Journal of Food Composition and Analysis 24 (2011) 615–619

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Short Communication

Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etlingera elatior* Jack.) inflorescence

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ARTICLE INFO

Article history:
Received 11 December 2009
Received in revised form 9 September 2010
Accepted 9 September 2010
Available online 7 December 2010

Keywords:
Antioxidants
Anthocyanins
Bioactive compounds
Bunga kantan
Phenolic compounds
Flavonoids
Tannins
Torch ginger
Food analysis
Food composition

ABSTRACT

In the present study, we investigated the effect of methanol, acetone (50%, 90% and 100%, v/v) and distilled water on the extractability of some of the antioxidant compounds (total phenols, tannins, flavonoids, anthocyanins) of bunga kantan inflorescence (*Etlingera elatior* Jack.). The antioxidant activity of each individual extract was also evaluated through DPPH (2,2-diphenyl-1-pic-rylhydrazyl) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay. Of all the solvents employed, 50% acetone extract showed highest amount of total phenols (687.0 mg GAE/100 g) and total flavonoids (1431 mg QE/100 g), while 50% methanol extract showed maximum (5.9 mg c-3-gE/100 g) recovery for anthocyanins. Tannin extractability was found to be highest with 100% methanol (467.8 mg CE/100 g). The results obtained suggest the use of bunga kantan inflorescence as a potential source of natural antioxidants for food and nutraceutical applications.

1. Introduction

During the normal metabolic process in aerobic cells, free radicals are generated that readily react with the lipids, nucleic acids, proteins, sugars and sterols (Lee et al., 2004), which might lead to the development of neurodegenerative diseases. To prevent the onset of these diseases, high amount of antioxidants need to be present in the body. The most applicable and healthy way to improve the antioxidant levels in the body is by consuming various naturally available food resources. Hence studies about commercial exploration and utilization of plant source as antioxidants are of increasing interest (Rice-Evans et al., 1997; Alothman et al., 2009).

Etlingera elatior (Jack.), sometimes called "Torch ginger", is a plant that belongs to Zingiberaceae family in which the ginger plant species are categorized. This plant is popularly known in Malaysia as "bunga kantan" (Jaafar et al., 2007), and is commonly found in the South Asian countries. This plant is considered to be a native of Sumatra, Indonesia and is referred to as "kecombrang" or as "kincung". Torch ginger plant has extensive traditional uses: the young shoots, flower buds or fruits are consumed by indigenous communities raw, as a condiment, or cooked. The inflorescences of this plant are very popular as a spice for food flavouring; because of them, the plant is also used as a garden ornamental. In some traditional foods of Malaysia (like asam laksa, nasi kerabu and nasi ulam) the inflorescence is a key ingredient (Chan et al., 2007). Earlier studies have reported (Mohamad et al., 2005; Abas et al., 2006; Chan et al., 2009) on the analysis of antioxidant activities of leaves and rhizomes of this plant. However, to our knowledge no detailed studies on the antioxidant activities of the inflorescence have been reported.

Generally, for the extraction of polyphenols or other bioactive compounds from plant materials, water and organic solvents (ethanol, methanol, acetone, diethyl ether) are used. Additionally, during the extraction process, the percent recovery depends mainly on the type of solvent and the extraction methods being adapted (Sun and Ho, 2005; Turkmen et al., 2006; Hayouni et al., 2007). Depending on the plant materials, the nature of the bioactive compound present also varies. Hence, in general, it is very difficult to recommend a suitable extracting solvent for individual plant materials. The main objective of the present study was to evaluate the effect of various solvents (methanol, acetone and water) on the extractability of antioxidant compounds such as total phenols, total flavonoids, tannins, anthocyanin and antioxidant activity (as percent DPPH inhibition activity and FRAP assay) of the inflorescence of bunga kantan. We therefore hope to provide

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doi:10.1016/j.jfca.2010.09.018

a suitable base for commercial exploitation as a source of natural antioxidant for food and nutraceutical applications.

2. Materials and methods

2.1. Standards and reagents

Folin's reagent (Folin-Ciocalteu reagent), gallic acid, quercetin, catechin, vanillin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich®, USA. Acetone, aluminium chloride, ferrous sulphate, ferric chloride, methanol, potassium chloride, sodium acetate, sodium carbonate and sodium hydroxide were purchased from R & M chemicals (Essex, UK). All the other chemicals used in the present study were of analytical grade quality.

2.2. Sample preparation

Inflorescences of bunga kantan (Fig. 1) were purchased from the local wet market in Pulau Penang, Malaysia. Fresh, unopened inflorescences selected were of equal maturity and uniform colour with no apparent physical defects. Soon after the collection, the samples were brought to the laboratory and surfaced cleaned with distilled water (to remove adherent dust particles). Further, the samples were cut into uniformly sized small pieces (approximately 0.5 cm × 0.5 cm) and were subjected to freeze drying for 48 h. (Model 7754511, Labconco Corporation, Kansas City, Missouri, USA). On completion of freeze drying, the samples were ground to a fine powder using a commercial kitchen blender (Model BL 335, Kenwood, Selangor, Malaysia) and stored at 4 °C in amber coloured glass bottles, covered with aluminium foil (to prevent direct exposure to light) until further analysis.

2.3. Solvent extraction

One gram of accurately weighed freeze dried sample powder was mixed with 40 ml of the desired solvent and extracted using the magnetic stirrer and hotplate at 1200 rpm (Model FSMQ 143551, Fisher Scientific, Kuala Lumpur, Malaysia) for one hour at room temperature (25 ± 1 °C). Extracts were then filtered under suction by using Whatman No. 1 filter paper. The remaining residue on the filter paper was transferred back into the same flask and re-extracted for two more times following the same procedure until the residue became colourless. Filtrates collected from all the three successive extractions were pooled and collected in reagent bottle covered with aluminium foil (to avoid light exposure). For the extraction, distilled water and different concentrations of methanol and acetone (50%, 90% and 100%, v/v) were used. Each of the extraction was carried out in replicates (n = 3).

Fig. 1. Inflorescence of *Etlingera elatior*.

2.4. Determination of total phenolic content

Determination of total phenols in the extract was done using Folin-Ciocalteu (FC) assay as described by Singleton and Rossi (1965) with slight modifications. Properly diluted (until the absorbance unit measured in the spectrophotometer was <1.2) extract of 450 μl was added to 2.25 ml of FC reagent (ten fold diluted with distilled water) and 1.8 ml of sodium carbonate (7.5%, w/v). Further, the contents were mixed and allowed to stand for 30 min at room temperature (25 ± 1 °C). The absorbance was measured at 765 nm using UV–vis spectrophotometer (Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan). Total phenol content was expressed as mg gallic acid equivalent (GAE), per 100 g wet weight.

2.5. Determination of total flavonoid content

Total flavonoid content was determined using the aluminium trichloride method as described by Liu et al. (2008). Briefly, 500 μl of the extract solution was added to a test tube with 2.5 ml of distilled water. Sodium nitrite solution (5%, w/v, 150 μl) was added to the mixture and maintained for 5 min. After that, 300 μl of aluminium chloride (10%, w/v) was added. After 6 min of incubation, 1 ml of 1 M sodium hydroxide (NaOH) was added. Then, the mixture was diluted with 550 μl of distilled water, and shaken vigorously. The absorbance of the mixture was measured at 510 nm (UV–vis spectrophotometer, Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan) immediately, and the total flavonoid content was expressed as mg quercetin equivalent (QE)/100 g sample.

2.6. Determination of total anthocyanin content

The spectrophotometric pH differential method as described by Giusti and Wrolstad (2001) was used to determine the total anthocyanin content in the extracts of the inflorescence. In brief, 0.5 ml of the extract was mixed thoroughly with 3.5 ml of 0.025 M potassium chloride buffer (at pH 1). The mixture was allowed to stand for 15 min and the absorbance was measured at 510 and 700 nm (UV–vis spectrophotometer, Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan) against a blank of distilled water. Following the same procedure, extract was then added to 0.025 M sodium acetate buffer at pH 4.5, and the absorbance was again measured at 510 nm and 700 nm after 15 min. The total anthocyanin content was calculated using the following equation:

$$\text{Total anthocyanin content (mg/l)} = \frac{A \times MW \times DF \times 1{,}000}{\varepsilon \times C}$$

where A is absorbance of the extract calculated as:

$$A = (A_{515} - A_{700})_{\text{pH 1.0}} - (A_{515} - A_{700})_{\text{pH 4.5}}$$

MW is the molecular weight for cyanidin-3-glucoside = 449.2; DF is the dilution factor of the samples, ε is the molar absorptivity of cyanidin-3-glucoside = 26,900; and C is the concentration of the buffer in mg/ml. Results were expressed as mg of cyanidin-3-glucoside equivalents (c-3-gE) for 100 g of sample.

2.7. Determination of tannin content

For the determination of tannins in the sample extracts, vanillin–HCl method described by Broadhurst and Jones (1978) was adapted. Briefly, 0.5 ml of the extract was added to 3 ml of vanillin reagent (4%, w/v, vanillin in methanol) and mixed thoroughly. To this mixture 1.5 ml concentrated hydrochloric acid was added and vortex mixed. Content was kept in the dark for