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Water soluble phenolics, flavonoids and anthocyanins extracted from jaboticaba berries using maceration with ultrasonic pretreatment

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ABSTRACT

Jaboticaba is healthy and delicious berries rich in phenolic compounds, particularly flavonoids, anthocyanins and ellagitannins. The natural occurring polyphenols have been getting interested by food and pharmaceutical industries, mainly due to their remarkable pharmacological actions. Therefore, this study was focused on the extraction of water soluble phenolics from jaboticaba berries using maceration technique with and without ultrasonic pretreatment. A short period of ultrasonic treatment (30 min) prior to maceration could improve the extraction to recover total phenolic and anthocyanin content, especially from its thick fruit peel for 75% and 61%, respectively. About 4.5% - 8.5% and 8.6% - 9.4% of the total phenolics was flavonoids and anthocyanins, respectively. The phenolics were statistically correlated to different colorimetric antioxidant assays to postulate the plausible antioxidant pathway. Hierarchical clustering revealed that phenolics in jaboticaba extracts were the dominant scavengers against free, cationic and peroxyl radicals, specifically flavonoids and anthocyanins were likely to be reducing agents. Jaboticaba extracts were also found to have higher scavenging capacity against peroxyl radicals, and higher reducing power than that of Trolox. Mass screening by UPLC-MS/MS detected many phenolics including glycosylated cyanidins and delphinidins, as well as galloylated flavonoids, ellagitannins and phospholipids in the peel extract.

Introduction

Jaboticaba (*Plinia cauliflora*) is a popular Brazilian fruit belonging to the Myrtaceae family. It is a grapelike berry grown on the trunks and branches. Each fruit may have 1 to 4 seeds having an average diameter of 2.8 cm and an average weight of 12 g (Gurak, De Bona, Tessaro & Marczak, 2014). Approximately, one third of the berry weight is attributed to its peel and another two third is contributed by its pulp and seeds. It has thicker peel than grape, and its jelly like pulp tastes sweet and sub-acid. The berry is green and turns into reddish-purple or magenta when it ripens. Recently, its popularity is extended from Brazil to other countries such as the United States, China and the European communities, mainly due to its health benefits reported in scientific studies. A recent review article reported that there are about 200 articles published on the nutritional, phytochemical and biological properties of jaboticaba berries, as well as the technologies used for processing jaboticaba derived products (Inada et al., 2021). The review emphasizes on the aspect of jaboticaba polyphenolic compounds, extraction techniques, compound metabolism in human body and challenges in the development of jaboticaba derived products. The common jaboticaba products include juices, jellies, vinegar, liqueurs and wines (Wu et al., 2013a). Since jaboticaba berries are perishable and seasonal, and converting fresh berries into products would be value added and expanding the product market for global commercialization.

The remarkable health benefits of jaboticaba is most probably attributed to the existence of phenolic compounds, particularly anthocyanins and ellagitannins (Inada et al., 2021). Ellagitannins or its monomer ellagic acid have been reported to exhibit numerous beneficial effects on human health including anti-inflammatory, anticancer, antioxidant, prebiotic, and cardioprotective properties (Evtyugin, Magina & Evtuguin, 2020). The occurrence of ellagitannins can only be detected in a few berries like strawberry, blackberry and raspberry. However, fruit

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processing may change the phytochemical composition of jaboticaba, especially the pH and heat sensitive anthocyanins. Anthocyanins are water soluble plant pigments and usually present in the form of glycosylated and/or acylated anthocyadinins. Its stability is strongly dependent on the pH of solution because of the ionic nature of its molecular structure having a positive charge at the oxygen atom of the C-ring of basic flavonoid structure called as flavylium (2-phenylchromenylium) ion (Turturică, Oancea & Râpeanu, 2015). The red colored pigments are stable in acidic pH, they would become purple in neutral pH and change to blue in alkaline pH. This is due to the hydration and deprotonation of flavylium ion to form anionic quinonoidal species when pH is increasing (Coutinho, Freitas, Maçanita & Lima, 2015). Glycosylation and acylation may also increase the color strength of anthocyanins. In addition, polymerization of anthocyanin-tannin could increase the color stability at a lower pH (Sims & Morris, 1985).

The conventional technique of maceration is preferable for the extraction of phenolic compounds from natural material at industrial scale (Ćujić et al., 2016; Deng et al., 2017; Putnik, Barba, Lucini, Rocchetti & Montesano, 2019). Maceration could avoid compound degradation because the technique does not apply the harsh extraction conditions such as high temperature and pressure. However, maceration takes longer extraction time and its performance is lower than other advanced extraction techniques such as supercritical fluid, subcritical water, ultrasound and microwave assisted extractions (Ju & Howard, 2005; King, Grabiel & Wightman, 2003; Nunes Mattos et al., 2022; Pitz et al., 2016).

In the present study, jaboticaba berries were sourced from a local plantation in Johor, Malaysia. The berries were smashed and macerated in water with intermittent shaking for 5 days. A short period of ultrasonic pretreatment was also applied to enhance the extraction performance prior to maceration. The similar experiment was carried out for jaboticaba peel and pulp separately for comparison. Statistical comparison was conducted using the data of total phenolics, flavonoids and anthocyanins, as well as antioxidant capacities in terms of radical scavenging and reducing power. Unsupervised multivariate data analytical approaches such as principal component analysis and hierarchical clustering were used to determine the similarity and relationship of the data in order to postulate the plausible antioxidant mechanism of water soluble phenolics in jaboticaba.

Materials and methods

Chemicals and reagents

The reagents such as 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and ferric 2,4,6-Tri(2-pyridyl)-*s*-triazine, as well as the standard chemicals such as gallic acid (97.5%), quercetin (95%), cyanidin-3-glucoside (≥95%) and 6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid (Trolox, 97%) were obtained from Sigma Aldrich (St Louis, MO, USA). The salts such as sodium carbonate, aluminum chloride, sodium acetate, ferric chloride and potassium persulfate were sourced from Merck (Darmstadt, Germany). Analytical grade of methanol, Folin-Ciocalteu reagent, ascorbic acid (97%), fluorescein (95%) and hydrochloric acid (1 N) were also purchased from Merck. Jaboticaba berries were harvested in the month of August 2022 from a plantation located at Senai (Johor, Malaysia) with the coordinate of 1.6062 and 103.6351.

Sample extraction

The whole fruit of jaboticaba (100 g) were cleaned, manually mashed and transferred into two different Schott bottles. Some fruit were peeled off into peel (100 g) and pulp (100 g), and then transferred into different bottles. Distilled water was added into the bottles in a ratio of 1:10 solid to liquid. One set of the samples was subject to the process of maceration for 24 h, and another set was pretreated with ultrasound at 40 kHz for 30 min prior to maceration for 24 h. The temperature of the extraction process was maintained at room temperature (28 °C). The supernatant of samples was filtered and concentrated by a rotary evaporator (4001 Efficient, Heidolph Instruments, Schwabach, Germany) after extraction. The concentrated supernatant was further dried in an oven at 40 °C until a constant weight was recorded. The pH and total soluble solid of samples were measured by dissolving and well mixing 0.5 g dried extracts in 10 mL distilled water using a digital pH meter (Laquatwin, Horiba, Kyoto, Japan) and Brix refractometer (Milwaukee MA871, California, USA), respectively.

Phenolic composition of samples

The phenolic composition of samples was colorimetrically assayed for the determination of total phenolic content (TPC), total flavonoid Content (TFC) and total anthocyanin content (TAC) using a UV–Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan).

TPC was determined using Folin-Ciocalteu reagent according to the procedures reported by Alezandro et al. (2013). A 0.5 mL sample, 0.3 mL Folin-Ciocalteu reagent and 2 mL sodium carbonate (15% w/v) was sequentially pipetted into a 5 mL centrifugal tube, and then the mixture was topped up with distilled water (2.2 mL) to make up the volume of the mixture into 5 mL. The mixture was incubated for 2 h at room temperature in a dark place and its absorbance was measured using the spectrophotometer at 798 nm. Gallic acid (Sigma-Aldrich, Burlington, USA) was used as the standard chemical and results were expressed as mg gallic acid equivalents (GAE) per gram sample. The equation of the calculation is presented in Eq. (1).

$$\text{Total Phenolic Content (TPC)} = \frac{\text{C.V}}{\text{m}} \quad (1)$$

C: Concentration of gallic acid or quercetin from calibration curve m: Weight of sample (g)

V: Volume of sample (mL)

TFC was measured using 5% aluminum chloride according to the procedures reported by Kung et al. (2022). A 2 mL sample and 3 mL aluminum chloride were pipetted into a 5 mL centrifugal tube. The solution was well mixed and incubated in a dark place for 30 min prior to the absorbance measurement at 510 nm using the spectrophotometer. Eq. (1) was used to calculate TFC using quercetin as standard chemical. The result is expressed as mg quercetin equivalent (QE) per gram sample.

TAC was determined using the pH differential method (Lee et al., 2005). Two types of buffer solution at different pH values were prepared in this assay. The aqueous buffer (1 L) was prepared by dissolving KCl (1.86 g) in distilled water and then pH adjusting to 1.0 ± 0.05 using HCl (0.01 M). The acetate buffer (1 L) was prepared by dissolving sodium acetate (54.43 g) in distilled water and pH adjusting to 4.5 ± 0.05 using HCl. One portion of sample (mg/L) was added into four portions of aqueous buffer at pH 1 and acetate buffer at pH 4.5 each. The absorbance was measured at 520 nm and 700 nm after 30 min of incubation. Cyanidin-3-glucoside was used as positive control. Eq. (2) was used to calculate TAC. The results were expressed as milligram cyanidin-3-glucoside equivalent per gram sample (mg C3GE/g).

$$\text{Total anthocyanin content (TAC)} = \frac{\text{A x MW x 1000}}{\varepsilon \text{ x p x s}} \quad (2)$$

Where

A: absorbance, $A = (A_{520} - A_{700})pH1.0 - (A_{520} - A_{700})pH4.5$MW: molecular weight of cyanidin-3-glucoside (449.2 g/mol)

ε: extinction coefficient $(26,900 \frac{L}{mol.cm})$P: pathlength (1 cm)

S: sample concentration (g/L)

Antioxidant capacity of samples

The antioxidant capacity of samples was compared using different colorimetric assays which were performed based on the capability of samples acting either as free radical scavenger, radical cation scavenger or reducing agent. The scavenging activity of samples was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and ORAC (2,2′-azobis(2-amidinopropane) dihydrochloride) reagents to generate free radicals, cationic radicals and peroxyl radicals, respectively. The reducing power of samples was measured based on the reduction of colorless Fe^{III}-TPTZ (ferric-tripyridyltriazine) into blue Fe(II)-TPTZ.

The DPPH assay was carried out according to the procedures reported by Alezandro et al. (2013). A 2 mL sample and 2 mL methanolic DPPH (0.1 mM) were mixed thoroughly, and then incubated in a dark room for 30 min prior to the absorbance measurement at 517 nm using the spectrophotometer. The purple colored DPPH radicals was changed to yellowish solution after scavenged by antioxidative compounds in samples. The inhibitory percentage of DPPH free radicals can be calculated using Eq. (3) and results were expressed as the effective concentration of sample to exhibit 50% of inhibition.

$$\text{Radical inhibition } (\%) = \frac{A_o - A_1}{A_o} \text{ x } 100 \quad (3)$$

A_0 is the absorbance of control and A_1 is the absorbance of sample.

The ABTS assay was performed according to the procedures reported by Chew et al. (2018). An equal volume of ABTS (7 mM) and potassium persulfate (2.45 mM) was added and incubated overnight in a dark place to generate radical cations. The solution was diluted with distilled water until its absorbance achieved the value of 1.00 at 734 nm. The diluted ABTS solution (2 mL) was added into different concentrations of sample solution (100 mL). The intensity of the blue colored solution was getting lighter or even colorless after scavenged by antioxidative compounds in samples for 6 min in a subdued light condition. The absorbance of sample was measured again at 734 nm using the spectrophotometer.

The ORAC (oxygen radical absorbance capacity) assay was carried out to determine total antioxidant capacity of samples according to the procedures described by Andrade et al. (2017) with modification. A 1.50 mL fluorescein working solution (6.30 mmol/L) and 0.75 mL samples (1 – 3 mg/mL) or standard Trolox (2.0 – 10.0 mg/L) were mixed and incubated at 37 °C for 15 min. Then, 0.75 mL AAPH working solution (153 mmol/L) was added into the mixture. The absorbance was recorded every 90 s at 520 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) for 1 hour. The ORAC value was calculated by substracting the reaction curve area of blank with the reaction curve area of sample. The reaction curve area was plotted based on the decreasing absorbance over the reaction time for 1 hour. The results are expressed as micromole Trolox equivalent per g sample (μmol TE/g).

The FRAP assay was determined according to the procedures reported by Muthukrishnan and Manogaran (2016). FRAP solution was freshly prepared by adding 300 mM acetate buffer (25 mL) at pH 3.6, 10 mM TPTZ (2.5 mL) in HCl (40 mL) and 20 mM ferric chloride (2.5 mL). An equal volume of FRAP solution (1 mL) was mixed with different concentrations of sample (1 mL) and the absorbance of the mixture was read at 593 nm. Trolox (0.004 – 2.000 mg/mL) was used as standard chemical. The reducing power was evaluated using Eq. (4).

$$\text{Ferric reducing antioxidant power } (\%) = \frac{A_1 - A_o}{A_s - A_o} \text{ x } 100 \quad (4)$$

A_o is the absorbance of control, A_S is the absorbance of ascorbic acid and A_1 is the absorbance of sample.

UPLC-MS/MS

A hyphenated analytical technique of UPLC-MS/MS (Waters Acquity, Milford, MA and Applied Biosystems 4000 Q TRAP; Carlsbad, CA) was used for compound separation and identification. Compound separation was performed by a C18 column (Acquity, 150 × 4.6 mm, 1.7 μm) using a programed mobile phase gradient consisted of solvent A (water acidified with 0.1% formic acid) and solvent B (acetonitrile). The gradient was: 0–5 min, 10% B; 5–15 min, 10–90% B; 15–20 min, 90% B; 20–25 min, 90–10% B; 25–30 min, 10% B with a flow rate of 0.20 mL/min. The separated compound was then flowed into an electrospray ionization (ESI) source for ionization. The ionized compounds were detected at the mass analyzer using both positive and negative ion modes.

The mass screening method of enhanced mass spectra (EMS) coupled to two parallel enhanced product ion (EPI) was used to detect masses from 100 to 2000 amu. The temperature of ion source was set at 400 °C. The voltage was maintained at 5.5 kV and −4.5 kV for positive and negative ion modes, respectively. Nitrogen was used as the nebulization (40 psi), solvent drying (40 psi) and curtain gas (10 psi) at the scan rate of 1000 amu/s. Data acquisition was performed using Analyst 1.4.2 (AB SCIEX, Foster City, CA).

Statistical analysis

All experiments were conducted in triplicate and the results were expressed as mean ± standard deviation. Principal component analysis (PCA) was performed to cluster jaboticaba extracts based on their similarity using experimental data from sample characteristics such as extraction yield, pH, Brix, total phenolics, total flavonoids, total anthocyanins, DPPH radical scavenging, ABTS radical cation scavenging, ORAC absorbance capacity and reducing power (Abriata, 2021). The relationship of sample characteristics was also examined using the Ward's method of hierarchical clustering after data normalization. The output was plotted in dendrogram using the online Statistics Software (Wessa, 2017).

Results and discussion

Brix and pH values of jaboticaba extracts

The extraction yield of jaboticaba extracts is presented in Fig. 1(a). The results showed the yield of extraction was ranged from 8.2 to 9.2% w/w and 9.4 to 11.2% w/w for samples without and with ultrasound pretreatment, respectively. A short period of 30 min ultrasonication did improve the yield of extraction for about 1 to 2%. The fruit peel exhibited higher yield than fruit pulp, particularly pretreated with ultrasound prior to maceration in water. Anyhow, a single factor ANOVA showed the insignificant difference of extraction yield at 95% confident level. The extraction yield of fruit peel was comparable with the data reported by Santos et al. (2010). They reported the extraction yield of jaboticaba peel was 11.93% using the technique of ultrasound assisted extraction in the similar solid to liquid ratio of 1:10 for 2 h. Even though ultrasonic treatment did not improve the extraction yield significantly, the quality of jaboticaba extracts was affected based on the sample characterization below.

A short duration of ultrasonic pretreatment slightly increased the Brix value of samples and the increment was about 1 Brix for fruit pulp (Table 1). Brix was used to estimate total sugar or water soluble content in samples. Fruit pulp is mostly composed of sugars which are not ionic compounds, and therefore, sugars do not release hydrogen ions in reducing the pH value of solution. The fruit pulp had higher pH explaining lower concentration of hydrogen ions. This also explains the significant difference of pH for pulp extracts compared to other samples. However, there is no significant difference of pH for whole fruit and its peel extracts. This study applied low energy ultrasound (40 kHz) which was not sufficient to change the pH of water. Previous studies reported that ultrasound at 1 MHz would only be able to decrease the pH of water resulted from the release of atomic hydrogen (Vikulin & Vikulina,

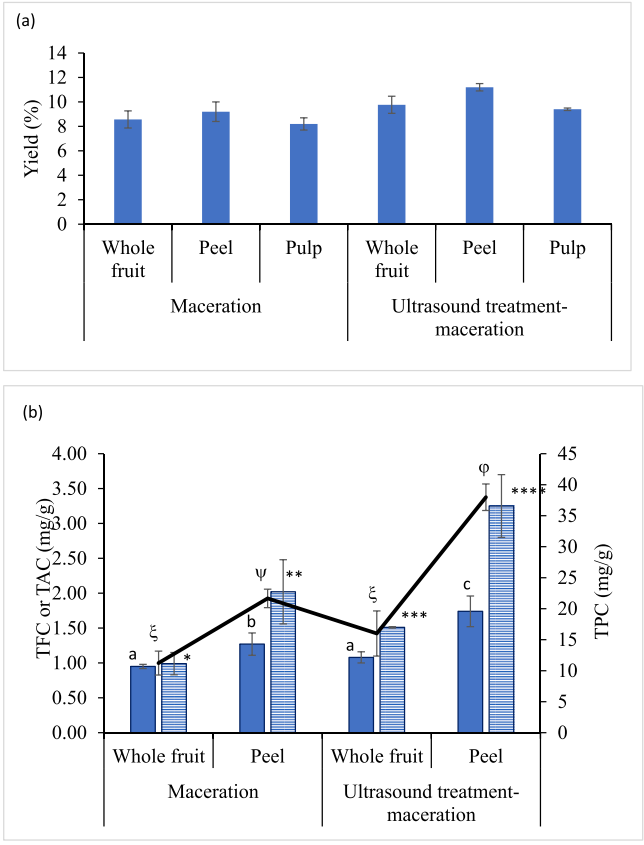

Fig. 1. (a). Extraction yield of jaboticaba whole fruit, its peel and pulp using maceration without and with ultrasound pretreatment. A single factor ANOVA revealed that there was no significant difference among samples. (b). Total phenolics (line), flavonoids (solid bar) and anthocyanins (line bar) of jaboticaba whole fruit, its peel and pulp extracts using maceration without and with ultrasound pretreatment. A single factor ANOVA followed by a post hoc Tukey-Kramer test was performed to determine the significant difference of samples in each assay. The significant difference of total phenolics is explained by different symbols. The significant difference of total flavonoids is explained by different alphabets. The significant difference of total anthocyanins is explained by different number of asterisk.

Table 1
pH and brix values of jaboticaba extracts at 50 mg/mL.

Extraction	Sample	pH	Brix
Maceration	Whole fruit	3.40 ± 0.00^{a}	3.43 ± 0.06^{a}
	Peel	3.40 ± 0.00^{a}	2.50 ± 0.00^{b}
	Pulp	3.53 ± 0.06^{b}	3.57 ± 0.06^{c}
Ultrasonic pretreatment -Maceration	Whole fruit	3.40 ± 0.00^{a}	3.87 ± 0.12^{d}
	Peel	3.40 ± 0.00^{a}	2.27 ± 0.11^{e}
	Pulp	3.50 ± 0.00^{ac}	4.53 ± 0.06^{f}

Different superscript letters in column denote for the significant difference of mean values from triplicate experiments based on a single factor ANOVA followed by a post hoc Tukey-Kramer test.

2020).

Phytochemical content in jaboticaba extracts

Spectrophotometric assays were able to give valuable qualitative and quantitative information about jaboticaba extracts. The technique had been used to measure different groups of polyphenols because of its simplicity, reliability, fast and cost effectiveness (Ignat, Volf & Popa, 2011; Guemari et al., 2022). This technique remains as a valuable method for natural product samples, even though it limits to compounds having UV–visible wavelength range of absorption ability. It has also low sensitivity since the concentration of sample typically ranges from a few micrograms per milliliter to a few milligrams per milliliter.

The methods using Folin-Ciocalteau reagent, aluminum chloride and pH differential technique were used to estimate the content of phenolics, flavonoids and anthocyanins, respectively in the whole fruit and its peel extracts. Phenolics are one of the most diverse group of phytochemicals that are widely distributed in plant samples including vegetables and fruits. Previously, Stafussa and his coworkers (2018) carried out intensive studies on the bioactive compounds of 44 traditional and exotic Brazilian fruit pulps. They reported that jaboticaba was among the top ranked fruits, besides panã, acerola and açaí, containing high TPC, TFC and TAC values. Phenolics such as phenolic acids, flavonoids and anthocyanins including their glycosylated and acylated derivatives would be considered in TPC. No such assays were performed for fruit pulp since it was mostly composed of sugar.

The colorimetric assay of TPC estimated phenolic compounds which were able to donate electrons to form blue molybdenum–tungsten complex for spectrophotometric detection at 760 nm. The results showed that TPC had the highest content (11.23 to 31.98 mg GAE/g) compared to TFC (0.95 to 1.74 mg QE/g) and TAC (0.99 to 3.25 mg C3GE/g) in this study (Fig. 1b). Fruit peel appeared to have higher TPC than its whole fruit extracts, even in the maceration without ultrasound pretreatment. The introduction of ultrasonic pretreatment did also improve TPC, particularly the TPC of fruit peel extract (from 21.66 to 37.98 mg GAE/g). The TPC of this study was higher than the TPC of jaboticaba peel extract (13 mg GAE/g) prepared by the sophisticated technique of high pressure carbon dioxide assisted extraction (Santos & Meireles, 2011). However, the result of the present study was lower than the data (92.8 mg GAE/g) reported by Rodrigues et al. (2015), who also used ultrasound assisted extraction. The good performance could be explained by the use of 46% aqueous ethanol in acidic medium (pH 1.0). Besides extraction technique, the solvent system also played an important role in phenolic extraction. This had been proven by the recent works of Fernandes et al. (2020) and Azman et al. (2020), who found that acidic solvent was superior to recover phenolics and anthocyanins jaboticaba and blackcurrant peels, respectively. Samples that subjected to ultrasonic treatment were found to significantly increase in TPC which was about 42% and 75% increment for whole fruit and its peel, respectively.

For the colorimetric TFC assay, the use of aluminum chloride would form stable acid complexes with C-4 keto and hydroxyl groups in the C-3 and C-5 of flavonols and flavones for spectrophotometric measurement. The salt also forms labile acid complexes with ortho-dihydroxyl groups either in the A- or B-ring of flavonoid skeleton. To the best of our knowledge, there are no studies to express the TFC in quercetin equivalent for jaboticaba samples for accurate comparison. Most studies expressed the TFC in catechin equivalent (CE). For instance, the TFC of jaboticaba peel extract was 33 mg CE/ 100 g and 53 mg CE/100 g reported by Abe et al. (2012) and Marquetti et al. (2018), respectively in their studies.

In the present study, flavonoids were covered for 4.6 to 8.5% of the TPC based on the ratio of TFC to TPC, whereas anthocyanins accounted for 8.6 to 9.4% of the TPC based on the ratio of TAC to TPC. The observation proved that jaboticaba berries were rich in anthocyanins. Anthocyanin which is one of the sub-groups of flavonoids, is water soluble plant pigments and its color is pH dependent. Hence, the pH differential method could be effectively used to estimate TAC in jaboticaba extracts. A comparable result (2.24 mg C3G/g) was reported by Santos and Meireles (2011). Somehow, the TAC (0.99–3.25 mg C3G/g) was lower than the value (7.33 mg C3G/g) reported by Leite-Legatti et al. (2012). Possibly, this could be due to different solvent system

(methanol: hydrochloric acid (99.9: 0.1 v/v) used by the researchers in the anthocyanin extraction. Another possible explanation was the sonochemical-induced hydrolysis resulted from ultrasonic energy. This was because Fernandes et al. (2020) ever reported that ultrasonication for more than 20 min would increase the extraction yield, but also would degrade anthocyanins. The presence of anthocyanins was also tentatively identified based on the fragmentation patterns of compounds detected from high throughput mass screening by UPLC-MS/MS (Table S1). Besides the commonly reported anthocyanins such as cyanidin 3-glucoside and delphinidin 3-glucoside in jaboticaba berries, there were also many other anthocyanin derivatives detected from this study (Wu et al., 2012). In particular, anthocyanins such as petunidin 3-glucoside, peonidin 3-glucoside, delphinidin pentoside, cyanidin-3-laminaribioside and etc., were detected from the fruit peel extract that pretreated with ultrasound. In agreement with the findings of Wu et al. (2013b), jaboticaba peel extract contained mostly anthocyanins derived from the aglycones of delphinidin and cyanidin.

Antioxidant capacity of jaboticaba extracts

The characterization of jaboticaba extracts was extended to the antioxidant capacity of samples via various potential pathways. The radical scavenging pathway was evaluated based on free radicals (DPPH•) and cationic radicals (ABTS•+) assays. Both assays show a dose dependent manner of radical inhibitory action until the scavenging activity reaches an equilibrium as presented in Fig. 2.

The figure clearly shows that peel extract was likely to be a good radical scavenger, particularly the fruit peel extract pretreated with ultrasound. The inhibition of free radicals was found to be faster than the inhibition rate of radical cations. Such observation was also recorded by Cefali et al. (2021) who plotted the inhibitory curves of jaboticaba peel extracts at different concentrations (5–40 mg/mL). In addition to DPPH and ABTS assays, the researchers also performed evaluation for solar protection factor and concluded that jaboticaba peel extract could be a good source antioxidant and wound healing promoter.

Another explanation was given by Xie and Schaich (2014) who revealed that HAT occurred more slowly than the rate of SET. DPPH assay was used to examine the antioxidant capacity of samples following the mechanisms of single electron transfer (SET) and hydrogen atom transfer (HAT), while ABTS assay was used to evaluate samples that followed the mechanism of HAT. Although slower rate of inhibition against radical cations, jaboticaba extracts could achieve maximum inhibition up to 90% at the concentration higher than 6 mg/mL. On the other hand, the maximum inhibition was about 80% at 6 mg/mL against free radicals in DPPH assay. Obviously, the performance of this study was higher than the results reported by Cefali et al. (2021) who required at least 20 mg/mL of jaboticaba peel extract to achieve a comparable maximum inhibition (85–95%). The difference of results was partly because of the variance in extraction techniques. Cefali and his coworkers (2021) prepared the peel extract by heating the dried ground peel samples in 60% aqueous ethanol at 50 °C for 3 h. The higher inhibitory action of samples, especially the peel extracts against ABTS•+ could be attributed to the presence of ellagitannins (Wu et al., 2012). Moreover, a wide range of both lipophilic and hydrophilic compounds was able to scavenge ABTS•+. Unlike DPPH assay, the absorbance interference, mostly contributed by anthocyanins, was also lesser since the measurement of ABTS assay was performed at 734 nm.

Based on the IC50 values in Table 2, it was found that jaboticaba extracts appeared to be better as free radical scavengers than cationic radical scavengers. The lower IC50 values were attributed to the antioxidative compounds which were prone to donate hydrogen atom to quench radicals. In particular, anthocyanins which were positively

Table 2
Antioxidant capacity of jaboticaba fruit, its peel and pulp extracts based on the effective concentration at 50% inhibition for scavenging DPPH free radicals and ABTS radical cations, and for total antioxidant capacity based on oxygen radical absorbance and ferric reducing power.

Extraction	Samples	DPPH, IC50 (mg/g)	ABTS, IC50 (mg/g)	FRAP (mol TE/g)	ORAC (μmol TE/g)
	Ascorbic acid	0.120 ±0.003^{a}	–	–	–
	Trolox	–	0.9502 ±0.0424^{a}	0.799 ±073^{a}	5.363 ±1.175^{a}
Maceration	Whole fruit	40.318 ±1.210^{b}	70.400 ±4.360^{b}	1.039 ±0.389^{bc}	6.651 ±1.559^{a}
	Peel	9.362 ±1.838^{c}	51.900 ±3.331^{c}	1.232 ±0.086^{b}	23.602 ±3.180^{b}
	Pulp	29.900 ±1.424^{d}	97.720 ±8.420^{d}	0.913 ±0.062^{bc}	20.9337 ±0.129^{b}
Ultrasonic pretreatment-Maceration	Whole fruit	42.998 ±1.050^{e}	69.654 ±4.935^{b}	1.248 ±0.519^{b}	21.898 ±2.200^{b}
	Peel	5.490 ±1.583^{f}	39.080 ±2.380^{e}	1.414 ±0.097^{d}	27.578 ±2.842^{c}
	Pulp	29.834 ±1.803 g	88.090 ±6.340^{f}	1.188 ±0.056^{bc}	26.802 ±1.825^{c}

Different superscript letters of samples in column denote the significant difference of mean values from triplicate experiments based on a single factor ANOVA followed by a post hoc Tukey-Kramer test.

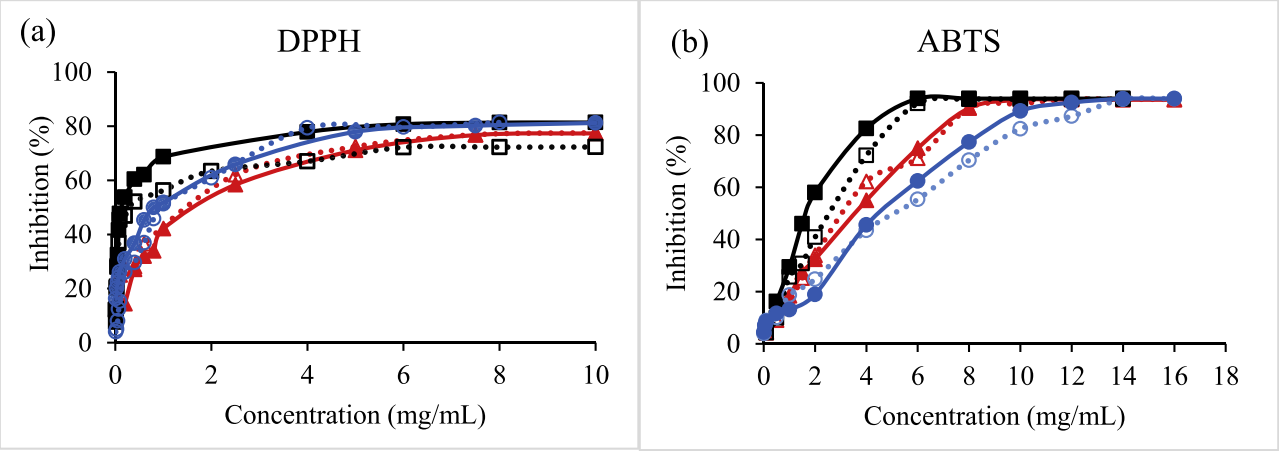

Fig. 2. A dose dependent manner of inhibition against (a) DPPH free radicals and (b) ABTS cationic radicals by different concentrations of Jaboticaba fruit, its peel and pulp extracts. Samples extracted with maceration is denoted by solid line (–) and samples pretreated with ultrasound followed by maceration is denoted by dashed line (—) in which triangle (▲) is assigned for whole fruit, square (■) is assigned for its peel and round (●) is assigned for pulp extracts.

charged compounds (flavylium cations) at the oxygen atom of the C-ring of flavonoid skeleton would encourage the transfer of hydrogen atom for radical inhibition. Possibly, the pulp extracts were likely to follow the mechanism of SET rather than HAT. SET could be the major mechanism because the pulp extracts exhibited higher pH values with lower concentration of hydrogen ions for HAT. The high throughput mass screening by UPLC-MS/MS revealed that flavonoids, anthocyanins, hydrolysable tannins and phospholipids were mostly detected in peel extract (Table S1). The compound detection explained the high antioxidant capacity of ultrasound pretreated jaboticaba peel extracts. A short period of ultrasonic treatment, 30 min was able to assist the release of anthocyanins and phospholipids from fruit peel samples. The acoustic cavitation of ultrasound could possibly damage the cell walls of plant matrices, and thereby accelerating the release of compounds (Tiwari, 2015).

The peroxyl radical scavenging activity was also analyzed for jaboticaba extracts. The results found that the peel extracts exhibited higher ORAC than that of Trolox which was used as the positive control in this experiment. Trolox is vitamin E analog which is well known for its antioxidant capacity. The ORAC of peel extracts (in μM TE/g) was comparable with the value reported by Leite-Legatti et al. (2012) who prepared the fruit peel powder from freeze drying. Besides the scavenging ability, the peel extracts also exhibited higher reducing power than the whole fruit and its pulp extracts. The ferric reducing power of jaboticaba extracts was better than Trolox. Therefore, jaboticaba extracts, especially its peel contained high antioxidative phenolic compounds, especially flavonoids, ellagitannins and anthocyanins to scavenge free, cationic and peroxyl radicals, as well as to reduce the oxidation state of compounds by donating electrons.

Data mining for jaboticaba extracts

Unsupervised multivariate data analysis techniques such as PCA and hierarchical clustering had been applied to view the similarity and dissimilarity of jaboticaba extracts prepared by different extraction methods using different sample matrices. Based on the score plot of PCA, it was found that jaboticaba extracts could be grouped into 3 clusters for whole fruit, peel and pulp extracts (Fig. 3(a)). Only small difference is observed for samples extracted with and without ultrasound treatment in the figure. The first principal component explains the majority of total variance as expressed in eigenvalues as presented in Fig. 3(b).

In the subsequent statistical analysis, the relationship of sample characteristics was established in hierarchical clustering. The dendrogram clearly groups samples into two distinct clusters based on the chemical properties and antioxidant potentials (Fig. 4). The first cluster explains the reducing power is closely depended on the flavonoids and anthocyanins. The second cluster illustrates the close relationship between phenolics and ORAC in samples. In addition to scavenging peroxyl radicals, phenolics are also the dominant compounds to scavenge free (DPPH) and cationic (ABTS) radicals. Therefore, this unsupervised pattern recognition technique reveals that phenolics, mostly ellagitannins are prone to transfer their hydrogen atoms to quench radicals. On the other hand, polyphenols like flavonoids and anthocyanins were likely to be reducing agents or electron donors to stabilize radicals. This is the first study to evaluate the plausible antioxidant mechanism followed by phenolic compounds in jaboticaba.

Conclusion

Jaboticaba is rich in phenolics, especially anthocyanins and ellagitannins, and they were mostly detected from its fruit peel. Maceration with ultrasonic pretreatment on jaboticaba samples could enhance the

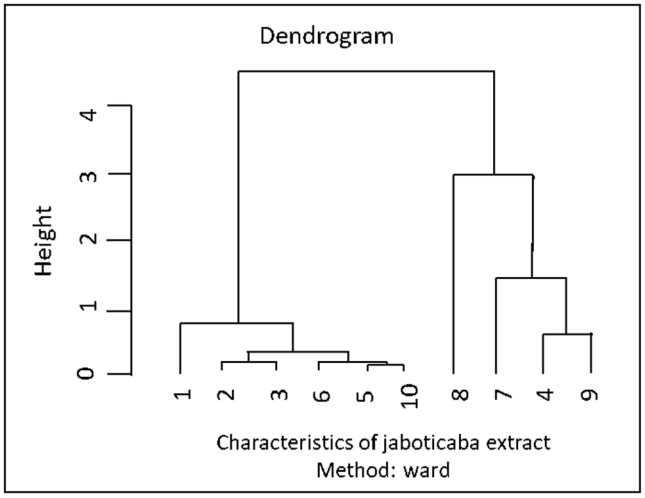

Fig. 4. Relationship of jaboticaba extracts based on the chemical properties and antioxidant potentials in which 1, extraction yield; 2, pH; 3, Brix; 4, total phenolic content; 5, total flavonoid content; 6, total anthocyanin content; 7, free radical scavenging capacity (DPPH assay); 8, cationic radical scavenging capacity (ABTS assay); 9, oxygen radical absorbance capacity (ORAC assay) and 10, ferric reducing power (FRAP assay).

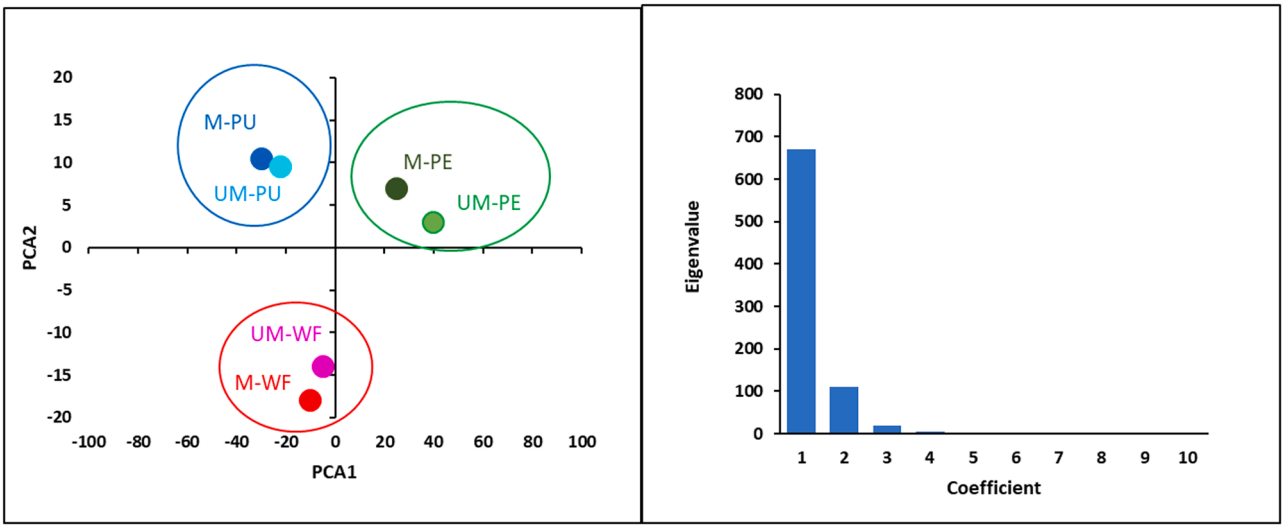

Fig. 3. (a) Score plot of principal component analysis and (b) its component eigenvalues.

extraction of water soluble anthocyanins and ellagitannins from its thick peel. This also explains higher radical scavenging and reducing power of the fruit peel extract than the whole fruit and its pulp extracts. Glycosylated cyanidin, delphinidin, peonidin and petunidin were detected from the positive ion mode, whereas galloylated flavonoids and ellagitannins were detected from the negative ion mode of UPLC-MS/MS. Dendrogram from hierarchical cluster analysis revealed that phenolics from jaboticaba extracts could be divided into two main clusters. Flavonoids and anthocyanins were found to be good reducing agents, whereas phenolics were prone to be radical scavengers. The benefits of jaboticaba could be explained by its antioxidative water soluble phenolics.

CRediT authorship contribution statement

Lee Suan Chua: Conceptualization, Data curation, Writing – review & editing, Supervision. **Nurul Syafiqah Abd Wahab:** Methodology, Investigation. **John Soo:** Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100387.

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