Pertanika J. Trop. Agric. Sci. 41 (3): 1367 - 1381 (2018)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Chemical Profile, Total Phenolic Content, DPPH Free Radical Scavenging and α-Glucosidase Inhibitory Activities of *Cosmos Caudatus* Kunth Leaves

Wan Nadilah Wan Ahmad[1], Khozirah Shaari[1]*, Alfi Khatib[2], Azizah Abdul Hamid[3] and Muhajir Hamid[4]

[1]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia
[2]Kuliyyah of Pharmacy, International Islamic University Malaysia (IIUM), 25200, Kuantan, Pahang, Malaysia
[3]Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia
[4]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT

Herbs and medicinal plants are major sources of traditional or folk medicines for many countries of the world, including Malaysia. This study evaluated the bioactive potential of the leaf ethanolic extract and solvent fractions of *Cosmos caudatus* Kunth, in scavenging free radicals and inhibiting the enzyme α-glucosidase. In addition, their metabolite profiles were also characterized using liquid chromatography–mass spectrometry. The bioactivity was found to be concentrated in the EtOAc and BuOH fractions which largely contained rutin, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinofuranoside, quercetin 3-*O*-rhamnoside, and quercetin 3-*O*-galactoside, as profiled by LC-MS/MS. It was further shown that the flavonoids glycosides contributed to the free radical scavenging and glucose lowering effects of *C. caudatus* leaves. The results indicated that the leaves of *C. caudatus* are a rich source of bioactive compounds and could be prospective materials for development of new anti-diabetic agents.

Keywords: α-glucosidase inhibition, *Cosmos caudatus*, free radical scavenging activity, LC-MS/MS

ARTICLE INFO

Article history:
Received: 11 Jan 2018
Accepted: 29 Mar 2018
Published: 29 August 2018

E-mail addresses:
wnadilahahmad@gmail.com (Wan Nadilah Wan Ahmad)
khozirah@upm.edu.my (Khozirah Shaari)
alfikhatib@iium.edu.my (Alfi Khatib)
azizahah@upm.edu.my (Azizah Abdul Hamid)
muhajir@upm.edu.my (Muhajir Hamid)
* Corresponding author

ISSN: 1511-3701
e-ISSN 2231-8542

INTRODUCTION

Cosmos caudatus Kunth, locally known as "ulam raja", is a small branched perennial aromatic herb that is 30-250 cm tall with finely dissected leaves (Bunawan, Baharum, Bunawan, Amin, & Noor, 2014). The plant belongs to the family *Asteracecae* and is commonly found in Southeast Asian countries, particularly Indonesia and Malaysia. A decoction of the leaves was recommended for use against diabetes, high blood pressure, arthritis and fever (Abas, Shaari, Lajis, Israf, & Kalsom, 2003; Burkill, 1966; Rasdi, Samah, Sule, & Ahmed, 2010) as well as for several other health benefits such as for longevity and aiding digestion (Bunawan et al., 2014; Ong & Norzalina, 1999). Some major chemical constituents of *C. caudatus* have been investigated and reported in scientific literature. Among these, the phenolic constituents were implicated in the antioxidant and antidiabetic properties of the plant (Kerem, Bilkis, Flaishman, & Sivan, 2006; Kunyanga, Imungi, Okoth, Biesalski, & Vadivel, 2012; Mai, Thu, Tien, & Chuyen, 2007; Ranilla, Kwon, Apostolidis, & Shetty, 2010). An increased production and ineffective scavenging of reactive oxygen species are also known to play a critical role in diabetes mellitus. *C. caudatus* has been reported to have an exceptionally high antioxidant capacity, mainly due to its polyphenolic content (Shui, Leong, & Wong, 2005). Polyphenol-rich foods are known to be potent antioxidants (Dai & Mumper, 2010), protecting cells against oxidative stress and reducing the risk of chronic disease (Art & Hollman, 2005). Thus the antioxidant property of *C. caudatus* has the potential to reduce the harmful effects of oxidative stress.

Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes. If left unmanaged, it will lead to serious damages to various systems of the body, especially the heart, eyes, nerves, blood vessels, and kidneys. Postprandial glucose is an indicator for glycemic control. Postprandial hyperglycaemia is strongly associated with an increased risk of cardiovascular disease in diabetic patients (Hanefeld et al., 1996). One way to assess this is through postprandial glucose. Postprandial hyperglycaemia can be decreased by delaying carbohydrate absorption in the gastrointestinal tract (Dehghan-Kooshkghazi & Mathers, 2004). This can be achieved by inhibiting carbohydrate-hydrolysing enzymes and slowing glucose uptake by intestinal α-glucosidase. Apart from being costly to produce and associated with a decline in efficiency with long term use, modern synthetic hypoglycemic agents have been reported to cause side effects (De Melo Junior, Raposo, Neto, & Diniz, 2002; Satyanarayana, Katyayani, & Latha, 2006). To a certain extent, these reasons have fuelled continued chemical and biological exploration of medicinal and edible plants for the discovery of new and alternative agents with antidiabetic properties. Natural product based α-glucosidase inhibitors are the key targets to identify new compounds for the therapeutic management of diabetes

(Franco, Rigden, Melo, & Grosside, 2002; Yin, Zhang, Feng, Zhang, & Kang, 2014). *C. caudatus* has been shown to have hypoglycemic property in both *in vitro* (Loh & Hadira, 2011) and in *in vivo* study (Perumal, Hamid, & Ismail, 2014). Thus, it is interesting to ascertain if this property could be further exploited for the management of diabetes and to identify the bioactive constituents of the plant. The free radical scavenging activity combined with α-glucosidase inhibitory activity of the ethanol extract and its different polar, non-polar fractions from *C. caudatus* have not been fully studied. Therefore, this study investigated the *in vitro* antioxidant and α-glucosidase inhibitory activities of the ethanolic extract of *C. caudatus* and profiled the probable bioactive chemical constituents in the ethanolic extract by LC-MS/MS technique.

MATERIALS AND METHODS

Materials

C. caudatus Kunth was obtained from UPM Agriculture Park. A voucher specimen SK 2511/14 was deposited in the mini herbarium of the Institute Bioscience, University Putra Malaysia. Taxonomic verification of the species was carried out by Dr. Shamsul Khamis, Biodiversity Unit, Institute Bioscience, UPM).

Plant Extraction and Solvent Fractionation

The leaves of plant material were washed under running tap water and dried under shade. The sample dried leaves were ground to a fine powder in a Waring blender (model 32 BL 80, New Hartford, CT, USA). The powder material was macerated in EtOH:water, 80:20, plant powder (g) to solvent (mL) ratio 1:3, at room temperature. To facilitate extraction, the mixture was sonicated for 1 h, at two intervals of 30 min with a break of 15 min to avoid solvent overheating. The extraction procedure was repeated three times, the pooled filtrates filtered through Whatman Filter No 1, and evaporated to dryness using a rotary evaporator to yield 41.2 g of total crude extract. About 40.0 g of the crude extract was resuspended in 2 L of water and partitioned sequentially with 2 L of hexane, 2 L of DCM, 2 L of EtOAc, 2 L of BuOH to yield the respective hexane (2.69 g), DCM (15.56 g), EtOAc (0.86 g), BuOH (0.73 g) and aqueous (6.09 g) fractions. The extract and solvent fractions were stored at -4°C before subjecting them to bioassay and LC-MS/MS analysis.

Measurement of Total Phenolic Content

The Folin-Ciocalteu assay was used to determine the total phenolic content (TPC) of the extracts based on the procedure described by Zhang et al. (2006), with minor modifications. In triplicate, 20 µL of each serial dilution (6.25, 12.5, 25, 50, 75, 100, 125, 250, 500 ppm) was introduced into test tube, alongside the same series of serial dilution for quercetin as the positive standard. Folin-Ciocalteu reagent (100 µL) was added to each well, mixed thoroughly using a vortex mixer, and allowed to rest for 5 minutes at room temperature. This

was followed by the addition of 80 µL of 7.5% (w/v) sodium carbonate solution and made up to a final volume of 200 µL with distilled water. The solutions in the test tubes were allowed to stand for 30 min prior to the measurement of the absorbance at 765 nm. TPC was expressed as the gallic acid equivalent (GAE) in milligrams per gram of extract.

In Vitro Biological Assays

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Free Radical Scavenging Assay. The DPPH free radical scavenging assay was conducted according to methods described by Li and Seeram (2011), and Wan, Yuan, Cirello and Seeram (2012) with slight modification. The assay was performed in 96-well microplate and performed in triplicates. Aliquots of 50 µL of each serial dilutions (6.25, 12.5, 25, 50, 100, 200, 400 and 500 ppm), of the test samples and quercetin (positive control), made from the stock solutions 0.5 mg/mL and 0.2 mg/mL, respectively, were placed in each well. This was followed by the addition of 150 µL 1,1-diphenyl-2-picryl-hydrazyl (DPPH) which was prepared beforehand at a concentration of 59 mg/L to each well. The microplate was then incubated in the dark at room temperature for 30 min. The analysis was performed in triplicates. The absorbance of the reaction mixtures was measured at 517 nm using a microplate reader. The percentage of inhibition of each test sample was calculated from the following formula, % Inhibition = $[(A_o - A_s)/A_o] \times 100$ where, A_o = absorbance of the reagent blank and A_s = absorbance of the test samples.

The IC_{50} value was then determined from a plot of % inhibition versus concentration of the test samples. The IC_{50} value (µg/mL), for the free radical scavenging activity refers to the concentration at which the scavenging activity was inhibited by 50%.

Alpha-glucosidase Inhibition Assay. The α-glucosidase inhibitory activity was tested following the method described by Collins, Ng, Fong, Wan and Yeung (1997) with slight modifications. The property was determined by measuring the release of *p*-nitrophenyl from the substrate *p*-nitrophenyl-α-D-glucopyranose (PNPG) (Sigma-Aldrich, N1377-1G). Release of *p*-nitrophenyl was observed as a formation of a yellow color upon addition of the reaction stopping reagent glycine (pH 10).

First, stock solutions of the test samples were prepared by dissolving 0.2 mg of the extract in 1 mL ethanol. Meanwhile, 0.4 mg of quercetin, the positive control, was dissolved in 1 mL ethanol (Subramaniam, Asmawi, & Sadikun, 2008). Aliquot of 10 µL of each serial dilutions (3.125, 6.25, 12.5, 25, 50, 100, 200 ppm) made from the stock solutions (0.2 mg/mL) and for quercetin (positive control), made from stock solution (0.4 mg/mL) were placed in each well. The substrate and enzyme were dissolved in 50 mM buffer. Briefly, 10 µL of the sample extract were added to 100 µL of α-glucosidase type 1 from *Saccharomyces*

cerevisiae (Sigma G5003) solution (0.02 U/well) in 30 mM phosphate buffer (pH 6.5). The sample mixture was then incubated for 5 min at room temperature (Deutschländer, Van de Venter, Roux, Louw, & Lall, 2009). In the meantime, 60 mg of 4-nitrophenyl-α-D-glucopyranoside (PNPG) which is the substrate, was dissolved in 20 mL of 50 mM phosphate buffer (pH 6.5). This solution has been reported to be comparable to that of intestinal fluid (Lee, Mediani, Nur Ashikin, Azliana, & Abas, 2014). The PNPG solution (75 µL) was added to each well and the reaction mixtures were incubated for 15 min at room temperature. The reaction was stopped by adding 50 µL of 2M glycine (pH 10) to each well. The optical densities (ODs) were then immediately read off at 405 nm using a microplate reader (Deutschländer et al., 2009). The analysis was performed in triplicates.

The α-glucosidase inhibition activity of the test sample was expressed as percentage (%) inhibition and calculated using the following formula, % inhibition = $[(A_c-A_e/A_c]$ X 100%, where A_c = difference in absorbance between the control (with enzyme) and the blank control (without enzyme) and A_e = difference in absorbance between a sample (with enzyme) and the blank sample (without enzyme). The percentage inhibition was plotted against the concentrations of each sample to determine the concentration required to inhibit 50% of the α-glucosidase enzyme (IC_{50} value) in µg/mL.

Analysis of Extract and Solvent Fractions by LC-MS/MS

Test samples were prepared by dissolving 1 mg of the ethanolic extract and each solvent fraction in 1 mL methanol, followed by ultrasonication for 30 min at room temperature. The test samples were then filtered and kept at 4°C prior to the LC-MS/MS analysis.

The LC column used for the LC-MS/MS analysis was a Dionex C18 Reversed-Phase column (Dionex, Sunnyvale, USA) with column dimensions 250 (l) × 2.0 (i.d) mm and 2.5 µm particle size. Analysis was performed on a Dionex Ultimate 3000 HPLC, at 26.9°C (thermostated column compartment). Mobile phase used was double distilled water containing 0.1% acetic acid (solvent A) and HPLC grade acetonitrile containing 0.1% acetic acid (solvent B). The addition of acetic acid to the mobile phase was to enhance compound peak sharpness by inducing ionization of metabolites (De Moraes, Gregório, Tomaz, & Lopes , 2009). Sample elution was performed in a gradient manner with 10 to 100 mL for solvent A, and 90 to 0 mL solvent B, respectively. The injection volume was 15 µL with constant flow rate of 1.00 mL/min. The flow was split to allow 200 µL/min of eluent into the mass spectrometer. The total LC run time was 35 min.

API-ESI (Atmospheric Pressure Ionization – Electrospray Ionization) mass spectral measurement of the sample was performed on a MicroTOF mass spectrometer (Bruker Daltonic GmbH,

Bremen, Germany). The source conditions were: nebulizer gas nitrogen (N_2) at 0.2 bar, dry gas (N_2) at 3.0 L/min, dry temperature at 180°C, capillary voltage at 4500 V and end plate offset at -500 V. Data acquisition was performed by HyStar Application version 3.2 while data processing was carried out with DataAnalysis Version 3.4 by Bruker Daltonik GmbH. The MS acquisitions were performed in the negative electrospray ionization mode, for the mass range of 50-1000 *m/z*. Additionally, MS/MS experiments were carried out in the automatic and multiple reaction monitoring (MRM) mode. Automatic MS/MS low-energy collision dissociation (CID) was performed at 5- 8 eV collision energy. For the MRM mode, MS/MS spectra were acquired manually by selecting the mass of the parent ion to be fragmented and by setting the amount of collision energy. Neutral loss spectra were extracted from the MS/MS fragment spectra. The neutral loss spectrum was calculated by subtracting the *m/z* value of each fragment ion present from the *m/z* of the precursor ion. The resulting difference values were exhibited with the intensity of the corresponding fragment ion. To add a neutral loss chromatogram, a neutral mass value was entered in the 'Edit' menu after a chromatogram of interest was chosen (DataAnalysis Help, Bruker Daltonik GmbH). The conventional nomenclature used by Domon and Costello (1988), and Wu, Yan, Li, Liu and Liu (2004), were used in representing the fragment ions of glycoconjugates.

Statistical Analysis

The results were presented as the mean ± standard deviation (SD). The statistical significance of the results was evaluated using one-way ANOVA with Duncan's post hoc test. Significant differences were based on p values where $p < 0.05$ are considered significantly different and vice-versa. All the bioassay analysis was performed in triplicates.

RESULTS AND DISCUSSION

Total Phenolic Contents

Table 1 shows the TPC of the *C. caudatus* leaf ethanolic extract and its various solvent fractions, expressed in terms of g gallic acid/g dry weight (GAE/g DW) extract. The TPCs of the solvent fractions ranged from 0.21-0.72 g GAE/g DW extract. The content was highest, and not significantly different from each other, in the EtOAc and BuOH fractions with 0.72 and 0.60 g GAE/g DW, respectively. Although the ethanolic extract consisted of more DCM solubles (37.7%), the nature of these metabolites were mainly non-phenolics since the TPC of the DCM fraction was only 0.22 g GAE/g DW and were not significantly different from the TPCs of the hexane (0.21 g GAE/g DW) or the aqueous (0.36 g GAE/g DW) fractions. Thus, it was concluded that the phenolic constituents of the *C. caudatus* leaf sample were present mainly in the EtOAc (2.1%) and BuOH (1.8%) soluble fractions.

The observed differences in the TPCs of the solvent fractions can be attributed to the difference in polarity of the solvents

used for the extraction. The more polar solvents have a higher ability of extracting phenolic constituents as compared to the less polar and moderate polarity solvents, such as hexane and DCM (Dehghan, Sarrafi, & Salehi, 2016; Fatin et al., 2012; Hatipoĝlu et al., 2013; Robya, Sarhan, Selim, & Khalel, 2013). Other studies have also demonstrated the efficiency of EtOAc in extracting phenolic constituents, for example from outenga (Abdille, Singh, Jayaprakasa, & Jens, 2005), pomegranate (Li et al., 2006), onion (Peschel et al., 2006) and citrus peel (Rehman, 2006). Meanwhile, Bonoli, Verardo, Marconi and Caboni (2004) reported that the maximum phenolic compounds were obtained from barley flour with mixtures of ethanol and acetone. Similarly, aqueous methanol was found to be more effective in recovering higher amounts of phenolic compounds from rice bran (Chatha, Anwar, Manzoor, & Bajwa, 2006) and *Moringa oleifera* leaves (Siddhuraju & Becker, 2003). Meanwhile, Anwar, Jamil, Iqbal and Sheikh (2006) extracted antioxidant compounds from various plant materials including rice bran, wheat bran, oat groats and hull, coffee beans, citrus peel and guava leaves, using aqueous 80% methanol (methanol:water, 80:20 v/v).

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity of the solvent fractions was determined in order to gauge their antioxidant potential. As seen in Table 1, the EtOAc and BuOH fractions exhibited very good DPPH free radical scavenging activity, with 84.5% and 71.5% inhibition, respectively, at the test concentration of 0.5 mg/mL. The inhibition percentages of the EtOAc and BuOH fractions were not significantly different ($p > 0.05$) from each other, with quercetin (91.1%) at a test concentration of 0.2 mg/mL. The DCM and hexane fractions showed lower percentage inhibitions (24.5-33.4%) and were not significantly different from each other. Similarly, the aqueous factions showed lower DPPH free radical scavenging activity of 44.4%. Thus, it was clearly seen that solvent fractions with higher TPCs (EtOAc and BuOH fractions) also exhibited higher free radical scavenging activity, and vice versa.

With respect to the IC_{50} values, the EtOAc (255.20 µg/mL) and BuOH (257.61 µg/mL) fractions exhibited lower values than the crude EtOH:water (80:20) extract (197.87 µg/mL). The IC_{50} values of the EtOAc and BuOH fractions were not significantly different ($p>0.05$) from each other but were both significantly different ($p < 0.05$) from the crude EtOH:water (80:20) extract. The bioactivity of the crude extract and solvent fractions were significantly lower ($p < 0.05$) than that of the positive standard, quercetin with 69.56 µg/mL. Therefore, it was concluded that, although *C. caudatus* was as efficient as quercetin in inhibiting DPPH free radical, it was, however, less potent, based on the higher IC_{50} value in comparison to the positive standard.

Alpha-glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of the solvent fractions was determined in order to gauge their glucose lowering potential. As shown in Table 1, the solvent fractions of the crude extract showed variable α-glucosidase inhibitory activities. The highest α-glucosidase inhibition percentage was exhibited by the EtOAc fraction (79.3%) which was as high as and not significantly different ($p > 0.05$) with that shown by the crude extract (81.6%) at a test concentration of 0.2 mg/mL, and by the positive standard, quercetin (95.1%) at a test concentration of 0.4 mg/mL. Meanwhile, both the BuOH and DCM showed significantly lower inhibition values than the EtOAc fraction. Their inhibition percentages of 57.2% and 55.6%, respectively, were moderate and not significantly different from each other. The hexane and aqueous fractions showed much lower percentage inhibitions among all the solvent fractions.

The crude extract and solvent fractions exhibited significantly more potent α-glucosidase inhibition (27.56-85.73 µg/mL) than quercetin (109.30 µg/mL). In particular, the EtOAc fraction, with an IC_{50} value of 40.90 µg/mL, seemed to be the solvent fraction that contained most

Table 1
Yield of extracts, TPC, DPPH free radical scavenging and α-glucosidase inhibitory activities of C. caudatus *Leaf EtOH:water 80:20 Extract, and its various solvent fractions*

Sample	Yield of extract (g)	Yield of extract (%)	Total phenolic content (g GAE/g DW)	DPPH free radical scavenging activity		α-glucosidase inhibitory activity	
				Inhibition (%) [Test conc: 0.5 mg/mL]	IC_{50} (µg/mL)	Inhibition (%) [Test conc: 0.2 mg/mL]	IC_{50} (µg/mL)
Crude extract	41.2	25.9	0.73 ± 0.13[a]	85.6 ± 4.41[a]	197.87 ± 13.59[a]	81.6 ± 6.89[a]	27.56 ± 6.31[a]
Hexane fraction	2.69	6.5	0.21 ± 0.04[b]	33.4 ± 3.86[b]	nd	29.2 ± 6.58[b]	nd
DCM fraction	15.56	37.7	0.22 ± 0.14[b]	24.5 ± 4.95[b]	nd	55.6 ± 5.53[c]	85.73 ± 5.94[b]
EtOAc fraction	0.86	2.1	0.72 ± 0.19[a]	84.5 ± 5.37[a]	255.20 ± 19.41[b]	79.3 ± 1.27[a]	40.90 ± 6.55[a]
BuOH fraction	0.73	1.8	0.60 ± 0.18[a]	71.5 ± 5.93[a]	257.61 ± 11.20[b]	57.2 ± 3.31[c]	74.84 ± 5.83[b]
Aqueous fraction	6.1	14.8	0.36 ± 0.03[b]	44.4 ± 4.05[c]	nd	31.4 ± 7.46[b]	nd
Quercetin	nd	nd	nd	91.1 ± 3.52[a]	69.56 ± 2.39[c]	95.1 ± 6.41[a]	109.30 ± 4.30[c]

Note: Values are expressed as mean ± standard deviation (n=3). The concentration of quercetin for DPPH radical scavenging activity and α-glucosidase inhibitory activity were 0.2 mg/mL and 0.4 mg/mL, respectively. Values with different superscript letters (a, b, c) are significantly different ($p < 0.05$).

of the bioactive metabolites. Its IC_{50} value was close and not significantly different ($p > 0.05$) from that of the crude extract. Previous studies on other plant extracts have also found that EtOAc is a suitable solvent for extracting α-glucosidase inhibitory compounds from a plant (Ablat et al., 2014; Ado, Abas, Ismail, Ghazali, & Shaari, 2014; Hyun, Hyoun-Chol, & Ju-Sung, 2014; Hyun, Hyoun-Chol, Yeong-Jong, & Ju-Sung, 2016; Moein, Moein, & Ahmadizadeh, 2008; You, Chen, Wang, Jiang, & Lin, 2012). Overall, results from this work have provided additional support to the conclusion that *C. caudatus* can potentially reduce postprandial hyperglycaemia by delaying carbohydrate digestion.

Metabolite Profiling of *C. caudatus* using LC-MS/MS

Since the EtOAc and BuOH fractions exhibited significant bioactivities, these solvent fractions were chemically profiled using API-ESI LC-MS/MS in order to gain better insight into the phenolic constituents that may be contributing to the observed biological activities. The negative ion mode was used in the analysis since most of the constituents in the solvent fractions exhibited a higher response in the negative mode rather than in the positive mode. Six and five compound peaks were observed in the total ion chromatograms (TIC) of the EtOAc (Figure 1) and the BuOH (Figure 3) fractions, respectively. The compounds were tentatively identified, based on their MS/MS fragmentation and subsequently supported by comparisons with literature data and/or MassBank Software (High Quality Mass Spectral Database). The compounds in these bioactive fractions were found to consist mainly of quercetin derivatives.

The MS/MS fragmentation of the individual peaks from the TIC of the EtOAc fraction are shown in Figure 2 and listed in Table 2. Peak **1** exhibited an $[M-H]^-$ ion at *m*/*z* 609, which was further fragmented to daughter ions at *m*/*z* 463 and 300, consistent with rutin (Mediani, Abas, Ping, Khatib, & Lajis, 2012; Shui et al., 2005; Stobiecki, Malosse, Kerhoas, Wojlaszele, & Einhorn 1999; Stobiecki, 2000). Peaks 2 and 3, both showing $[M-H]^-$ ions at *m*/*z* 463, were identified as quercetin 3-*O*-galactoside and

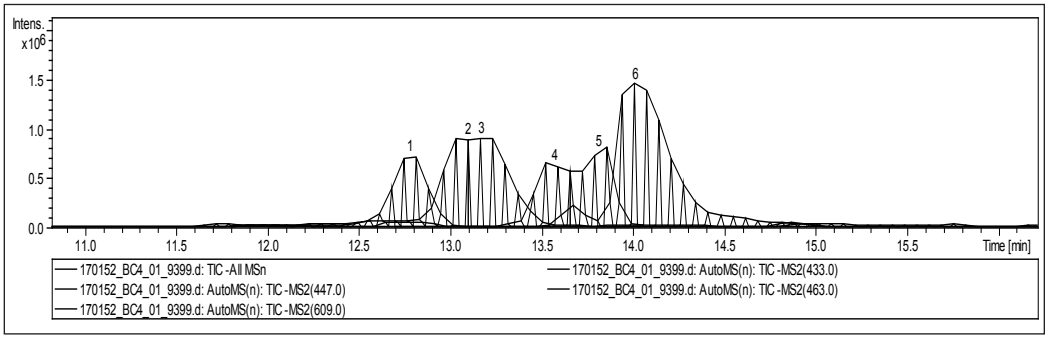

Figure 1. Total ion chromatogram (TIC) of the EtOAc fraction of leaves extract of *C. caudatus*. **1**, rutin; **2**, quercetin 3-*O*-galactoside; **3**, quercetin 3-*O*-glucoside; **4**, quercetin 3-*O*-xyloside; **5**, quercetin 3-*O*-arabinofuranoside; **6**, quercetin 3-*O*-rhamnoside.

quercetin 3-*O*-glucoside, respectively, due to the characteristic MS^2 ions at *m*/*z* 300, 179 and 151 (Qu, Liang, Luo, & Wang, 2004; Tahir et al., 2013). Peak 4 and 5 with their $[M-H]^-$ ions at *m*/*z* 433, which were also fragmented further to the characteristic ions at *m*/*z* 300, 179 and 151, were identified as quercetin 3-*O*-xyloside (Shui et al., 2005) and quercetin 3-*O*-arabinofuranoside (Mediani et al., 2012), respectively. Similarly, peak **6** with an $[M-H]^-$ ion at *m*/*z* 447 was identified as quercetin 3-*O*-rhamnoside, as previously reported by Mediani, Abas, Khatib and Tan (2013).

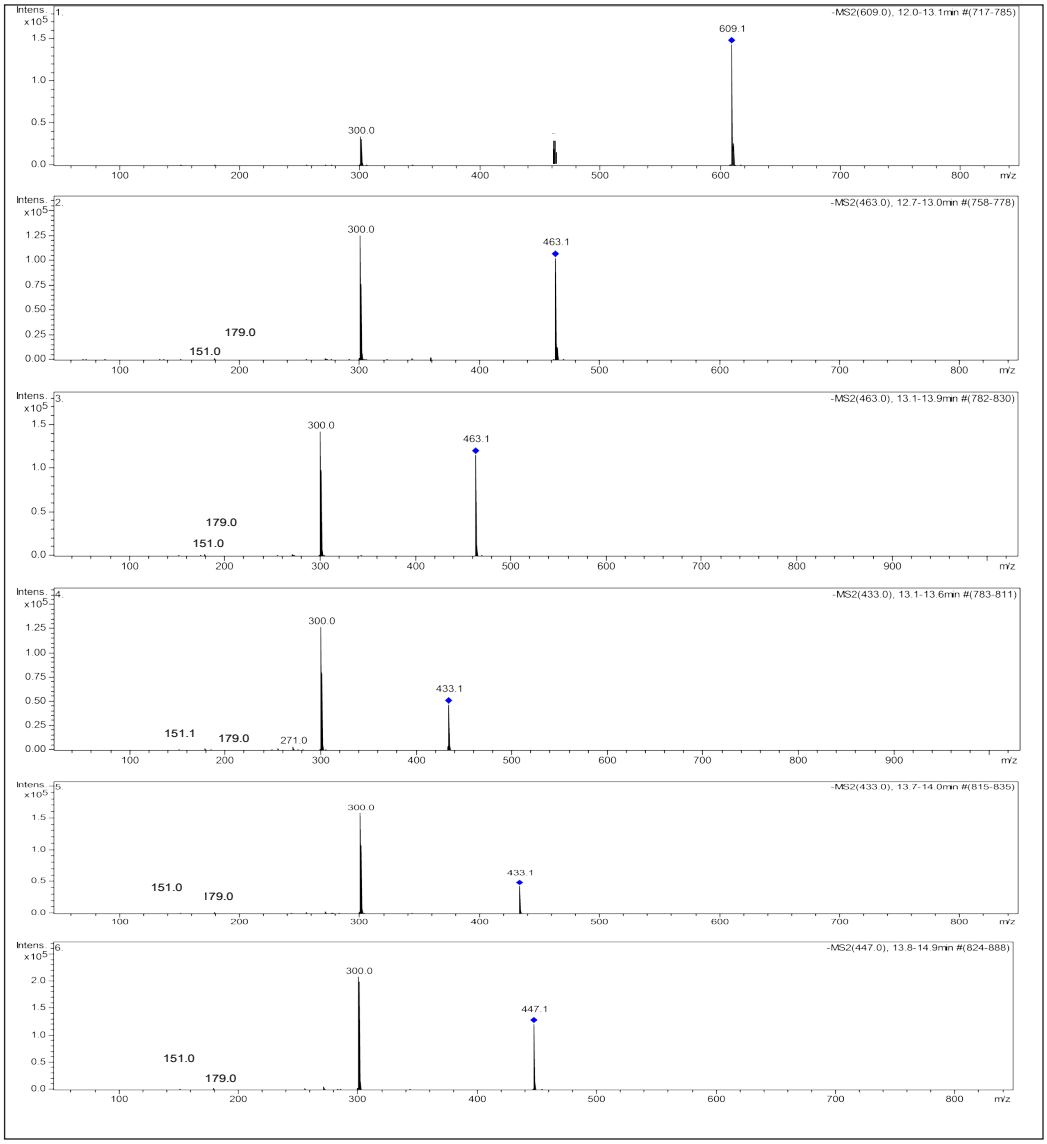

Figure 2. Negative mode API-ESI MS spectra of compounds 1-6. **1**, rutin; **2**, quercetin 3-*O*-galactoside; **3**, quercetin 3-*O*-glucoside; **4**, quercetin 3-*O*-xyloside; **5**, quercetin 3-*O*-arabinofuranoside; **6**, quercetin 3-*O*-rhamnoside.

Table 2
Tentative identification of phenolic compounds in the EtOAc Fraction of leaves extract of C. caudatus

Peak no	Retention time (min)	$[M-H]^-$ ion	MS/MS fragment ions	Tentative identification
1	12.8	609	463, 300	Rutin
2	13.1	463	300, 179, 151	Quercetin 3-*O*-galactoside
3	13.2	463	300, 179, 151	Quercetin 3-*O*-glucoside
4	13.6	433	300, 179, 151	Quercetin 3-*O*-xyloside
5	13.8	433	300, 179, 151	Quercetin 3-*O*-arabino-furanoside
6	14.1	447	300, 179, 151	Quercetin 3-*O*-rhamnoside

The TIC (Figure 3) and MS/MS data of the BuOH fraction was similarly analyzed and found to contain the same quercetin derivatives, tentatively identified as compounds 1- 5.

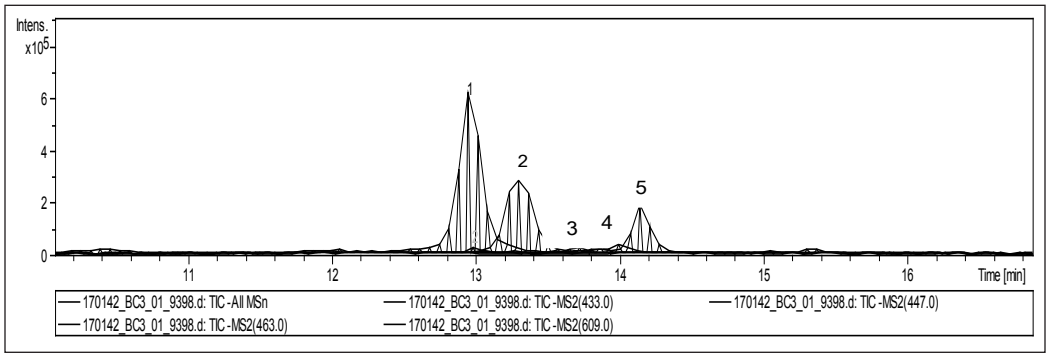

Figure 3. Total ion chromatogram (TIC) of the BuOH fraction of *C. caudatus*. **1**, rutin; **2**, quercetin 3-*O*-glucoside; **3**, quercetin 3-*O*-xyloside; **4**, quercetin 3-*O*-arabinofuranoside; **5**, quercetin 3-*O*-rhamnoside.

Table 3
Tentative identification of phenolic compounds in the BuOH Fraction of leaves extract of C. caudatus

Peak	Retention time (min)	$[M-H]^-$ ion	MS/MS fragment ions	Tentative identification
1	13.0	609	463, 300	Rutin
2	13.3	463	300, 179, 151	Quercetin 3-*O*-glucoside
3	13.7	433	300, 179, 151	Quercetin 3-*O*-xyloside
4	14.0	433	300, 179, 151	Quercetin 3-*O*-arabinofuranoside
5	14.1	447	300, 179, 151	Quercetin 3-*O*-rhamnoside

CONCLUSION

This study demonstrated that the EtOAc and BuOH fractions, derived from the ethanolic extract of *C. caudatus* leaves, have good antioxidant and antidiabetic activities based on the significant DPPH radical scavenging and α-glucosidase inhibition activities, respectively. The phytoconstituents that could be responsible for the antioxidant and α-glucosidase inhibitory activities, presumably acting in synergy, included rutin, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinofuranoside, and quercetin 3-*O*-rhamnoside. The chemical information and biological activity obtained are relevant as a starting point for studies on plant phytochemicals, which can contribute to further development of the medicinal plant into functional food, nutraceutical, and medicinal preparation.

ACKNOWLEDGEMENT

The authors wish to thank Universiti Putra Malaysia (UPM), Agro Biotechnology Institute (ABI) and Ministry of Science, Technology and Innovation (MOSTI) for the provision of research grant (grant no: 637007). The authors are also grateful to Mr Alefee Ayatillah from Centre Research Institute Malaysia (CRIM), Universiti Kebangsaan Malaysia (UKM), Malaysia, for LC-MS/MS analysis.

REFERENCES

Abas, F., Shaari, K., Lajis, N. H., Israf, D. A., & Kalsom, Y. U. (2003). Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. *Natural Product Sciences, 9*(4), 245-248.

Abdille, M. H., Singh, R. P., Jayaprakasa, G. K., & Jens, B. S. (2005). Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chemistry, 90*, 891-896.

Ablat, A., Mohamad, J., Awang, K., Jamil, A., Shilpi, & Arya, A. (2014). Evaluation of antidiabetic and antioxidant properties of *Brucea javanica* seed. *The Scientific World Journal*, *786130*, 2-10.

Ado, M. A., Abas, F., Ismail, I. S., Ghazali, H. M., & Shaari, K. (2014). Chemical profile and antiacetylcholinesterase, antityrosinase, antioxidant and α-glucosidase inhibitory activity of *Cynometra cauliflora* L. leaves. *Journal of the Science of Food and Agriculture, 95*(3), 635-642.

Anwar, F., Jamil, A., Iqbal, S., & Sheikh, M. A. (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. *Grasas Aceites Sevilla, 57*, 189-197.

Art, I. C., & Hollman, P. C. (2005). Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition, 81*(1), 317-325.

Bonoli, M., Verardo, V., Marconi, E., & Caboni, M. F. (2004). Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic acids. *Journal of Agricultural and Food Chemistry*, *52*, 5195-5200.

Bunawan, H., Baharum, S. N., Bunawan, S. N., Amin, N. A., & Noor, N. M. (2014). *Cosmos caudatus* Kunth: A traditional medicinal herb. *Global Journal of Pharmacology, 8*(3), 420-426.

Burkill, I. H. (1966). *A Dictionary of the Economic Products of the Malay Peninsula, Vol. 1 and 2*. Kuala Lumpur, Malaysia: Ministry of Agriculture Cooperative.

Chatha, S. A. S., Anwar, F., Manzoor, M., & Bajwa, J. R. (2006). Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. *Grasas Aceites Sevilla, 57*, 328-335.

Collins, R. A., Ng, T. B., Fong, W. P., Wan, C. C., & Yeung, H. W. (1997). Inhibition of glycohydrolase enzymes by aqueous extracts of Chinese medicinal herbs in a microplate format. *IUBMB Life, 42*(6), 1163-1169.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*(10), 7313-7352.

Dehghan, H., Sarrafi, Y., & Salehi, P. (2016). Antioxidant and antidiabetic activities of 11 herbal plants from Hyrcania region. *Iran Journal of Food and Drug Analysis, 24*, 179-188.

Dehghan-Kooshkghazi, M., & Mathers, J. C. (2004). Starch digestion, large-bowel fermentation and intestinal mucosal cell proliferation in rats treated with the α-glucosidase inhibitor acarbose. *British Journal of Nutrition, 91,* 357-365.

Deutschländer, M. S., Van de Venter, M., Roux, S., Louw, J., & Lall, N. (2009). Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology, 124*(3), 619-624.

De Melo Junior, E. J. M., Raposo, M. J., Neto, J. L., & Diniz, M. F. A. (2002). Medicinal plants in the healing of dry socket in rats: Microbioligical and microscopic analysis. *Phytomedicine*, *9*(2), 109-116.

De Moraes, S. L., Gregório, L. E., Tomaz, J. C., & Lopes, N. P. (2009). Rapid screening and identification of polar constituents from Brazilian Arnica *Lychnophora* sp. by LC-UV/DAD-ESI-MS and LC-UV/DAD-ESI-MS/MS analysis. *Chromatographia, 69,* 157-165.

Domon, B., & Costello, C. E. (1988). A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate Journal, 5 (4),* 397-409.

Fatin, R. J, Wahab, R., Daud, J. M., Sudin, M., Rasat, M. S., & Sulaiman, O. (2012). Study on methanol extracts of *Nauclea subdita* (Korth) Steud. heartwood parts for the total phenolic contents and free radical scavenging activities. *Current Research Journal of Biological Sciences, 4*(5), 600-607.

Franco, O. L., Rigden, D. J., Melo, F. R., & Grosside, S. M. F. (2002). Plant α-amylase inhibitors and their interaction with insect alpha-amylases. *European Journal of Biochemistry, 269*, 397-412.

Hatipoğlu, G., Sökmen, M., Bektaş, E., Daferera, D., Sökmen, A., Demir, E., & Şahin, H. (2013). Automated and standard extraction of antioxidant phenolic compounds of *Hyssopus officinalis* L. ssp. angustifolius. *Industrial Crops and Products, 43*, 427-433.

Hanefeld, M., Fischer, S., Julius, U., Schulze, J., Schwanebeck, U., Schmechel, H., ... & DIS Group. (1996). Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. *Diabetologia*, *39*(12), 1577-1583.

Hyun, T. K., Hyoun-Chol, K., & Ju-Sung, K. (2014). Antioxidant and antidiabetic activity of *Thymus quinquecostatus* Celak. *Industrial Crops and Products, 52*, 611-616.

Hyun, T. K., Hyoun-Chol, K., Yeong-Jong, K., & Ju-Sung, K. (2016). Antioxidant, α-glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowberry (*Empetrum nigrum var. japonicum*). *Saudi Journal of Biological Sciences, 23*, 181-188.

Kunyanga, C. N., Imungi, J. K., Okoth, M. W., Biesalski, H. K., & Vadivel, V. (2012). Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT-Food Science and Technology, 45*(2), 269-276.

Kerem, Z., Bilkis, I., Flaishman, M. A., & Sivan, L. (2006). Antioxidant activity and inhibition of α-glucosidase by trans-resveratrol, Piceid, and a novel trans-stilbene from the roots of Israeli *Rumex bucephalophorus* L. *Journal of Agricultural and Food Chemistry, 54*(4), 1243-1247.

Lee, S. Y., Mediani, A., Nur Ashikin, A. H., Azliana, A. B. S., & Abas, F. (2014). Antioxidant and α-glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants. *International Food Research Journal, 21*(1), 165-172.

Li, L., & Seeram, N. P. (2011). Further investigation into maple syrup yields 3 new lignans, a new phenylpropanoid, and 26 other phytochemicals. *Journal of Agricultural and Food Chemistry, 59*, 7708-7716.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry, 96*, 254-260.

Loh, S. P., & Hadira, O. (2011). *In vitro* inhibitory potential of selected Malaysian plants against key enzymes involved in hyperglycemia and hypertension. *Malaysian Journal Nutrition, 17*, 77-86.

Mai, T. T., Thu, N. N., Tien, P. G., & Chuyen, N. V. (2007). Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of Nutritional Science and Vitaminology, 53* (3), 267-276.

Mediani, A., Abas, F., Ping, T. C., Khatib, A., & Lajis, N. H. (2012). Influence of growth stage and season on the antioxidant constituents of *Cosmos caudatus*. *Plant Foods for Human Nutrition, 67*, 344-350.

Mediani, A., Abas, F., Khatib, A., & Tan, C. P. (2013). *Cosmos caudatus* as a potential source of polyphenolic compounds: Optimisation of oven drying conditions and characterisation of its functional properties. *Molecules, 18*(9), 10452-10464.

Moein, M. R., Moein, S., & Ahmadizadeh, S. (2008). Radical scavenging and reducing power of *Salvia mirzayanii* subfractions. *Molecules, 13*, 2804-2813.

Ong, H. C., & Norzalina, J. (1999). Malay herbal medicine in Gemencheh, Negeri Sembilan, Malaysia. *Fitoterapia, 70*(1), 10-14.

Perumal, V., Hamid, A. A., & Ismail, A. (2014). Effect of *Cosmos caudatus* Kunth leaves on the lipid profile of a hyperlipidemia induced animal model. *Journal of Food Chemistry and Nutrition, 2* (1), 43-51.

Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzía, I., Jiménez, D., ... & Codina, C. (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chemistry*, *97*(1), 137-150.

Qu, J., Liang, Q., Luo, G., & Wang, Y. (2004). Screening and identification of glycosides in biological samples using energy-gradient neutral loss scan and liquid chromatography tandem mass spectrometry. *Analytical Chemistry, 76,* (8), 2239-2247.

Ranilla, L. G., Kwon, Y., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes, relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource Technology, 101*(12), 4676-4689.

Rasdi, N. H. M., Samah, O. A., Sule, A., & Ahmed, Q. U. (2010). Antimicrobial studies of *Cosmos caudatus* Kunth. (Compositae). *Journal of Medicinal Plant Research, 4*(8), 669-673.

Rehman, Z. U. (2006). Citrus peel extract- A natural source of antioxidant. *Food Chemistry, 99*, 450-454.

Robya, M. H., Sarhan, M. A., Selim, K. A., & Khalel, K. I. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products, 43*, 827-831.

Satyanarayana, T, Katyayani, B. M., & Latha, H. E. (2006). Hypoglycemic and antihyperglycemic effect of alcoholic extract of *Euphorbia leucophylla*. *Pharmacognosy Magazine, 2*, 244-253.

Shui, G., Leong, L. P., & Wong, S. P. (2005). Rapid screening and characterisation of antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass spectrometry. *Journal of Chromatography B, 827*(1), 127-138.

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry, 5*(8), 2144-2155.

Subramanian, R., Asmawi, M. Z., & Sadikun, A. (2008). In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta Biochimica Polonica, 5* (2), 391-398.

Stobiecki, M., Malosse, C., Kerhoas, L., Wojlaszele, P., & Einhorn, J. (1999). Detection of isoflavonoids and their glycosides by liquid chromatography/ electrospray ionization mass spectrometry in root extracts of lupin (*Lupinus albus*). *Phytochemical Analysis, 10* (4), 198-207.

Stobiecki, M. (2000). Application of mass spectrometry for identification and structural studies of flavonoid glycosides. *Phytochemistry, 54*(3), 237-256.

Tahir, N. I., Shaari, K., Abas, F., Parveez, G. K. A., Ishak, Z., & Ramli, U. S. (2013). Characterization of apigenin and luteolin derivatives from oil palm (*Elaeis guineensis* Jacq.) leaf using LC–ESI-MS/MS. *Journal of Agricultural and Food Chemistry, 60*(45), 11201-11210.

Yin, Z., Zhang, W., Feng, F., Zhang, Y., & Kang, W. (2014). α-Glucosidase inhibitors isolated from medicinal plants. *Food Science Human Wellness, 3*, 136-174.

You, Q., Chen, F., Wang, X., Jiang, Y., & Lin, S. (2012). Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. *Food Science and Technology, 46*, 164-168.

Wan, C., Yuan, T., Cirello, A. L., & Seeram, N. P. (2012). Antioxidant and α-glucosidase inhibitory phenolics isolated from highbush blueberry flowers. *Food Chemistry, 135*, 1929-1937.

Wu, W., Yan, C., Li, L., Liu, Z., & Liu, S. (2004). Studies on the flavones using liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of Chromatography A, 1047*(2), 213-220.

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D. A., & Barrow, C. J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology, 18*(3), 445-450.