Antioxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides* L.

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Hyperglycemia causes oxidative stress, which further exacerbates the progression of diabetes mellitus and its complications. The aim of the present study was to evaluate the antidiabetic and antioxidant properties of the 80% methanolic extract of the aerial parts of *Carpesium abrotanoides* L. (CAME) through various *in vitro* models. The extract exhibited potent α-glucosidase inhibitory activity with IC₅₀ value of 44.22 µg/ml. Kinetic studies revealed non-competitive inhibition of CAME on α-glucosidase enzyme. The antioxidant activities of CAME, measured in terms of IC₅₀ values using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferrous ion chelating (FIC) and nitric oxide scavenging (NOS) were 111.2, 15.6, 150.2 and 798.5 µg/ml, respectively. CAME also showed significant reducing capability by ferric reducing antioxidant power (FRAP) assay. Inhibition of lipid peroxidation in CAME was found to be dose-dependent. It possessed considerable amounts of phenolics (88 mg gallic acid equivalent/g of extract) and flavonoids (12 mg quercetin equivalent/g of extract). Based on its strong α-glucosidase inhibitory and antioxidant activities, CAME appears to be a potential herb for the treatment of diabetes and can be further explored as a functional medicinal plant for isolating the active ingredient(s) along with animal studies *in vivo*.

**Key words:** *Carpesium abrotanoides* L., phenolic compounds, flavonoid compounds, α-glucosidase, ferric reducing antioxidant power, antioxidant, free radical.

INTRODUCTION

Oxidative stress is due to an imbalance between pro-oxidant/free radical production and opposing antioxidant defences (Cui et al., 2004). It causes biochemical damage in cells and tissues, which results in aggravating the progression of many clinical diseases, including diabetes (Cheng et al., 2003; Yen et al., 2008). The increased free-radical production and reduced antioxidant defense may partially mediate the initiation and progression of diabetes-associated complications (Yao et al., 2010). Therefore, supplementation of antioxidants can be beneficial for diabetic patients, not only to maintain antioxidant levels in the body but also to treat the long-term complications that can arise (Iwai, 2008).

Nature has been a potential source of many therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from natural resources, e.g. plants (Cragg and Newman, 2001). Major phytochemicals, e.g. phenolic acids, flavonoids, coumarin derivatives, etc., are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants. In addition, many efforts have been made to search for more effective and safe inhibitors of α-glucosidase from natural materials to treat diabetes. Moreover, the combination of α-glucosidase inhibitors and antioxidants will become more effective for the prophylaxis of type 2 diabetes (Shibano et al., 2008).

*Carpepsism abrotanoides* L. (Asteraceae) is an erect perennial herb, widely available in South Europe to East Asia, particularly Korea, China, Japan and the Himalayas. The aerial parts of *C. abrotanoides* L. (CAL) have been used in Korean and Chinese medicines as an insecticide and to treat bruises (Wang et al., 2009a). In addition, the whole plant possesses antipyretic, detoxifying and diuretic properties. It treats stomach ulcers, tonsillitis,
bronchitis and boils. The chemical constituents of this medicinal plant exhibited antifungal, antibacterial and cyto-toxic activities (Wang et al., 2009a; Lee et al., 2002). As CAL has been reported to a limited extent regarding its antioxidant potential (Lee et al., 2010), and possesses no earlier reports related to its α-glucosidase inhibitory prospective, the present study was undertaken to investigate its antidiabetic and antioxidant properties in detail using various in vitro models. Additionally, total phenolic and flavonoid contents were also determined.

MATERIALS AND METHODS

Plant material

The dried aerial parts of C. abrotanoides were obtained from "Korean Collection of Herbal Extracts", a Biotech company in Korea. A voucher specimen is available from the company (Korea Collection of Herbal Extracts, 2000).

Preparation of extracts

The dried aerial parts of C. abrotanoides (500 g, dry weight) were extensively extracted with 80% aqueous methanol at room temperature for 3 days and filtered through a Whatman No. 1 filter paper. The extract was concentrated using rotary vacuum evaporator to give a residue. The residue was freeze-dried to obtain a dry powder (14 g) and kept in a glass bottle (stored at 4°C until used). For evaluating the extract through various in vitro assays, the powder was first dissolved in methanol to obtain CAL methanolic extract (CAME) with different concentrations depending on the study type.

Chemicals

α-Glucosidase (from Saccharomyces cerevisiae type I), 4-nitrophenyl-α-D-glucopyranoside (PNPG), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ferrozine, ferric chloride, sodium nitrite, Griess reagent, L-ascorbic acid, Folin-Ciocalteu reagent, gallic acid, citric acid, aluminium chloride and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other commercially available reagents and solvents were used as received.

Determination of total phenolic content (TPC)

TPC of the extract was determined using Folin-Ciocalteu assay as described by Zhang et al. (2006) with minor modifications. In this assay, 10 µl of extract, 60 µl of methanol, 10 µl aluminium chloride (10% w/v), 10 µl of potassium acetate (1 M) and 120 µl of distilled water were mixed well and incubated at room temperature for 30 min, followed by absorbance measurement at 415 nm. TPC was expressed as quercetin equivalent (QE) in mg per gram dry extract.

α-Glucosidase inhibitory assay

The enzyme inhibition activity for α-glucosidase was assessed according to the method reported by Sancheti et al. (2010) with minor modifications. The reaction mixture contained 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of 0.5 mM 4-nitrophenyl α-D-glucopyranoside, 10 µl of test sample at various concentrations and 25 µl of α-glucosidase solution (0.2 Unit/ml). This reaction mixture was then incubated at 37°C for 30 min. The reaction was terminated by adding 100 µl of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using microplate reader. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. Controls were conducted in an identical manner replacing the plant extracts with methanol. Acarbose was used as positive control. All experiments were carried out in triplicates. The inhibition percentage of α-glucosidase was assessed by the following formula:

% Inhibition = \([1 - (\text{sample absorbance/control absorbance})\] \times 100

Kinetics of inhibition against α-glucosidase

To evaluate the inhibition type against α-glucosidase, increasing concentration of PNPG was used as a substrate in the absence or presence of CAME at different concentrations. The inhibition type of the data was determined by Lineweaver-Burk plot analysis of the data, which were calculated from the results according to Michaelis-Menten kinetics.

DPPH free radical scavenging assay

The free radical scavenging activity of CAME at different concentrations was measured by the method of Blois (1958) with minor modifications. In this assay, 50 µl of 0.5 mM DPPH in methanol, 10 µl of test sample at different concentrations and 50 µl of 0.1 M tris HCl buffer (pH 7.0) were added in the 96-well microplate and the change in absorbance was measured at 517 nm 30 min later. The positive control contained 10 µl of methanol instead of test sample. L-Ascorbic acid was used as a reference standard. All experiments were carried out in triplicates. The percentage (%) scavenging activity was calculated by the following formula:

% scavenging activity= \([1 - (\text{sample absorbance/control absorbance})\] \times 100

ABTS radical cation decolorization assay

The ABTS radical cation decolorization assay was carried out using the method reported by Re et al. (1999) and Ling et al. (2009) with slight modifications. ABTS+ was generated by oxidation of ABTS with potassium persulfate in 20 ml of distilled water. The ABTS stock solution was prepared by adding 0.0768 g of ABTS salt and 0.0132 g of potassium persulfate in 20 ml of distilled water. Stock solution was kept in dark
for 12 - 16 h (overnight) at room temperature prior to use. The ABTS*⁺ solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 200 µl of diluted ABTS solution (A₇₃₄ nm = 0.700 ± 0.020) to 10 µl of the sample at various concentrations, the absorbance was read at 734 nm at 30°C using microplate reader exactly after 6 min after initial mixing. The positive control contained 10 µl of methanol instead of test sample. L-ascorbic acid was served as a reference standard. All experiments were carried out in triplicates. The percentage scavenging activity was calculated as described for the DPPH assay.

**Ferrous ion-chelating assay**

The ferrous ion chelating potential of the extract at different concentrations was investigated according to the method of Decker and Welch (1990) and Wang et al. (2009b) with minor modifications. In this, 2 mM ferrous sulfate (FeSO₄) solution and 5 mM ferrozine solution were prepared and diluted 20 times at the time of experiment. 50 µl of diluted FeSO₄ and 50 µl of the extract were mixed in a microplate and the reaction was initiated by the addition of 50 µl of diluted ferrozine. The solutions were well mixed and allowed to stand at 25°C for 10 min. After incubation, the absorbance was measured at 562 nm. Methanol was used as positive control instead of sample. Distilled water was used as blank instead of ferrozine, which was used for error correction. Citric acid was used as reference standard. The percentage scavenging activity was calculated as described for the DPPH assay. All determinations were carried out in triplicate.

**Ferric (Fe³⁺) reducing antioxidant power (FRAP) assay**

The reducing power of the CAME was determined according to the method of Ak and Gülçin (2008) with slight modifications. Briefly, 10 µl of sample was mixed with 15 µl of 0.1 M sodium phosphate buffer (pH 6.6) and 15 µl of potassium ferricyanide (1% w/v). This reaction mixture was incubated at 50°C for 20 min. After 20 min incubation, the reaction mixture was acidified with 15 µl of trichloroacetic acid (10%) and mixed well. To this, 55 µl of distilled water and 110 µl of ferric chloride (0.1% w/v) were added and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing capability. L-ascorbic acid was taken as a reference standard. All experiments were carried out in triplicate.

**Nitric oxide scavenging assay**

The nitric oxide (NO) scavenging activity of the extract was measured according to the method described by Ho et al. (2010). This method consisted addition of 50 µl of sample solution with 50 µl of 10 mM sodium nitroprusside solution into a 96-well flat-bottomed plate and the plate was incubated under light at room temperature for 90 min. Finally, an equal volume of Griess reagent (1% of sulphanilamide and 0.1% of naphthylenenediamine in 2.5% HPO₃) was added to each well to measure the nitrite content immediately at 546 nm. L-ascorbic acid was evaluated as a reference standard. All experiments were carried out in triplicate. The percentage scavenging activity was calculated as described for the DPPH assay.

**Determination of lipid peroxidation**

In this, male wister rats were fasted overnight and anaesthetized using diethyl ether. Their livers were quickly removed and cut into small pieces and homogenized in phosphate buffer (50 mM, pH 7.4), to give a 10% w/v liver homogenates. Each homogenate was then centrifuged at 5000 g for 15 min at 4°C. The oxidant pair Fe²⁺/ascorbate was used to induce oxidative stress in the rat liver homogenate (Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009). The reaction mixture was composed of 0.5 ml of each liver homogenate, 0.9 ml phosphate buffer (50 mM, pH 7.4), 0.25 ml FeSO₄ (0.01 mM), 0.25 ml ascorbic acid (0.1 mM) and 0.1 ml of different concentrations of CAME. The reaction mixture was incubated for 30 min at 37°C. The extent of lipid peroxidation of the rat liver homogenate in the presence and absence of CAME was evaluated by measuring the product of thiobarbituric acid reactive substances (TBARS) using the Cayman’s TBARS assay kit (Seoul, Korea). The (MDA) malondialdehyde-TBA adducts formed by the reaction of MDA and TBA under high temperature and acidic conditions was measured colorimetrically at 532 nm. The amount of TBARS formed was calculated using the MDA standard curve. Quercetin was used as a reference standard.

**Statistical analysis**

All assays were performed at least three times with triplicate samples. All results are expressed as mean ± SD. IC₅₀ values were determined by plotting a percent inhibition versus concentration curve for all the assays (excluding FRAP and lipid peroxidation), in which the concentration of sample required for 50% inhibition was determined and expressed as IC₅₀ value.

**RESULTS AND DISCUSSION**

**Total phenolics and flavonoid compounds**

The results indicated that CAME contains significant amounts of total phenolic compounds equivalent to 88 mg gallic acid /g and flavonoid compounds equivalent to 12 mg quercetin /g. Both these classes of compounds generally possess good antioxidant and antidiabetic potentials. Phenolic compounds are very important plant constituents as their hydroxyl groups confer scavenging ability and flavonoids act through scavenging or chelation. They are known as powerful chain breaking antioxidants. The hydroxyl group present in the phenolic compound confers scavenging ability and also plays an important role in preventing lipid peroxidation. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. They are known to inhibit lipid peroxidation in model system, such as autoxidation of linoleic acid, methyl linolenate or lecithin liposomes and in biological systems, such as liver microsomes treated with Fe²⁺ (Brown et al., 1998; Ferrali et al., 1997; Sugihsara et al., 1999). It is also reported that flavonoids may preserve beta cell function by reducing oxidative stress-induced tissue damage and therefore protect against the progression of insulin resistance to type 2 diabetes (Song et al., 2005).

**α-glucosidase inhibitory activity**

In this study, the hypoglycemic potential of CAME was
evaluated by the α-glucosidase inhibition assay. The optimal concentration of CAME required for the 50% inhibition (IC$_{50}$) against alpha-glucosidase was 44.22 µg/ml. Acarbose was used as positive control with IC$_{50}$ value of 2.5 µg/ml. This assay presented dose dependent inhibition (Figure 1). Kinetic studies were performed to determine the mode of inhibition by Lineweaver–Burk plot analysis. This showed non-competitive inhibition against α-glucosidase (Figure 2), which indicated that CAME binds to a site other than the active site of the enzyme and combines with either free enzyme or the enzyme-substrate complex, possibly interfering with the action of both. The results elucidated that, the inhibitory potential of CAME against the starch hydrolyzing enzyme-α-glucosidase was comparatively lesser as compared to acarbose. This is because crude extracts contain

**Figure 1.** α-glucosidase inhibitory effect of CAME (25 - 250 µg/ml). Each value is mean of three observations in triplicate, IC$_{50}$ value of CAME = 44.22 µg/ml. Acarbose was taken as reference standard, IC$_{50}$ value = 2.5 µg/ml.

**Figure 2.** Lineweaver-Burk plots of kinetic analysis of α-glucosidase inhibition by CAME, α-glucosidase was treated with various concentrations of PNPG in the absence (■) or presence of CAME at two different concentrations [(□) 47.62 µg/ml and (▲) 95.23 µg/ml].
non-active components along with the active ones, therefore to isolate the active compound(s) from this plant will help identify the potenti nal inhibitor(s) of carbohydrate hydrolyzing enzyme, in turn helping prevent and/or treat diabetes. Inhibition of α-glucosidase by different classes of phenolic compounds is described in the literature (Kim et al., 2005; Shim et al., 2003; Tadera et al., 2006). α-Glucosidase was effectively inhibited by naringenin, kaempferol, luteolin, apigenin, (+)-catechin/(-)-epicatechin, diadzein and epigallocatechin gallate (Tadera et al., 2006). These flavonoids exhibited a mixed and close to non-competitive type of inhibition on yeast α-glucosidase.

**Antioxidant activities**

In this study, the detailed antioxidant potential of the CAME, with various antioxidant assay techniques were evaluated. DPPH (deep violet color) is a very stable organic free radical. It receives proton from any hydrogen donor and converts it into a colorless α-α-diphenyl-β-picryl hydrazine. The amount of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm (Kaur et al., 2006). CAME significantly reduced the DPPH radicals in a dose dependent manner (Table 1). This pronounced activity might be due to the presence of phenolic compounds in the CAME.

ABTS assay is another excellent tool for determining the antioxidant capacity of hydrogen donating antioxidants. ABTS•⁺ is a blue chromophore produced by the reaction between ABTS salt and potassium per-sulfate. Addition of CAME to this pre-formed radical cation reduced it to ABTS in a concentration dependent manner (Table 2). In the literature, it has been observed that, the ABTS reducing property is directly proportional to the amount of phenolics. Therefore, the potent ABTS reduction observed in this assay might be due to the phenolics compounds present in the extract.

The chelation of ferrous ions by CAME was estimated using ferrozine. Ferrozine can quantitatively form complex with ferrous ion. In the presence of other chelating agents, the complex formation is disrupted with the reduced red coloration of the complexes formed. Therefore, measurement of the color reduction allows estimation of chelating activity of the coexisting chelator. In this assay, CAME interfered with the formation of ferrous and ferrozone complex, suggesting that it has chelating activity and captures ferrous ion before ferrozone. The results for ferrous ion chelating assay were dose dependent and revealed that CAME has an effective capacity for ion binding, suggesting that its action as an antioxidant may be related to its ion binding capacity. The data with IC₅₀ value is given in Table 3. For the measurement of reductive ability, we investigated the ferric ion-ferrous ion transformation in the presence of CAME in the ferric reducing antioxidant power (FRAP) assay. The reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity. Figure 3 shows the reductive capabilities of CAME as compared to that of ascorbic acid. However, the reducing power of ascorbic acid was relatively more pronounced than that of CAME.

Nitric oxide (NO) is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, cardiovascular dilation and blood pressure. Despite these possible beneficial effects of NO•+, its contribution to oxidative damage is increasingly becoming evident. This is due to the fact that NO• can react with superoxide to form the peroxynitrite anion, which is a potential strong oxidant that can decompose to produce •OH and NO₂•. NO• released from sodium nitroprusside (SNP) has a strong NO• character which can alter the structure and function of many cellular components (Awah et al., 2010). In this study, CAME in SNP solution decreased levels of nitrite, a stable oxidation product of NO• liberated from SNP in a dose dependent manner and the data along with IC₅₀

### Table 1. DPPH radical scavenging activity of CAME.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>IC₅₀ Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAME</td>
<td>56.82</td>
<td>30.2 ± 0.34</td>
<td>111.2</td>
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<tr>
<td></td>
<td>113.64</td>
<td>51.0 ± 0.02</td>
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<tr>
<td></td>
<td>227.28</td>
<td>73.7 ± 0.11</td>
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<tr>
<td></td>
<td>454.56</td>
<td>91.6 ± 0.27</td>
<td></td>
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<tr>
<td>L-Ascorbic acid</td>
<td>7.55</td>
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</table>

### Table 2. ABTS radical cation decolorization activity of CAME.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>IC₅₀ Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAME</td>
<td>7.45</td>
<td>30.43 ± 0.35</td>
<td>15.6</td>
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<td></td>
<td>14.9</td>
<td>44.19 ± 0.84</td>
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<td></td>
<td>29.8</td>
<td>67.31 ± 0.56</td>
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<tr>
<td></td>
<td>59.6</td>
<td>99.12 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>6.9</td>
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</table>

### Table 3. Ferric ion-chelating activity of CAME.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>IC₅₀ Value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAME</td>
<td>52.08</td>
<td>32.91 ± 0.23</td>
<td>150.2</td>
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<td></td>
<td>104.16</td>
<td>43.00 ± 0.18</td>
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<td></td>
<td>208.33</td>
<td>58.73 ± 0.62</td>
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<td></td>
<td>416.66</td>
<td>70.41 ± 0.33</td>
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<tr>
<td></td>
<td>833.33</td>
<td>77.36 ± 0.54</td>
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<tr>
<td>Citric acid</td>
<td>9764</td>
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</tbody>
</table>
Figure 3. Reducing power of CAME and L-ascorbic acid (50-200 µg/ml) by FRAP assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
<th>IC₅₀ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAME</td>
<td>156.25</td>
<td>21.63 ± 0.55</td>
<td>798.5</td>
</tr>
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<td></td>
<td>312.5</td>
<td>26.09 ± 0.27</td>
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<tr>
<td></td>
<td>625</td>
<td>43.81 ± 0.80</td>
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<tr>
<td></td>
<td>1250</td>
<td>71.26 ± 0.81</td>
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</tr>
<tr>
<td>L-ascorbic acid</td>
<td></td>
<td></td>
<td>220.0</td>
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</table>

The lipids in membrane are continuously subjected to oxidant challenges. Oxidant induced abstraction of a hydrogen atom from an unsaturated fatty acyl chain of membrane lipids initiates the process of LPO, which propagates as a chain reaction. In the process, cyclic peroxides, lipid peroxides and cyclic endoperoxides are generated which ultimately fragment into aldehydes like MDA. MDA forms a pink chromogen with TBA that absorbs at 535 nm (Kaur et al., 2006). Incubation with Fe²⁺ ascorbate system produced a noteworthy increase in MDA formation in rat liver microsomes (Table 5). CAME dose dependently inhibited the amount of MDA generated and thus lipid peroxidation in liver microsomes, consistent with Visavadiya et al. (2009) who studied the free radical scavenging and antiatherogenic activities of Sesamum indicum seed extracts in chemical and biological model systems (Visavadiya et al., 2009). In the present study, lipid peroxidation inhibition can be positively correlated with the high level of total phenolic content.

In summary, it could be concluded that CAME bear a potent antioxidant and α-glucosidase inhibitory activities. Its constituents exhibited different levels of antioxidant activities in the various models through scavenging free radicals, metal ion chelation, ferric reducing power and inhibiting lipid peroxidation. Preliminary chemical examination indicated the presence of polyphenols and flavonoids, which may be responsible for the antioxidant and antidiabetic (α-glucosidase inhibitory) activities. Further studies on the isolation of active constituent(s) along with the animal studies in vivo need to be investigated in detail to explore its pharmaceutical/neutraceutical use.

ACKNOWLEDGEMENT

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REFERENCES


