LWT - Food Science and Technology 56 (2014) 181–186

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Antioxidant activity of phenolics–saponins rich fraction prepared from defatted kenaf seed meal

Kim Wei Chan [a,*], Shahid Iqbal [b], Nicholas M.H. Khong [a], Der-Jiun Ooi [a], Maznah Ismail [a]

[a] Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Chemistry, University of Sargodha, Sargodha 40100, Pakistan

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form
16 October 2013
Accepted 19 October 2013

Keywords:
Defatted kenaf seed meal
Hibiscus cannabinus L.
Phenolic compounds
Saponins
Antioxidant activity

ABSTRACT

The current study is aimed to determine the antioxidant properties of crude ethanolic extract (CEE) of defatted kenaf seed meal (DKSM) and its derived n-butanol (BF) and aqueous (AqF) fractions. Spectrophotometric assays showed that BF contained the highest amount of phenolic compounds and saponins, followed by CEE and AqF ($p < 0.05$). Similarly, HPLC-DAD analysis revealed that level of all the detected predominant phenolic compounds was significantly higher in BF ($p < 0.05$). Through multiple antioxidant assays, BF exhibited higher antioxidant activity than CEE and AqF, except for iron chelating activity ($p < 0.05$). Antioxidant activity of CEE and fractions were strongly correlated to their phenolic and saponin contents. This study showed that phenolic compounds and saponins could be extracted and partially purified simultaneously from DKSM by employing a simple alcoholic extraction–fractionation procedure. High antioxidative phenolics–saponins rich fraction from DKSM is a potential active ingredient that could be applied in nutraceuticals, functional foods as well as natural food preservatives.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) is a biennial and short-day cordage crop, a member of Malvaceae family, which is closely related to cotton (*Gossypium hirsutum* L.) and okra (*Abelmoschus esculentus* L.). Stem fiber of kenaf tree is reported to be possessing characteristics similar to jute fiber and have been widely applied in the production of non-wood paper, rope, twine, sack cloth, fiber board, biocomposite materials, absorbents, building materials, and livestock feed (Webber III, Bhardwaj, & Bledsoe, 2002). Currently, kenaf is mainly cultivated in India, Bangladesh, United States, Indonesia, Malaysia, South Africa, Vietnam and Thailand.

Kenaf seed is an agricultural by-product of kenaf plantation, which yields up to 1000 kg/hectare, depending on the variety and agroclimatic conditions (Scott & Cook, 1995). Previous studies have shown that kenaf seed oil possesses substantially high antioxidant activity (Chan & Ismail, 2009). Besides, oil extracted from kenaf seeds by supercritical fluid extractor showed higher anticancer properties against cancerous cell lines and colon cancer-induced rats (Ghafar et al., 2013, Ghafar, Yazan, Tahir, & Ismail, 2010; Yazan et al., 2010). Large scale extraction of oil from kenaf seeds produces huge amount (80% of the seed) of secondary by-product, DKSM, which might still contain considerable amounts of health promoting bioactives such as phenolic compounds and saponins. Recently, Chan, Khong, Iqbal, Mansor, and Ismail (2013) suggested that DKSM could be potentially served as an alternative edible flour with high nutritive value and antioxidant activity. To-date, no value-added products have been manufactured from DKSM, rather it is usually discarded as waste. Within the aim of adding value to this kenaf seed by-product as a potential source of bioactive compounds, its composition in phenolic compounds and saponins was examined.

Phenolic compounds are secondary metabolites of plants that play an important role in determining the sensory quality and nutraceutical potential of a plant (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). Phenolic compounds are highly correlated with antioxidant activity of plant and thus can be used as the major determinants in evaluating antioxidant potential of foods (Chan, Iqbal, Khong, & Babji, 2011; Ismail, Chan, Mariod, & Ismail, 2010). Besides exhibiting a wide range of physiological properties like cardioprotective, anti-cancer, and neuroprotective activities; phenolic compounds are effective natural food preservatives against oxidative deterioration and microbial contamination (Crozier, Jaganath, & Clifford, 2009; Freeman, Eggett, & Parker, 2010). Saponins are glycosides which consist of a steroidal or terpenoid aglycone connected to one or more sugar chains. This

Abbreviations: AqF, aqueous fraction; BF, n-butanol fraction; CEE, crude ethanolic extract; DKSM, defatted kenaf seed meal.

* Corresponding author. Tel.: +60 389472145; fax: +60 389472116.
E-mail addresses: chankim@upm.edu.my, chankw_antioxidant@yahoo.com (K. W. Chan).

0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.10.028

unique structure enables saponins to exhibit multiple biological and food preservatory properties, such as antioxidant, antimicrobial, hypoglycemic, anti-inflammatory, cytotoxic and antitumor activities (Man, Gao, Zhang, Huang, & Liu, 2010). From the toxicological aspect, most saponins possess hemolytic properties and very toxic to most of the cold-blooded animals. However, their oral toxicity to mammals is considerable low (Sparg, Light, & van Staden, 2004).

Limited studies on phenolic compounds, saponins or antioxidant activity of DKSM have been reported so far. The study is mainly aimed to explore the potential of this underutilized agro-industrial waste as a source of natural food preservatives, nutraceuticals and functional food ingredients. In the present study, an ethanolic fractionation-based procedure has been developed and optimized for the simultaneous extraction of saponins and phenolics with high recovery from DKSM. Saponins and phenolic contents of DKSM extract and fractions have been determined by spectrophotometer and High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). Multiple assays based on different principles have been employed to evaluate the antioxidative properties of DKSM extract and its derived fractions.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of analytical reagent or HPLC grade and were given as: methanol, chloroform and Tween 20 (Fisher Scientific, Loughborough, Leicestershire, UK); linoleic acid, gallic acid, β-carotene (Type I synthetic, 95%), anisaldehyde, sodium bicarbonate, diosgenin, vanillin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium persulfate, ferrous sulfate, hydrogen peroxide, ferrous chloride, ethylenediaminetetra acetic acid (EDTA), vanillin, sulfuric acid, anisaldehyde, ethyl acetate (EA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol reagent and ferrozine (Sigma–Aldrich Co., St. Louis, MO, USA); n-hexane, n-butanol, absolute ethanol, sulfuric acid, ethyl acetate, acetonitrile, acetic acid and dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) and 5,5-dimethyl-*N*-oxide pyroline (DMPO) (Labotec Ltd., Tokyo, Japan).

2.2. Preparation of DKSM

Kenaf seeds (variety V 36) were purchased from Malaysian Kenaf and Tobacco Board, Pasir Puteh, Kelantan, Malaysia. Seeds were thoroughly washed with tap water for 10 min, rinsed twice with distilled water and air-dried in an electric oven (FD 115, Fisher Scientific, Loughborough, Leicestershire, UK) at 50 °C overnight. From the dried sample, 200 g seeds were pulverized and homogenized (Ultra-turax T25 basic, IKA®-WERKE GmbH & Co. KG, Staufen, Germany) at 9500 rpm with 400 mL of n-hexane for 15 min at room temperature. Subsequently, the mixture was filtered through Whatman filter paper No. 2 and the residue was re-extracted twice following the same procedure. Finally, the residue obtained (DKSM) was collected, dried in an oven at 50 °C for 3 h to remove any residual solvent and passed through a 30 mesh sieve followed by storage at −20 °C prior to extraction. Moisture and fat contents of DKSM were 9.34 ± 0.04 g/100 g sample and 0.73 ± 0.05 g/100 g sample, respectively.

2.3. Extraction and fractionation of DKSM

The extraction and fractionation of DKSM were performed according to the procedures described by Chan, Khong, Iqbal, and Ismail (2013). One hundred grams of DKSM were refluxed in aqueous ethanol (50 mL ethanol in 100 mL solution) at the ratio of 1:15 (g:mL) for 3 h to obtain crude ethanolic extract (CEE). The mixture was then was filtered through Whatman filter paper No. 2 and solvent was removed from the filtrate under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland) followed by lyophilization (Virtis Benchtop K Freeze Dryer, SP Industries, Warminster, PA, USA).

For fractionation, 1 g of CEE was dispersed in 25 mL of distilled water and partitioned with 125 mL of *n*-hexane to remove residual lipids from CEE. After the removal of *n*-hexane layer, aqueous layer was further partitioned with 125 mL of *n*-butanol. This partitioning procedure was repeated thrice and *n*-butanol fraction (BF) was pooled and concentrated to dryness under reduced pressure (Rotavapor R210, Buchi, Postfach, Flawil, Switzerland). The aqueous fraction (AqF) left was subjected to lyophilization (Virtis Benchtop K Freeze Dryer, SP Industries, Warminster, PA, USA). The extraction and fractionation procedures were conducted in triplicate. Yields of CEE, BF and AqF were measured prior to storage at −80 °C till further analyses.

2.4. Determination of total phenolic, saponin and steroidal saponin contents

Total phenolic content (TPC) of CEE, BF and AqF was individually determined by Folin–Ciocalteu reagent assay as described by Iqbal et al. (2012). In brief, 0.1 mL of tested extract/fractions (5 mg/mL) was serially reacted with 0.5 mL of 10 folds-diluted Folin–Ciocalteu reagent and 0.4 mL of 7.5 g/100 mL sodium bicarbonate solution. After incubation at 40 °C for 30 min, absorbance of reaction mixtures was recorded at 760 nm spectrophotometrically (Pharmaspec uv-1700, Shimadzu, Kyoto, Japan). Gallic acid was used as standard and TPC of tested extract/fractions was expressed as milligram gallic acid equivalents (mg GAE)/g extract or fraction.

Furthermore, total saponin (TSC) and steroidal saponin (TSSC) contents of CEE, BF and AqF were determined following the procedures described by Hiai, Oura, & Nakajima (1976) and Baccou, Lambert, and Sauvaire (1977), respectively. For the determination of TSC, 0.l mL of tested extract or fraction was sequentially reacted with 0.1 mL of vanillin solution (8 g/100 mL) and 1 mL of diluted sulfuric acid (72 mL sulfuric acid in 100 mL solution). After incubation at 60 °C for 10 min, the absorbance of reaction mixtures was recorded at 540 nm spectrophotometrically (Pharmaspec uv-1700, Shimadzu, Kyoto, Japan). In order to determine the TSSC of CEE, BF and AqF, 0.5 mL of tested extract or fraction was reacted with 0.25 mL of anisaldehyde solution (0.5 mL/100 mL, in EA) and 0.25 mL of diluted sulfuric acid (50 mL/100 mL, in EA) consecutively. Then, the reaction mixtures were left at room temperature for 30 min before measurement of their absorbance at 430 nm spectrophotometrically (Pharmaspec uv-1700, Shimadzu, Kyoto, Japan). Diosgenin was used as the standard in both assays. TSC and TSSC of all the tested extract and fractions were expressed as milligram diosgenin equivalents (mg DE)/g extract or fraction.

2.5. HPLC-DAD analysis of major phenolic compounds

HPLC-DAD analysis was performed to identify and quantify major phenolic compounds in CEE, BF and AqF. Sample was injected using an Agilent G1310A auto-sampler into an Agilent 1200 series HPLC (Agilent, Stevens Creek Blvd Santa Clara, USA) system equipped with DAD 1300 diode array detector. Chromatographic separations were performed on an Agilent Zorbax SB C-18 column (5 μm, 150 × 4.6 mm). The solvent system used was a gradient of water–acetic acid (94:6, mL/mL) (A) and methanol–acetonitrile–acetic acid (95:5:1, mL/mL/mL) (B) as: 0–5 mL B in 100 mL mobile

Download English Version:

https://daneshyari.com/en/article/4563895

Download Persian Version:

https://daneshyari.com/article/4563895

Daneshyari.com