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Evaluation of antioxidant potential of *Artocarpus heterophyllus* L. J33 variety fruit waste from different extraction methods and identification of phenolic constituents by LCMS

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ABSTRACT

Artocarpus heterophyllus J33 (AhJ33) fruit is a popular and valuable jackfruit variety in Malaysia. For export, the pulp has to be separated from the skin which is usually discarded. Hence, the conversion of the fruit waste to food products with economic value needs to be explored utilizing the waste to wealth concept. This paper reports the evaluation of antioxidant potential of AhJ33 fruit waste (rind and rachis) extracts from three different extraction methods (maceration, percolation and Soxhlet). The antioxidant potential was assessed by DPPH radical scavenging, FRAP and β-carotene bleaching assays. The total phenolic and total flavonoid contents were estimated by TPC and the TFC assays. For both rind and rachis, the maceration technique yielded extracts with the strongest antioxidant activities which correlated with the highest TPC and TFC values. TOF LCMS analyses identified two phenolic acids as the major constituents responsible for the antioxidant activity of the active extracts.

1. Introduction

Artocarpus heterophyllus J33 (AhJ33) variety is the most economically important variety among Artocarpus species in Malaysia. In 2010 under the Economic Transformation Program, the government has come out with Entry Point Project 7 (NKEA) in which the agricultural sector focuses on improving the export capacity of fruits and vegetables to the premium level in order to increase local fruit exports. Until 2014, up to 5000 hectares of land has been planted with this *A. heterophyllus* variety. In 2014, the production of fruits was reported to be 33,788 tonnes with a productivity of 11.2 (tonne/hectare) generating a total income of about Ringgit Malaysia 107 million (Ministry of Agricultural and Agro-Based Industry Malaysia, 2015). The fruits from this species are exported widely throughout the world market mostly to Singapore, China, Macau, Hong Kong, Middle East and Europe. For export purpose, fruits usually undergo minimal processing to prolong the postharvest storage life and maintain freshness (Punan et al., 2000). For the export of AhJ33 variety, only the pulps (with the seed) are isolated while the rest of the fruit parts are usually discarded as waste. Hence, the aim of our study was to convert AhJ33 fruit waste to a food product with economic value based on the reported nutritional properties of the fruit (Baliga, Shivashankara, Haniadka, Dsouza, & Bhat, 2011).

Previous reports on *A. heterophyllus* found that extracts (including the pulp, leaf and root bark) possess moderate to high antioxidant activities. In general, the methanolic, ethanolic and aqueous crude extracts of *A. heterophyllus* showed more effective antioxidant activity compared to the acetone extract (Baliga et al., 2011; Biworo, Tanjung, Iskandar, & Suhartono, 2015). For the pulp, the major chemical constituents which contributed to the antioxidant activity in the methanol extract were reported to be β-carotenes (Biworo et al., 2015). From the leaf, root and bark methanol extracts, flavonoids have been reported to contribute significantly to the antioxidant activities which were evaluated by the DPPH assay (Ko, Cheng, Lin, & Teng, 1998). A preliminary phytochemical study on *A. heterophyllus* has been reported for the skin of the fruit

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(Sharma, Gupta, & Verma, 2015). However, to date, there has been no specific reports on the antioxidant activities of the rind and rachis extracts.

The extraction process is extremely important as the first step in the treatment of plant materials for further phytomedicinal screening and phytochemical investigations. The basic process for extraction included steps such as pre-washing, drying of plant materials, grinding and other steps to the kinetics of extraction and increase the contact of sample surface with the solvents (Sasidharan, Chen, Saravanan, Sundram, & Yoga Latha, 2011). Solid-liquid extraction known as leaching or lixiviation has been widely used for recovering secondary metabolite from plants using solvents (Luque de Castro & Priego-Capote, 2010). The extraction process usually involved thermal and non-thermal methods. For thermal extraction, the Soxhlet method has been accepted as a standard technique and remains as a primary reference in the development of modern thermal extraction methods such as microwave-assisted Soxhlet extraction and ultrasound-assisted Soxhlet extraction. Traditionally, the non-thermal extraction process has been carried out through maceration and percolation especially to extract non-volatile phytochemical (Sarker, Latif, & Gray, 2006). It has been reported that extraction methods may affect the phytochemical constituents of a plant (Pothitirat, Chomnawang, Supabphol, & Gritsanapan, 2010). Hence, for the first phase of this study, this paper reports the evaluation of antioxidant potential of AhJ33 fruit waste (rind and rachis) extracts from three different extraction methods (maceration, percolation and Soxhlet) evaluated by DPPH, FRAP and β-carotene assays. The phenolic and flavonoid contents estimated by the TPC and TFC assays were then correlated against these antioxidant activities. However, the interpretation of the values took into account the limitation of these assays, as reported by Pekal and Pyrzynska (2014). Finally, the major antioxidant constituents which may be responsible for the activities were identified using LCMS-TOF.

2. Materials and methods

2.1. General instrumentation

Solvents were evaporated using Buchi Rotavapor R210 at 45 °C. The UV–Vis was recorded on ELISA spectrophotometer Spectramax Plus (Molecular Devices). Mass of compounds was obtained from mass analyzer 6224 TOF LC/MS Agilent Technologies consisting of an electrospray (ESI) source system run in negative mode.

2.2. Plant material

Non-seasoning type of Malaysian *Artocarpus heterophyllus* fruit (AhJ33) for antioxidant and LCMS analysis was obtained from commercial source at Taman Kekal Pengeluaran Makanan Lancang Pahang, Pahang, Peninsular Malaysia. The rachis and rind of the fruits were separated, cut into small pieces (2.0 mm) and oven-dried at 40 °C for 48 h.

2.3. Extraction process

The extraction approach was carried out using maceration, percolation and Soxhlet techniques. A mixture of ethanol and water was used as the extracting solvent since numerous studies have reported on its efficiency in extracting phenolic and polyphenolic constituents (Laghari, Memon, Nelofar, & Laghari, 2011; Vongsak et al., 2013). Ethanol-water mixtures are suited to penetrate the hydrophobic areas of the vegetable matrix and help to precipitate soluble proteins, facilitating further processing. The extraction approach adopted in this study employs the method of Vongsak et al. (2013) with some minor modifications.

2.3.1. Maceration

The sample (1.0 kg) was macerated with 2 L 70% ethanol for 72 h at room temperature (25 °C) with occasional shaking. The extract was filtered through a Whatman No. 1 filter paper and the marc was re-extracted with the same amount of solvent. The solvent was evaporated off to yield the crude rind maceration extract (RDM) and crude rachis maceration extract (RCM).

2.3.2. Percolation

Solvent (70% ethanol) was added to the sample (1.0 kg) and the mixture was allowed to stand for 1 h. The mixture was then transferred gradually (400 ml volume at a time until 4 L) into a fabricated glass percolator (40 cm × 6 cm) set at a flow rate of 1 ml/min at room temperature (25 °C) until the extraction completed. The extract obtained was filtered and the solvent was evaporated off to yield the crude rind percolation extract (RDP) and crude rachis percolation extract (RCP).

2.3.3. Soxhlet

The sample (1.0 kg) was placed into a thimble and extracted with 70% ethanol (4 L) in a Soxhlet apparatus at boiling temperature for 5 h. The extract obtained was filtered through a Whatman No. 1 filter paper. The solvent was evaporated off to yield the crude rind Soxhlet extract (RDS) and crude rachis Soxhlet extracts (RCS).

2.4. Determination of scavenging activities

The chemical assay was based on method described by Ahmad et al. (2005). The antioxidant potential of the crude extract of AhJ33 fruit waste was assessed on the basis of their scavenging activity on the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. All test samples were prepared by dissolving 1.0 mg of samples into 1.0 ml 70% ethanol. A solution of DPPH was prepared by dissolving 5.0 mg DPPH in 2.0 ml of methanol and the solution kept in the dark at room temperature. Different concentrations of the test samples were prepared in 96-well microtitre plates. Five μl of methanolic DPPH solution was added to each well. The plate was shaken to ensure thorough mixing before being placed in the dark and wrapped with aluminum foil. After 30 min, the absorbance of the solution was analyzed using an ELISA reader at a wavelength of 517 nm. Percentage inhibition by sample treatment was determined by comparison with 70% ethanol treated control group. All test analyses were run in triplicates and the readings were averaged. Quercetin (Sigma, USA) was used as positive control. In the DPPH method, the antioxidant activity is described by percent inhibition;

$$\text{Percent inhibition} = [(\text{Abs control} - \text{Abs sample})/\text{Abs control})]100$$

2.5. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried according to method of Dian, Noriham, Nooraain, and Azizah (2015). The FRAP reagent was freshly prepared by mixing 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM 2-4-6-tripyridyl-s-triazine (TPTZ) was made to 40 mM hydrochloride at a ratio of 10:1:1. All test samples were prepared by dissolving 1.0 mg of sample into 1.0 ml 70% ethanol. Briefly, 0.1 ml sample/standard was mixed with 3 ml FRAP reagent and 3 ml distilled water. The mixture was incubated in the dark at 37 °C for 8 min and the absorbance at 595 nm was then read using UV–Vis spectrophotometer.