	0.45 ± 0.080^{c}	63.41 ± 6.509^{C}
Vitamin C	1.02 ± 0.056^{d}	16.75 ± 4.577^{D}
Methanolic extract	0.83 ± 0.056^{e}	32.50 ± 4.594^{E}
Chloroformic extract	1.14 ± 0.036^{f}	7.52 ± 2.914^{F}

Data represents mean ± SD. Each experiment was executed in triplicates and repeated three times. Different lowercase and uppercase letters in the same column indicate significant difference at P ≤ 0.05.

respectively). The control had the highest value throughout the study. The absorbance values for all the samples were maximal 7 day and then, dropped at 8 day due to malondialdehyde (MDA) compounds from linoleic acid oxidation, in which peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment.

The percentage of inhibition of methanolic extract at 7 and 8 day (40.10 ± 1.432% and 34.31 ± 3.064%, respectively) was significantly (P < 0.05) increased compared to chloroformic extract (12.36 ± 2.740 and 6.08 ± 2.563%, respectively). The percentage of inhibition of methanolic extract at 7 and 8 day was significantly (P < 0.05) higher than the percentage of inhibition of vitamin C (32.91 ± 1.773 and 28.546 ± 3.266%, respectively) and lower than vitamin E (65.76 ± 3.803 and 62.30 ± 4.739%, respectively). Chloroformic extract had a significantly (P < 0.05) lower percent of inhibition at 7 and 8 day when compared with vitamins C and E. Furthermore, the percent of inhibition of methanolic extract and chloroformic extract was significantly (P < 0.05) lower than the percent of inhibition of BHT at 7 and 8 day (95.48 ± 0.312 and 96.14 ± 0.376%, respectively) (Figure 2).

In this study, lipid peroxidation was elevated through 8 day (optical density of the control was maximal at 7 day). The control had increasing absorbance values from 0 day until the absorbance reached the maximal level at 7 day and the absorbance value dropped at 8 day.

Thiobarbituric acid (TBA) method

Table 1 shows the absorbance and percentage of inhibition of all samples. The absorbance values of methanolic extract and chloroformic extract were significantly (P < 0.05) higher than the absorbance value

Table 2. Evaluation of IC_{50} value of extracts.

Sample	IC_{50} value (µg/ml)
Vitamin C	25.55 ± 0.404[a]
Methanolic extract	306.53 ± 1.083[b]
Chloroformic extract	583.82 ± 13.386[c]

Data represents mean ± SD of duplicate analysis. Different lowercase letters within column indicate significant difference at P ≤ 0.05.

Table 3. DPPH-scavenging activity of extracts at various concentration.

Concentration (µg/ml)	DPPH scavenging (%Inhibition)	
	Methanolic extract	Chloroformic extract
15.625	1.95 ± 0.371	1.12 ± 0.593
31.25	5.57 ± 0.420[a]	3.70 ± 0.478[b]
62.5	13.10 ± 0.468[a]	6.62 ± 0.457[b]
125	21.95 ± 0.288[a]	12.36 ± 0.579[b]
250	40.34 ± 0.132[a]	23.46 ± 1.026[b]
500	81.22 ± 0.706[a]	42.26 ± 0.898[b]

Data represents mean ± SD of triplicate analysis. Different lowercase letters within rows indicate significant difference at P ≤ 0.05.

of BHT and vitamin E. Meanwhile, the absorbance value of methanolic extract was significantly (P < 0.05) lower than absorbance value of chloroformic extract. Similarly, the percentages of inhibition of methanolic extract and chloroformic extract were significantly (P < 0.05) lower than the BHT and vitamin E. However, the percentages of inhibition was significantly (P < 0.05) higher in methanolic extract than in chloroformic extract. The TBA results were in agreement with the FTC results.

Free radical scavenging activity

The 50% inhibitory concentration (IC_{50}) in methanolic extract was significantly (P < 0.05) lower than chloroformic extract (Table 2). These MC extracts had a lower scavenging activity than vitamin C.

Table 3 shows that methanolic extract contained significantly (P < 0.05) more antioxidant and free radical scavenger activity than chloroformic extract. These data showed that, the percentage of free radical inhibition increased as the concentration increased in both extracts. However, the percentage of radical inhibition was higher in methanolic extract (approximately 2 fold) when compared with chloroformic extract.

Total phenolic and flavanoid assay

Figure 3 shows the total phenol and flavonoid contents of methanolic extract and chloroformic extract of MC. Total phenol compounds were reported as gallic acid equivalents by reference to a standard curve ($y = 0.003\times + 0.077$; $r^2 = 0.997$). In addition, the total flavonoid contents were reported as mg quercetin equivalent/g of extract by reference to a standard curve ($y = 0.002\times + 0.014$; $r^2 = 0.992$). The results showed that, the total phenol and flavonoid contents of methanolic extract (10.18 mg GAE ± 0.501 mg GAE for total phenols and 7.63 mg QE ± 1.013 mg QE for total flavonoids) were significantly (P < 0.05) higher than the contents of chloroformic extract (0.66 mg GAE ± 0.333 mg of GAE for total phenols and 0.4 mg QE ± 0.145 mg QE for total flavonoids).

Correlation of phenol assay and DPPH assay

Figures 4 and 5 show linear correlations of the total phenolic content with the DPPH scavenging ability (IC_{50}) in both MC extracts. Significant and negative correlations were observed between the total phenolic content and IC_{50} values for the DPPH assay in extract A ($y = -2.159\times + 328.5$; $r^2 = 0.993$ and P = 0.05) and extract B ($y = -39.95\times + 610.3$; $r^2 = 0.999$ and P = 0.012). These results suggest that the decrease in radical scavenging ability may be related to the increase in phenolic compounds. Furthermore, a correlation between the IC_{50} and flavonoid compounds was not found in methanolic extract (R = -0.910 and P = 0.272) or chloroformic extract (R = -0.739 and P = 0.470) in this study.

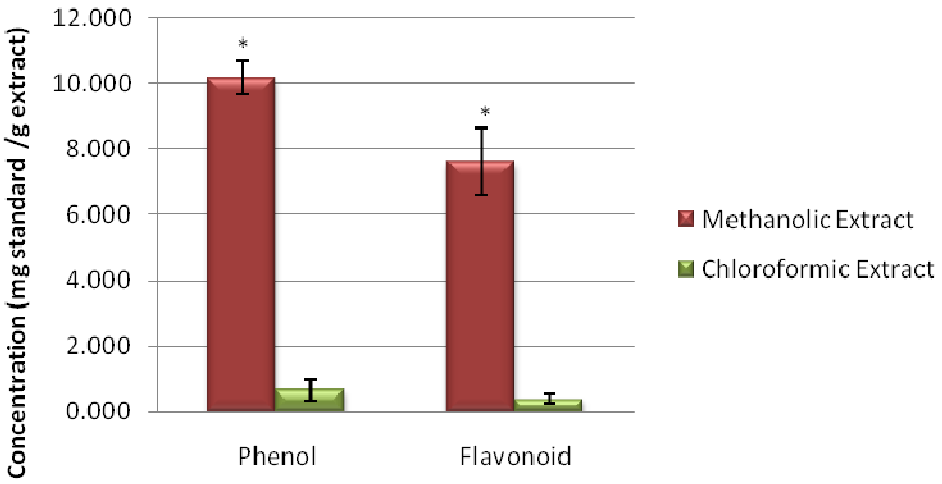

Figure 3. Total phenols and flavonoids contents obtain with different types of MC extracts. The asterisk indicates a significant difference between methanolic and chloroformic extracts at $P \leq 0.05$ as analyzed by student's t-test (mean ± SD).

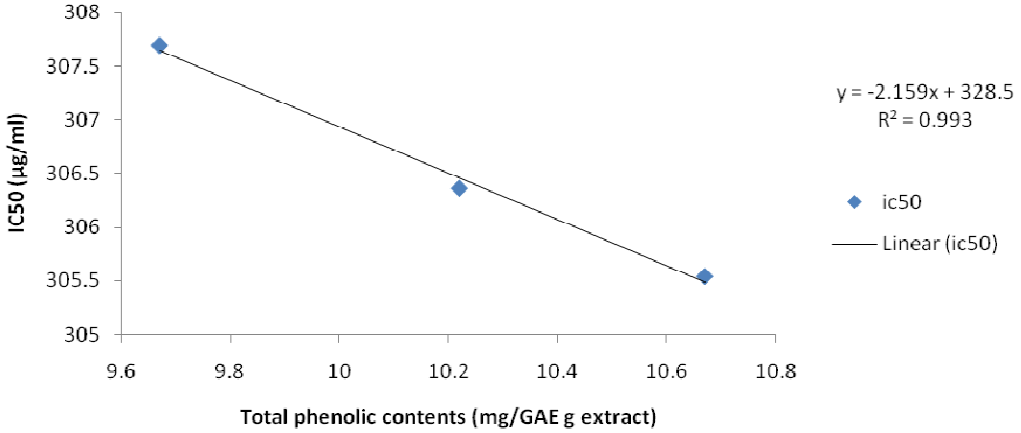

Figure 4. Relationship between total phenolic contents and DPPH (IC_{50}) in methanolic extract of MC. Data represents mean ± SD (n = 3).

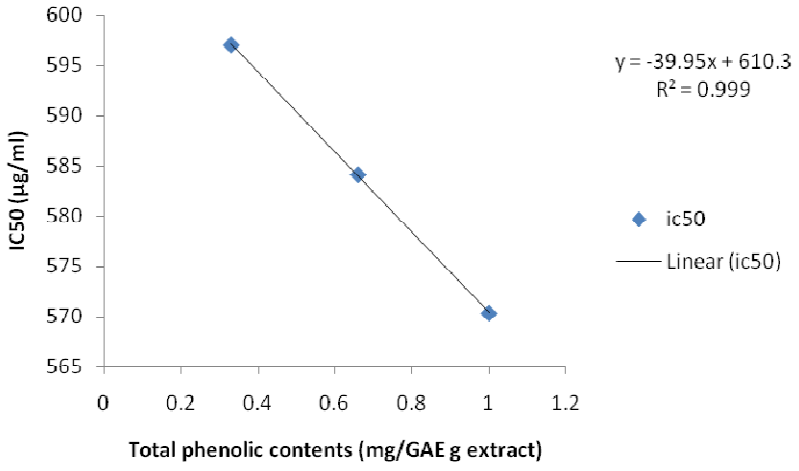

Figure 5. Relationship between total phenolic contents and DPPH (IC_{50}) in chloroformic extract of MC. Data represents mean ± SD (n=3).

DISCUSSION

Plants were identified as useful sources of natural antioxidants that can protect against oxidative stress and therefore, have a main role to protect against injuries from lipid peroxidation (Repetto and Llesuy, 2002). *M. omordica charantia* (MC) contains strong antioxidant and free radical scavenging activities, which can be extracted from compounds such as flavonoids and phenols (Wu and Ng, 2008). Unripe fruits of MC are also identified as a source of vitamins C and A (Raj et al., 2005).

The ferric thiocyanate (FTC) results showed that methanolic extract had greater antioxidant activity when compared with chloroformic extract. Previous studies have claimed that, the aqueous extract of herbal plants has high antioxidant activity against lipid peroxidation. The reaction in the FTC method is due to the MDA compounds from the linoleic acid oxidation in which, peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment (Al-Naqeeb et al., 2009). Peroxides are slowly decayed to lower molecular compounds during the oxidation course (Behbahani et al., 2007). Methanol has stronger polarity than the chloroformic solvent (Rahmat et al., 2003). In this study, however, the high antioxidant activity of *M. omordica charantia* methanolic extract may be due to the majority of the active compounds in the MC fruit being dissolved in the methanolic solvent (strong polar) instead of the chloroformic solvent (relatively-nonpolar).

In this study, the results of the thiobarbituric acid (TBA) test were confirmed with the FTC data. Based on these results, methanolic extract had higher antioxidant activity when compared with chloroformic extract (approximately 7 fold). The antioxidant activity of the ferric thiocyanate method is higher than the antioxidant activity of the TBA assay in chloroformic and ethanolic extracts of *Barringtonia racemosa* leaves (Behbahani et al., 2007). After time, malonaldehyde a secondary product of lipid peroxidation changes into alcohol and acid. The alcohol and acid cannot be detected with a spectrophotometer (Rahmat et al., 2003). The FTC method indicates the amount of peroxide in the initial stages of lipid peroxidation (Saha et al., 2004; Rahmat et al., 2003). Whereas, the thiobarbituric acid method shows the amount of peroxide in the secondary stage of lipid peroxidation (Rahmat et al., 2003). Therefore, the higher antioxidant activity found from the ferric thiocyanate method indicated that, the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage. Thus, these data suggest that methanolic extract has a better beneficial effect against lipid peroxidation when compared with chloroformic extract.

The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts (Elmastas et al., 2007). The results of this study indicated that, the IC_{50} in methanolic extract was significantly lower than the IC_{50} in chloroformic extract suggesting that the methanolic extract had better scavenging activity than the choloroformic extract. In this study, we found a dose dependent relationship in the DPPH assay. The activity increased as the concentration increased for both extracts. The increased formation of free radicals was associated with the increase in lipid peroxidation. One of the important roles of antioxidants is to inhibit the chain reaction of lipid peroxidation (Jain et al., 2008). Ansari et al. (2005) reported that, a heated methanol, water extract of MC showed higher free radical antioxidant activities than a cold extract of *M. omordica charantia*. High free radical antioxidant activity of wild fruit (Wu and Ng, 2008) and Momordica dioica Roxb. Leaves have been found in *M. omordica charantia* ethanolic extracts (Jain et al., 2008). Therefore, these results suggest that the difference in radical scavenging of these two extracts may be due to differential solubility of the MC compounds in the solvents. We conclude that, the methanolic extract (polar extract) with maximal inhibition of free radicals is a more potent extract when compared with the chloroformic extract (non-polar).

The present study estimated the phenolic and flavonoid contents of methanolic extract and chloroformic extract of *M. omordica charantia*. Our results showed that methanolic extract contained significantly higher phenol (approximately 15 fold) and flavonoid (approximately 19 fold) contents than chloroformic extract. MC is a powerful source of phenolic compounds, which may be a good source of antioxidants in the food system (Horax et al., 2005). Both aqueous and ethanolic extracts of MC contain phenol and flavonoid components (Wu and Ng, 2008). MC also has higher contents of other phenolic acids such as Gallic acid, gentisic acid, catechin and epicathecin (Horax et al., 2005). Previous studies have reported that solvent extractions are important in antioxidant activity depending on the phenolic content (Erkan et al., 2008). The different levels of antioxidant activities in plants may be due to not only differences in their phenolic contents, but also in their phenolic acid components (Horax et al., 2005). Liu et al. (2007) have also indicated that, phenolic and flavonoid contents in methanolic extracts (polar solvent) of endophytic Xylaria sp. are higher than the contents in hexane extracts (non-polar solvent). Because of the hydroxyl groups in the phenolic compounds, they may directly contribute to the antioxidant activity and have a critical role in scavenging free radicals (Sathishsekar and Subramanian, 2005; Elmastas et al., 2007). Recent studies have shown that, fruit and vegetable phenols and polyphenols such as flavonoids are one of the major groups that indicate a large spectrum of biological activities that are principally ascribed to their antioxidant property. They prevent free radical damage and lipid peroxidation (Bernardi et al., 2008; Akhila et al., 2009). The high content of total phenolic components in the methanolic extract may have

led to the better results found in the total antioxidant activity and free radical scavenging ability when compared with the chloroformic extract. In addition, the differential activities may have been due to the higher solubility of phenol components in the methanol solvent than in the chloroformic solvent.

In this study, the radical scavenging ability was significantly increased in methanolic extract when compared with chloroformic extract. The reason for the increase in radical scavenging ability may have been due to the increase in total phenolic compounds. Thus, it was important to calculate the correlation between the total phenol contents and total antioxidant activity. A good correlation was found between the total phenol contents and DPPH scavenging ability for methanolic extract and chloroformic extract. Our results are in accordance with previously published results that indicated a high correlation between antioxidant activity and total phenols (Gheldof and Engeseth, 2002; Holasova et al., 2002; Aljadi and Kamaruddin, 2004). Thus, this indicates that the antioxidant activity of many plant extracts is related to their phenolic components.

Furthermore, the methanolic extract containing the higher concentration of phenols had significant free radical scavenging activity in this study. The results suggest that, phenolics are important components of *M. omordica charantia* and that some of its pharmacological effects may be attributed to the presence of these valuable constituents.

Conclusions

In this study, all antioxidant methods (ferric thiocyanate, thiobarbituric acid, and DPPH) showed that the methanolic extracts of *M. charantia* contain more antioxidant activities than the chloroformic extract. Moreover, this study demonstrated that, MC is an important source of phenolic compounds, which are a good source of antioxidant activity. The phenolic component of MC has a high inhibitory effect that prevents lipid peroxidation. However, the solvent type has an important role in detecting phenolic compounds and antioxidant factors. Thus, we concluded that MC might react via its free radical scavenging to prevent lipid peroxidation. Therefore, natural plant antioxidants and phenolic compounds in MC have the capability to been used in food systems to preserve food quality.

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