Food Chemistry 181 (2015) 248–255

Contents lists available at ScienceDirect

Food Chemistry

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

Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from Northeastern Thailand

Sujitar Jorjong, Luchai Butkhup, Supachai Samappito *

Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand

ARTICLE INFO

Article history:
Received 21 May 2014
Received in revised form 6 January 2015
Accepted 18 February 2015
Available online 25 February 2015

Keywords:
Phenolic compound
Cultivars
Antidesma bunius
Antioxidant activity

ABSTRACT

Phenolic compounds extracted from fourteen Mao-Luang (*Antidesma bunius*) cultivars grown in Northeastern Thailand were quantified using HPLC analysis and the antioxidant capacity of the extracts was determined by $ABTS^{\cdot+}$, DPPH radical, and FRAP assays. This study showed that there were differences ($p < 0.05$) in phenolic compounds and antioxidant activities among Mao-Luang cultivars. Gallic acid, (−)-epicatechin, (+)-catechin, and cyanidin-3-*O*-glucoside were the major polyphenolic components in all Mao-Luang cultivars. The highest contents were found in 'Kumlai' with values of 281.30, 949.73, 127.60 and 54.67 mg/100 g DW, respectively. 'Kumlai' also exhibited the highest antioxidant activity with the values of 103.04 mmol VCEAC/g DW (DPPH assay), 35.35 mmol Fe(II)/g DW (FRAP assay), and 46.37 mmol TE/g DW ($ABTS^{\cdot+}$ assay). Correlation analyses revealed that gallic acid, ferulic acid and some anthocyanins, mainly cyanidin-3-*O*-glucoside, were responsible for the antioxidant capacity of Mao-Luang fruits.

1. Introduction

Antidesma bunius (Linn) Spreng is one of the tropical fruits, a wild plant in the Euphorbiaceae family. It is more popularly known in Thailand as "Mao-Luang". It is widely grown in Northeastern Thailand. The entire plant is of medicinal value acting as antioxidant (Belina-Aldemita, Sabularse, Dizon, Hurtada, & Torio, 2013), anticancer (Pan et al., 2011) and antidiabetic (Lawag Ivan, Aguinaldo Alicia, Naheed, & Mosihuzzaman, 2012). The fruit is edible and nutrient-rich and is prepared either as a healthy juice drink, juice concentrate, and excellent red wine. Phytochemical analysis of the different species of *A. bunius* has confirmed the presence of varying amounts of phenolic acids, flavonoids and anthocyanins (Lim, 2012). This finding confirms our previously reported study of different cultivars of *A. bunius* grown in Northeastern Thailand showing that all cultivars contain three major flavonoids namely catechin, procyanidin B1 and procyanidin B2 (Butkhup & Samappito, 2008). Flavonoids and phenolic acids are widely distributed bioactive compounds found in plant foods and can be grouped in several structural classes including anthocyanins, flavones, flavan-3-ols, flavanones, flavonols, and tannins. Flavonols (kaempferol, quercetin and myricetin) and phenolic acids (*p*-coumaric, caffeic, ferulic and ellagic acids) and anthocyanins (pelargonidin, cyanindin, peonidin, petunidin, malvidin, and delphinidin) have been demonstrated to have beneficial effects on human health as antioxidant (Koponen, Happonen, Mattila, & Törrönen, 2007; Rodrigues et al., 2011), antibacterial, anti-inflammatory and anticarcinogenic agents (Nohynek et al., 2006; Seeram, 2008).

It has been demonstrated that a wide range of phytochemical levels and antioxidant activities exist within and across genera of small fruits (Cordenunsi, Oliveira do Nascimento, Genoves, & Lajolo, 2002). Furthermore, accumulating evidence suggests that plant cultivar may have a profound influence on the content of bioactive compounds in berries (Primetta et al., 2013; Rodrigues et al., 2011). The Phu Phan National Park, which is located in Northeastern Thailand, is well-known for its diverse natural resources, in particular the biodiversity of Mao-Luang cultivar. The cultivars of Mao-Luang grown in mountainous areas and cultivated by local people around the Phu Phan mountain represent a wide genetic diversity. The Mao-Luang germplasm collection of the Rajamangala University of Technology Isan, Thailand, provided documentation, evaluation and utilization of Mao-Luang germplasm resource in Northeastern Thailand.

The selection of Mao-Luang cultivars is important for processing of red wine as novel quality functional drink rich in phenolic antioxidants. Differences in anthocyanin, flavonoids and phenolic acids from tropical Mao-Luang fruits harvested from different geographical regions and locations were documented (Butkhup &

* Corresponding author. Tel./fax: +66 4375 4333.
E-mail address: samappito@msu.ac.th (S. Samappito).

http://dx.doi.org/10.1016/j.foodchem.2015.02.093
0308-8146/

Samappito, 2008), except for some cultivars and locations. Furthermore, cultivars can also affect wine composition and not all may be useful for optimal fermentation purposes. However, there are insufficient information on the polyphenolic composition of these Mao-Luang cultivars and their antioxidant capacity. The correlation between their polyphenolic content and antioxidant capacity has not yet been examined. Consequently, the purpose of this work was to select the best Mao-Luang cultivar rich in phytochemicals including anthocyanin, phenolic acids and flavonoids for the production of red wine with enhanced health-promoting properties. *In vitro* biological activities in terms of antioxidant capacity of each extract were also investigated.

2. Materials and methods

2.1. Chemicals

2,2′-Diphenyl-1-picryl hydrazyl hydrate (DPPH) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox) were obtained from Fluka (Buchs, Switzerland). Folin–Ciocalteu phenol reagent and TPTZ (2,4,6-tripyridyl-*s*-triazine) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Methanol, acetonitrile and phosphoric acid were of HPLC grade from Merck (Darmstadt, Germany). Deionized water was prepared by a Milli-Q Water Purification system (Millipore, MA, USA). Phenolic standards [(+)-catechin, (−)-epicatechin, rutin, *tran*-resveratrol, myricetin, quercetin, naringenin, kaempferol, gallic acid vanillic acid, chlorogenic acid, caffeic acid, ferulic acid, sinapinic acid, cinnamic acid, pelargonidin, cyanindin, cyanidin-3-*O*-glucoside, cyanidin 3-rutinoside, malvin, malvidin 3,5-diglucoside, and delphinidin] were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Sample preparation

Fourteen cultivars of Mao-Luang fruits harvested from the Mao-Luang germplasm collection of the Rajamangala University of Technology Isan, Northeastern Thailand namely 'Phuphanthong', 'Lungplain', 'Kumlai', 'Thepnimit', 'Sorwannarsung', 'Sang krow No. 1', 'Sang krow No. 2', 'Huoibang', 'Nongwai', 'No. 120', 'Faprathan', 'Lungkumlar', 'Phuchong', and 'Yaikumtar' were studied. Fruits were selected at early morning based on the uniformity of the color and stored at −4 °C immediately after collection. Fruit samples (50 g) of each Mao-Luang cultivars were lyophilized (Heto power dry PL 3000 Thermo Electron Corporation, Czech Republic) at −40 °C. After drying the samples were ground and stored at −4 °C until analyzed.

Extraction of the polyphenol was as described by Oszmiański, Wojdylo, and Kolniak (2009) with some modifications. The precisely weighed (1 g) amount of freeze-dried Mao-Luang samples was extracted with 10 ml of methanol in a sonicator bath (KuDos Model 621 OPH, China). Sonication was carried out at ambient temperature for 20 min. The mixture was centrifuged at 19,000×*g* for 10 min, solvent was decanted, and the residue was re-extracted with a fresh 10 ml solvent. The two extracts were combined, and the volume was adjusted to 20 ml with the same solvent. Appropriate aliquots of extracts were filtered through a 0.45 μm membrane filter and stored at −18 °C for future analysis.

2.3. HPLC analysis

HPLC–DAD system (Shimadzu, Japan), comprising of Shimadzu LC-20AC pumps, a SPD-M20A diode array detector and a Apollo C-18 column (Alltech Associates, Deerfield, IL, USA) (4.6 mm × 250 mm, 5 μm) protected with guard column Inertsil ODS-3 (4.0 mm × 10 mm, 5 μm; GL Science Inc., Tokyo, Japan) were used for the analysis of flavonoids, phenolic acids and anthocyanins. The mobile phase for flavonoids used acetonitrile/deionized water (2/97.8, v/v) containing 0.2% phosphoric acid (solvent A) and acetonitrile/deionized water (97.8/2, v/v) containing 0.2% phosphoric acid (solvent B) at a flow rate of 0.6 ml/min and 40 °C column temperature. The UV–Vis spectra were detected at 254 nm. Gradient elution started with 20% solvent B, 50% solvent B at 30 min, 60% solvent B at 35 min, 20% solvent B at 40 min at isocratic elution until 55 min (Butkhup & Samappito, 2008). The mobile phase for phenolic acids consisted of acetonitrile (solvent A) and phosphoric acid in deionized water pH 2.58 (solvent B) at a flow rate of 0.8 ml/min and 40 °C column temperature. The following gradient was used: 0–15 min, 9% B, 15–22 min, from 9% B to 11% B, 22–38 min, from 110% B to 18% B, 38–43 min, 18% B to 23% B, 43–45 min, from 23% B to 30% B, 45–46 min, from 30% B to 80% B, 46–55 min, 80% B, 55–60 min, from 80% B to 5% B. The UV–Vis spectra were detected at 280 nm for hydroxybenzoic acids and 320 nm for hydroxycinnamic acids (Kubola, Siriamornpun, & Meeso, 2011). The mobile phase for anthocyanins used phosphoric acid 4% in deionized water (solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min and 40 °C column temperature. The UV–Vis spectra were detected at 520 nm. The linear gradient started with 94% solvent B at 0 min, 75% solvent B at 55 min, 75% solvent B at 65 min at isocratic elution until 70 min (Wrolstad, Decker, Penner, Reid, & Schwartz, 2005, Chapter F. pigments and colorants). Quantification was carried out by comparing the retention times and the spectra as well as by the addition of standards. The concentrations of phenolic compounds were calculated using a corresponding external standard.

2.4. Determination of total phenolic content (TPC)

The determination of TPC was carried out using a previously reported procedure after some modification (Singleton & Rossi, 1965). The absorbance of the samples was determined at 760 nm using a microplate reader spectrophotometer (Synergy HT, BiotTek instruments, USA). The equation obtained for the calibration curve of gallic acid (0.5–100 mg/l) was $Y = 0.006X + 0.111$ ($r = 0.9967$). The results were expressed as milligram gallic acid per 100 g dry weight (mg GAE/100 g DW).

2.5. Determination of total flavonoid content (TFC)

The determination of TFC was performed using a colorimetric assay (Mahmood, Anwar, Abbas, & Saari, 2012) with some modifications. The absorbance of the samples was determined at 510 nm using a microplate reader spectrophotometer (Synergy HT, BiotTek instruments, USA). The equation obtained for the calibration curve of (+)-catechin (0.5–150 mg/l) was $Y = 0.005X + 0.093$ ($r = 0.9980$). The results were expressed as milligram (+)-catechin equivalent per 100 g dry weight (mg CE/100 g DW).

2.6. Determination of free radical scavenging capacity using DPPH method

The antioxidant activity of the extract was evaluated through the free radical scavenging effect on 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical as previously reported by Akowuah, Ismail, Norhayati, and Sadikun (2005). One hundred microliters of 0.2 mM DPPH methanolic solution was added to 100 μl of the sample extracts. The mixture was thoroughly mixed and kept in the dark for 1 h. The control was prepared by mixing 100 μl of DPPH and 100 μl of methanol. The absorbance was measured at 520 nm using a microplate reader spectrophotometer (Synergy HT, BiotTek instruments, USA). The equation obtained for the calibration curve of vitamin C (0–200 μm) was $Y = -0.001X + 635$