Concern looks into underutilised tropical fruit: Quantification and identification of phenolic compounds in methanol extracts of *artocarpusaltilis.*

Tara K. Jalal*

Department of Biomedical Science, International Islamic University, Kuantan, Pahang, Malaysia

Abstract

Diet richin fruits and vegetables is highly important in the maintenance of best possible general health. *Artoarpusaltilisconsider* an underutilizedfruit which is rich in phenolic compounds. Preliminary studies revealed that high quantities of phenoliccompounds actually exist in A. altilisfruit extracts. However,so far, the specific phenolic constituents in *A. altilisfruit* have not been wellidentified or studied. Therefore,the objective of this study was to identify and quantify some phenolic compounds in the methanol extracts of *A. altiliswhole* fruit, peel and pulp. By using the Ultra-High-Performance Liquid Chromatography-tandem mass spectrometry (UHPLC/MSMS) based approach, a total of 9 compounds were detected and characterized on the basis of their chromatographic retention time, UV-vis spectra and mass spectra in the negative-ion mode and data from the literature.The identified phenolic compounds were classified into flavonoids and phenolic acids. Two of the compounds detected were identified as flavonoids (quercetin and rutin), seven as phenolic acids such as, p-coumaric acid, ferulic acid, gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, sinapic acid and ascorbic acid. The hypothesis of the present study was asfollows: *A. altilisfruit* extracts contain phenolic compounds that could addcommercial value to this fruit.

Keywords: *Artocarpusaltilis*, Phytochemicals, Identification, Quantification.

Accepted on 02 August, 2021

Introduction

The world is becoming increasingly dependent on application of ethno-medicinal knowledge for curingvarious diseases. Application of traditional medicine as source of potentially useful compounds. Yet, many underutilized fruits that are yet to be fully recognized have the potential to play a much more vital role in contributing to food security, dietary, nutrition, health and income generation. The search for natural antioxidants, especially of plantorigin, has notably increased in recent years. In view of this, various plant fruits and vegetablesare under investigation for the detection of these bioactive compounds. Artocarpus species are used traditionally in numerous ailments. *Artocarpusaltilis* (Family-Moraceae) commonly known as breadfruit is originated from New Guinea grows extensively in the Southern parts of India. Breadfruit (*Artocarpusaltilis*) is a multipurpose agroforestry tree crop which is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus. Several reported are anti-inflammatory, antioxidant, antifungal, immuno-modulatory effect, antidiabetic effect, antibacterial effect, anti-cholinergic, chelating activity, nutritional assessment, cosmetic agent, toxicity to cancer cell, anthelmintic effect, protease inhibitors, regulation of oestrogens and inhibition of melanin biosynthesis. The varied uses of breadfruit include food, medicine, clothing and animal feed. The main objective of the present study is to identify and quantify the phytochemicals in *A. altilis* crude fruit extracts.

Materials and Methods

Sample extraction

The parts of *A. altilis* fruits were prepared for extraction by washing off all dirt and soil residues then, the dried pulp, peel and whole fruit of *A. altilis* were ground individually to a fine powder, and then stored in a cold room at 4°C until further analyses. A 250 g of pulp, peel and whole fruitwere extracted by filling the absorbent cellulose thimble and placing it in the thimble chamber of the Soxhlet apparatus. Then the solvents were removed by rotary evaporation at 60°C in vacuo.

Identification and quantification of phenolic compounds using UHPLC-MS/MS

Thetest was carriedonby advanced chemistry services. The analyses of phytochemicals components of *A. altilis* of MP, ML and MW extracts were performed on an AB Sciex 3200QTrap liquid chromatography-tandem mass spectrometry (LCMS/MS) coupled to Ultra-High-Performance Liquid Chromatography (UHPLC) system (Massachusetts, USA), operated by AB Sciex analyst software. A 0.22 μm pore size membrane filter wasusedtofilter the samples before injection. The injection volume was 20 μL.The mobile phasecontained of two solvent groups: Solvent group A, water with 0.1% formic acid and 5 mM ammonium formate and Solvent group B, acetonitrile with 0.1% formic acid and 5 mM ammonium formate. Phenolic compounds were eluted under the following conditions: interchanging 800 μl/minute to 1 mL/minute flow rate and the

temperature was set at 40°C, rapid screening at 15 minutes run time, gradient conditions from 10% B to 90% B from 0.01 minute to 8 minutes, held for 2 minutes and back to 10% B in 0.1 minute and re-equilibrated for 5 minutes. Ionization was performed in negative mode, the full mass spectrum ranges from m/z 100-1200 and mass fragmentation spectrum from m/z 50-1200. The identification of phenolic compounds in the fruit extracts was achieved by using the full mass spectrum and its unique mass fragmentation spectrum (which is a unique fingerprint for a compound) and searched against the mass spectrum fragmentation library. For further confirmation and identification, the standards were used as references by matching the retention times of the extract. The external standard method was used for the quantification of the individual phenolic compounds. Studies of 9 phenolic compounds such as quercetin, rutin, ascorbic acid, p-coumaric acid, ferulic acid, gallic acid, 4-hydroxybenzoic acid, protocatechuic acid and sinapic acid, were calculated with the regression equations from the standard curves. The standard calibration curve of each standard was used to quantify the number of phenolic compounds present in the crude extracts.

Results and Discussion

Identification of various phenolic compoundsin A. altilis of MP, ML and MW extracts using UHPLC-MS/MS

The chromatograms of nine compounds that were identified in MP, ML and MW of *A. altilis* extracts by UHPLC-LCMS/MS analysis at different retention times present in Figure 1. The UV-spectra and m/z channels used to identify the major compounds. The data of the retention times, UV-vis spectra, mass spectra and fragment ions in negative-ion modes) and the compounds identification were carried out by comparing them with mass spectral library, reference standards, as well as available literature to support the identification. The retention time is the time that takes the compound to elute on the column after it has been injected.

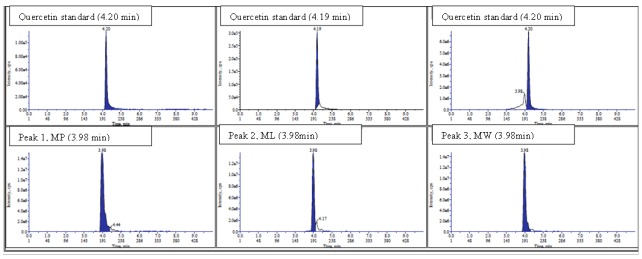

Figure 1. *The chromatogram of quercetin presents in MP, ML and MW of A. altilis methanol fruit extracts.*

A photodiode array detector (DAD) allowed the recording of the UV-vis spectrum of each peak of the chromatogram. The Mass Spectrometry detection (MS) optimized the identification and separation of many phenolic compounds through their mass spectra. Each compound has its unique mass spectra that can be compared with databases, literature or standards for identification. The standards of the phenolic compounds were used not only for verifying but also for supporting the method and quantitative analyses. In this present study, nine phenolic compounds were identified in different fruit parts (MP, ML, and MW). The identification of phenolic compounds was categorized into flavonoids and phenolic acids. The results of the current study showed the identification of two compounds classified as flavonoids and seven compounds as phenolic acids (Table 1).

Table 1. *Identification of phenolic compounds in A. altilis*

Compound identity	Standard RT (min)	UV (nm)	M-H$^-$ (m/z)	MS^2 (m/z)$^-$
Quercetin	4.2	360	301	151
Ferulic acid	3.55	232	193	178
4-Hydroxybenzoic acid	2.9	280	137	93
Sinapic acid	3.57	323	223	193
Protocatechuic acid	2.53	219	153	109
Rutin	3.38	255, 352	609	301
p-Coumaric acid	3.34	223, 309	163	119
Gallic acid	2.06	271	169	125
Ascorbic acid	1.31	265	175	87

Flavonoids

Flavonoids have attracted worldwide attention lately because of their broad range of valuable effects on human health. The best-characterized features of essentially every group of flavonoids are their ability to remove free radicals and inhibit other oxidation reactions. Compounds that belonging to various flavonoid groups in MP, ML and MW of *A. altilis* extracts were detected andanalysed. All the compounds eluted before 5 minutes. The peaks 1, 2 and 3 presented spectral characteristics of quercetin. Table 1 presents quercetin's spectral characteristics are UV−vis spectrum at 360 nm, M-H$^-$ at m/z 301 and fragment ion at m/z 151. And the standard chromatograph of quercet in is shown in Figure 1. Various phenolic compounds have been shown to exhibit anti proliferative and cytotoxic effects on numerous tumour cells and shown toxic effects that precisely target cancer cells rather than normal cells. Quercetin is known to have a very powerful antioxidant, chemo-preventive and chemotherapeutic (anticancer) potential which is the most abundant in dietary flavanols. It is found in abundance in fruits, vegetables, beverages and berries. The second compound that identified asrutin presents in Table 1 based on its M-H$^-$ at m/z 609 and fragment ion at m/z 301.

Phenolic acids

The most Phenolic acids that identified in *A. altilis* extracts were(p-coumaric acid, ferulic acid, gallic acid, 4-

hydroxybenzoic acid, protocatechuic acid, sinapic acid and ascorbic acid).Figure 1 (b) showed peaks 4, 5 and 6 in with M-H$^-$ at m/z 193 and fragment ion at m/z 178 (Table 1) were identified as ferulic acid according to the mass spectral library and reference standard. Furthermore, the mass spectrum peaks in agreement with study reported mentioned that ferulic acid-rich dates has been shown to have antioxidant, anti-microbial, anti-inflammatory,hepatoprotective, neuroprotective, anticarcinogenic, anti-diabetic and anti-cholesterolemic properties.

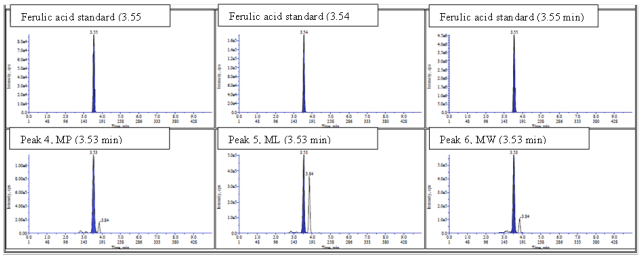

Figure 2. *The chromatogram of ferulic acid presents in MP, ML, and MW of A. altilis methanol fruit extracts.*

Spectrum 280 nm clearly identified it as 4-hydroxybenzoic acid. This is based on a similar compound reported in Taraxacum Formosa num, a Chinese medicinal herb grown in Taiwan. The retention time and fragment ion (m/z 93) were also identical of 4-hydroxybenzoic acid standard. In a study by Seidel et al., 2014, 4-hydroxybenzoic acid did not only arrest cell cycle progression but also triggered apoptotic cell death in cancer growth. Peaks 10, 11 and 12 shown in Figure 2 were characterized as sinapic acid based on UV-vis spectrum at 323 nm and M-H$^-$ at m/z 223. Also, the fragment ion at m/z 193 as provided in a reference standard. Sinapic acid had been reported for its various biological activities such as anti-inflammatoryand can be found in many fruits, vegetables, and cereal grains, medicinal plants, and spices, antibacterial and antidiabetic activities. It has been shown to acquire anticancer effects in different cancer cell lines such as antiproliferative, antiapoptotic properties and is also able to arrest the cell cycle. This compound was previously detected in abundance in edible fruits and vegetables and is thus one of the antioxidative components of normal human diet. Protocatechuic acid isolated from the Chinese herb Salvia miltiorrhiza, played a crucial role against inflammatory cytokines of atherosclerosis. Gentisic acid, p-coumaric acid, ferulic acid and gallic acid elicited a significant increase in the activities of several antioxidant enzymes such as Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) in rats.

Quantification of phenolic compounds INMP, ML and MW extracts

The identification of phenolic compounds in the extracts was generated from calibration curves after the reference standards. Linear regression equation obtained from the calibration curve of each standard applied for the quantification of phenolic compounds of *A. altilis* extracts. Concentrations of individual and total phenolic compounds. The concentrations of flavonoids differed from 12.20 to 44.10 mg/kg dry weight in MP extract. While in ML extract it varied from 22.80 to 47.40 mg/kg dry weight and in MW extract it was between 30.60 to 44.10 mg/kg dry weight. In the present study the range for phenolic acids were from 0.17 to 11.40 mg/kg dry weight for MP, 0.12 to 2.97 mg/kg dry weight for ML, and 0.14 to 17.00 mg/kg dry weight for MW, respectively. While protocatechuic acid was the most concentrated phenolic acid delivers43.36 %, 46.33 % and 49.09 % of the total phenolic acid concentration in MP, ML, and MW extracts, respectively. Followed by p-coumaric acid in both MP and MW was 31.70% and 26.96% of the total phenolic acid concentration, respectively. Ferulic acid was mainly concentrated in ML followed by MP and MW at 18.56%, 12.5%, and 4.09%, respectively. Gallic acid was concentrated in MW of 15.77%, followed by MP and ML at 4.00% and 1.71%, respectively. While the percentage of 4-hydroxybenzoic was 5.93%, 1.87% and 3.17% in MP, ML, and MW, respectively.

Conclusion

The biological activity of quercetin derivatives focused mostly on glycosides,in line with study that shown the quercetin aglycone and glycosides are absorbed from the gastrointestinal tract to a different extent; furthermore, absorption of quercetin glycosides depends on the position and nature of sugar substitutions. The hydroxylation structure of the B-rings and C-rings of the flavonoids play a crucial role in their cytotoxic activities, mainly the inhibition of protein kinase antiproliferation activity.

Conflict of Interest

There is no conflict of interest.

References

1. Abad-Garcia B, Berrueta LA, Garmon-Lobato S, et al. A general analytical strategy for the characterization of phenoli compounds in fruit juices by high-performance liquid chromatography with diode array detection coupled to electrospray ionization and triple quadrupole mass spectrometry. J Chromatogr A. 2009;1216:5398-415.
2. Bilbao MLM, Andres-Lacueva C, Jauregui O, et al. Determination of flavonoids in a Citrus fruit extract by LC-DAD and LC-MS. Food Chem. 2007;101:1742-7.
3. Cornwell T, Cohick W, Raskin I, et al. Dietary phytoestrogens and health. Phytochemistry. 2004;65:995-1016.
4. Fresco P, Borges F, Marques MPM, et al. The anticancer properties of dietary polyphenols and its relation with apoptosis. Curr Pharm Des. 2010;16:114-34.
5. Gomez-Romero M, Segura-Carretero A, Fernandez-Gutierrez A, et al. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry. 2010;71:1848-64.

Citation: *Jalal TK. Concern looks into underutilised tropical fruit: Quantification and identification of phenolic compounds in methanol extracts of artocarpusaltilis. J Biochem Biotech 2021;4(6):1-4.*

*Correspondence to

Dr. Tara K Jalal

Department of Biomedical Science

International Islamic University
Kuantan

Pahang

Malaysia

E-mail: tarajalal@gtmail.Com