Phytochemical composition and antioxidant activity of Cinnamomum burmannii Blume extracts and their potential application in white chocolate

Highlights

- *C. burmannii* extracts contained various phenolic compounds.
- The effect of the different extraction method was revealed.
- Cryo-SEM analysis showed the cavitation effect of ultrasonic extraction.
- Significant improvement on the antioxidant activity of white chocolate was found.

ESSENTIAL TITLE PAGE

Title

Phytochemical composition and antioxidant activity of *Cinnamomum burmannii* Blume extracts and their potential application in white chocolate

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Abstract

This study aims at determining the potentials of cinnamon (*Cinnamomun burmannii*) extracts to improve the health-promoting properties of white chocolate. LC-HRMS analysis was employed to obtain information regarding the phytochemical content while the phosphomolybdenum, FRAP and DPPH assays were used to determine antioxidant activity of cinnamon extract. Furthermore, the cinnamon extract was loaded into nanoparticles before adding it to white chocolate. The results show that cinnamon extracts contained phenols up to 310 mg EE and possessed antioxidant activity up to 260 mg TAE per gram of dry extract depending on the extraction mode (*i.e.,* traditional and ultrasonic-assisted method) and the solvent type. The cinnamon extract contained catechin, epicatechin, procyanidin B2, quercitrin, 3,4-dihydroxybenzaldehyde, protocatechuic acid and cinnamic acid at levels of 51, 53, 1396, 13, 1138, 228 and 934 μg/g of dry extract, respectively. The encapsulated cinnamon extract increased the phenolic content of white chocolate from 47.6 to 1060.6 μg EE/g.

Keywords: cinnamon, extraction, ultrasound, antioxidant, phytochemicals, UPLC-HRMS, chocolate, oleoresin

Chemical compounds studied in this article

Epicatechin (PubChem CID:72276); Catechin (PubChem CID:73160); Cinnamic Acid (PubChem CID: 444539); Procyanidin B1 (Pubchem CID: 11250133); Procyanidin B2 (Pubchem CID:

122738); 3,4-dihydroxybenzaldehyde (PubChem CID 8768); p-hydroxybenzaldehyde (Pubchem CID: 126); Protocatechuic acid (Pubchem CID: 72); Apigenin (Pubchem CID: 44257831); Rutin (Pubchem CID: 6728944); Quercitrin (Pubchem CID: 5280459); Coumarin (Pubchem CID: 323).

1. Introduction

The potential use of cinnamon (genus *Cinnamomum*, family *Lauraceae*) nowadays is not only as a flavouring agent, but also as an antimicrobial and antioxidant agent (Muhammad et al., 2017; Ju et al., 2018; Sun et al., 2018). Furthermore, cinnamon and its constituents were proven to have a great potency as anticancer, antitumor, anti-inflammatory, antidiabetic and antiviral agents (Ribeiro-Santos et al., 2017). Phytochemicals commonly found in cinnamon, such as polyphenols and cinnamaldehyde, have been reported to play important roles in these bio-activities (Muhammad & Dewettinck, 2017; Muhammad et al., 2020a). Approximately 250 different species of cinnamon were discovered in the world (Thomas & Duethi, 2001). However, till now, the identification and quantification of these bioactive compounds as well as the demonstration of the bio-activity of most cinnamon species are still under-investigated.

Cinnamomum burmannii, also known as Cassiavera or Korintje cinnamon, is an endemic species in Indonesia and native to Sumatra (Elliott & Brimacombe, 1987; Menggala et al., 2019). Data from Food and Agricultural Organization (2018), shows that in the last decade, the country produced approximately 90,000 tonnes of *C. burmannii* per year. In 2017, more than 50,000 tonnes were exported to 76 countries for a trade value of 100 million USD (International Trade Centre, 2018). Therefore, the investigation of its biologically active compounds is of great importance for the industrial application of *C. burmannii*. According to Dvorackova et al. (2015), intensive studies on chemical composition of cinnamon have been conducted. Nevertheless, identification of

86 phenolic substances in the cinnamon extract is insufficient and incomplete, and this is particularly
87 the case for *C. burmannii*. It is well-known that ultra-high-performance liquid chromatography –
88 high resolution mass spectrometry (UPLC-HRMS) is an analytical technique suitable for studying
89 the non-volatile phytochemical composition of a plant extract. However, no single study exists
90 which dealt with the use of UPLC-HRMS for phenolic profiling of *C. burmannii*.

91 Extraction is the primary essential step for the isolation of bioactive compounds. It has been
92 reported that the extraction method has a significant influence on the polyphenol composition and
93 also on the antioxidant capacity of cinnamon extracts (Jayaprakasha et al., 2007; Przygodzka et
94 al., 2014). For this reason, a suitable approach for extracting cinnamon to obtain desired
95 compounds is needed. Recently, ultrasonic extraction has gained interest for assisting in the
96 removal of targeted compounds from various plants (Klejdus and Kováčik, 2016; Qiu et al., 2019;
97 Belwal et al., 2019). Ultrasound causes cell disruption and particle size reduction resulting in a
98 greater contact area between the solid and the liquid phase. This further led to better access of
99 solvent within the material to be extracted as compared with conventional methods (Li et al., 2005).
100 Therefore, this study investigated the phytochemical properties and antioxidant activity of *C.*
101 *burmannii* bark originating from Kerinci, Indonesia extracted by traditional and ultrasonic
102 methods.

103 Recently, the use of cinnamon in the food and pharmaceutical industries has considerably
104 increased due to its potential health benefits. According to the report of the European Medicines
105 Agency (2011), there is no major concern about the safety of cinnamon including the herbal
106 substance, extract, oleoresin and essential oil. In addition, cinnamon and its derivatives are
107 generally recognised as safe (so-called "GRAS' status in the USA) (Muhammad & Dewettinck,
108 2017). Due to its potential health benefits, it has been used to improve the health-promoting

properties of foods, such as coffee, chocolate and cocoa drinks (Durak, et al., 2014; Muhammad et al., 2018; Muhammad et al., 2019). Its incorporation in chocolate has significantly improved the total phenolic content and the antioxidant activity of white chocolate (Muhammad et al., 2018). However, the phenolic profile of the cinnamon enriched-white chocolate has no proper investigation till date. This study was therefore designed to investigate the potential use of cinnamon extract to increase the phenolic content of white chocolate.

White chocolate was used in this work because its polyphenol content and antioxidant activity is significantly lower than milk and dark chocolate (Meng et al., 2009). The incorporation changes the sensory characteristics of chocolate, particularly the odour and taste and subsequently reduce consumer's acceptance of the cinnamon-enriched chocolate (Ilmi et al., 2017), as a result, the encapsulation of cinnamon extract as described in Muhammad et al. (2018) is required to reduce the alteration of chocolate's odour and flavour. According to Muhammad et al. (2020b), nanoencapsulation can also improve the thermal stability of cinnamon extract.

2. Materials and methods

2.1. Materials

Cinnamon barks (*C. burmannii* Blume) were collected from Kerinci, Sumatra (Indonesia). The cinnamon barks were ground and filtered using a mill and an ASTM standard sieves respectively, to acquire a particle size of 0.25-1.00 mm. In the fabrication of cinnamon nanoparticles shellac SSB 55 Astra FP obtained from SSB Stroever GmbH & Co. KG Bremen (Germany) and xanthan gum Satiaxane CX 931 produced by Cargill France SAS (France) were used. Also, white chocolate discs were obtained from Barry Callebaut Belgium NV (Belgium). Analytical grade acetone, methanol and ethanol were provided by Thermo Fisher Scientific (Belgium). Furthermore,

Ultrapure water purified by a Milli-Q filtration system (0.22mm) (Millipore Corp., Bedford, MA, USA) was used for extraction and analyses of cinnamon. Also, acetic acid, n-hexane, Folin-Ciocalteu reagent, aluminium chloride, formic acid, sulphuric acid, sodium phosphate, ammonium molybdate, potassium ferricyanide, trichloroacetic acid, 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), tannic acid, epicatechin and quercetin were purchased from Sigma Aldrich BVBA (Belgium).

2.2. Preparation of *C. burmannii* oleoresins by traditional and ultrasonic extraction

The extraction of polyphenols from the *C. burmanni* bark was conducted using two different methods. The first method (traditional extraction) was adopted from Udayaprakash et al. (2015). Briefly, the cinnamon powder (5 g) was subjected to 50 mL of different types of solvents (acetone, methanol, ethanol, and water), and then stirred using a magnetic stirrer for 48h. The extraction was carried out at laboratory temperature (~20 °C). The second extraction was carried out by ultrasonication according to the method of Klejdus and Kováčik (2016). The same solvents as used in the traditional method were tested. The sample solution containing cinnamon powder and the solvent was placed in an ultrasonic bath (Elmasonic P30H, Elma Schmidbauer GmbH, Singen, Germany) for 1 h at a frequency of 80 kHz and at 30 °C and then manually stirred for 3 min until a homogenous solution was obtained. In each case, the residue was separated by filtration using a Whatman® paper and vacuum pump (Laboport, KNF Neuberger Inc., New Jersey, USA). Also, all extractions were carried out in triplicate. The aqueous extract was dried further using a freeze-drier (VaCo 5-D, Zirbus technology GmbH, Harz, Germany), whereas the other extracts were concentrated by a rotary evaporator (Laborota 4000, Heidolph Instruments GmbH, Schwabach, Germany) to obtain cinnamon oleoresins (concentrated cinnamon extract).

155

156 **2.3. Preparation of chocolate enriched with cinnamon oleoresin**

157 Cinnamon oleoresin was added into white chocolate in nano-encapsulated form as described in
158 our previous work (Muhammad et al., 2017). Dried cinnamon nanoparticles were made by the
159 anti-solvent precipitation method using shellac and xanthan gum as the wall materials. Briefly, 1
160 g of cinnamon oleoresin and 1 g of shellac fine powder were solubilised in 98 g of ethanol using
161 a magnetic stirrer. The mixture was injected to 0.2% (w/w) xanthan gum aqueous solution in the
162 ratio of 1:3. The mixture was maintained in a rotary evaporator to remove the solvent and then
163 lyophilised to obtain dry cinnamon nanoparticles. Its image is shown in the Supplementary
164 Information (S1).

165 The cinnamon nanoparticles were added into the molten white chocolate at a concentration of 2%
166 (w/w) using a Stephan Universal Machine UMC 5 (Stephan Food Service Equipment GmbH,
167 Hameln, Germany) at 35 °C. The chocolates were then manually tempered by a trained chocolatier,
168 and then moulded into chocolate bars. Furthermore, the chocolate bars were cooled at 12 °C for 2
169 h before storing at 20 °C for 14 days to ensure proper maturation. Also, white chocolate without
170 cinnamon extract was prepared as a control.

171 Prior to the polyphenol and antioxidant analysis, the chocolates were washed three times with *n*-
172 hexane with a ratio of 1:5 (m/V) to remove fat content following the protocol of Tuenter et al.
173 (2020). The samples were sonicated in a Branson 3510 ultrasonicator (Danbury, USA) for 10 min,
174 followed by centrifugation for 5 min at 3500 rpm using a Mega Star 1.6R centrifuge (VWR, Oud-
175 Heverlee, Belgium), and the supernatant was discarded. The defatted white chocolates were air-
176 dried for 24 h at room temperature in dark condition. The extraction of 0.5 g of the samples was
177 carried out with 10 ml of a mixture of acetone (70%), distilled water (29.8%) and acetic acid

178 (0.2%). Samples were vortex mixed (IKA MS1 Minishaker, IKAWerke GmbH & Co. KG,
179 Staufen, Germany) and then submitted to ultrasound-assisted solid–liquid extraction for 1 h, with
180 repetition of the vortex mixing after 30 min. Furthermore, the chocolate samples were centrifuged
181 for 5 min at 3500 rpm and the supernatant was collected. The extraction was repeated once more
182 and supernatants were combined. All extracts were prepared in triplicate per sample.

183

184 **2.4. Total phenolic content analysis**

185 The total phenolic content of the samples (cinnamon oleoresins and white chocolates) was
186 estimated according to the method described by Udayaprakash et al. (2015). The diluted sample
187 (200 µl) was added to 1 mL distilled water and 200 µL Folin-Ciocalteu reagent. The solution was
188 maintained at room temperature for 6 min before the addition of 2.5 mL 7% Na_2CO_3 solution and
189 2.1 mL distilled water. In order to obtain a stable coloured complex, the solution was incubated
190 for 90 min at room temperature (~20 ℃). The absorbance was measured at 760nm using a UV-
191 visible spectrophotometer (Varian Cary 50 Bio, Agilent Technology). The total phenolic content
192 was expressed as milligrams of epicatechin equivalents per gram of the plant extract (mg ECE/g
193 extract).

194

195 **2.5. Total flavonoid content analysis**

196 The determination of the total flavonoid content of cinnamon oleoresins was carried out according
197 to Udayaprakash et al. (2015). The extract (200 µL) was mixed with a solution of aluminium
198 chloride (0.1 M, 5 ml) and kept at room temperature for 40 mins. After incubation, the absorbance
199 value was measured at 415 nm and the total flavonoid content was expressed as milligrams of

quercetin equivalent per gram of the plant extract (mg QE/g extract) using a standard plot of quercetin.

2.6. UPLC-HRMS analysis of cinnamon oleoresins

Cinnamon oleoresins were analysed on an Acquity UPLC with XEVO G2-XS QTOF MS system. Its separation was achieved on an UPLC BEH Shield RP18 column (2.1 x 100 mm, 1.7 µm) and H_2O + 0.1% formic acid (A) and CH_3OH + 0.1% formic acid (B) as mobile phase. The following gradient was applied: 0-1 min 5% B, 5 min 15% B, 12 min 25% B, 15-17 min 30% B, 27-29 min 100% B, 31-36 min 5% B and the flow rate was 0.4 mL/min. Detection was carried out in both ESI- and ESI+ mode, *m/z* scan range 50-1500. V_{cap} 0.8 kV (ESI-) or 1.0 kV (ESI+), V_{cone} 40 V, source offset 80, T_{source} 120 °C, $T_{desolvation}$ 550 °C, cone gas 50 L/h, desolvation gas 1000 L/h. A set of 33 reference compounds (including several products, previously reported as constituents of cinnamon), were analysed under the same conditions, in concentrations ranging from 1 ng/ml to 10 µg/ml, and calibration curves were developed in order to allow a quantitative determination of these compounds in the extracts. A list of these reference compounds is presented in Supplementary Information (S2). Furthermore, data processing was done with MassLynx V4.1 and GraphPad Prism 6.

2.7 UPLC-HRMS analysis of white chocolates

The chocolate extracts were analysed with the UPLC-HRMS system described above based on the protocol of Tuenter et al. (2020). Separation was achieved on an UPLC HSS T3 column (2.1 x 100 mm, 1.8 µm), kept at 40 °C, and H_2O + 0.1% formic acid (A) and CH_3CN + 0.1% formic acid (B) as mobile phase. The following gradient was applied: 0-1 min 3% B, 7 min 15% B, 14 min 22%

B, 17 min 30% B, 22-24 min 100% B, 26-30 min 3% B; and the flow rate was 0.4 mL/min. Detection was carried out in ESI+ mode, *m/z* scan range 50-1500. V_{cap} 1.0 kV, V_{cone} 40 V, source offset 80, T_{source} 120 °C, $T_{desolvation}$ 550 °C, cone gas 50 L/h, desolvation gas 1000 L/h. A set of 53 reference compounds (including a wide range of compounds, previously reported in cocoa and/or cinnamon), were analysed under the same conditions, in concentrations ranging from 610 pg/mL to 5 µg/mL, and calibration curves were developed to allow a quantitative determination of these compounds in the samples.

2.8. Microstructural observation

Cryo-scanning electron microscopy (cryo-SEM) was used to observe the microstructural properties of the cinnamon powder before extraction and its residue after extraction. A JEOL JSM 7100F SEM equipped with a PP3010T Cryo-SEM Preparation System (Oxford Instruments, Oxfordshire, UK) was employed. The samples were placed on the cryo-specimen holder, and then cryo-fixed in slush nitrogen (-210 °C). Furthermore, the sample was transferred to the cryo-unit in the vitrified state, sublimated (20 min at -140 °C) and sputter coated with platinum (4 min, 0.5 mbar) to prevent charging of specimens with an electron beam. Finally, the sample was transferred into the microscope where it was observed at -140 °C.

2.9. Determination of antioxidant activity

2.9.1. Phosphomolybdenum method

The phosphomolybdenum method based on the protocol of Udayaprakash et al. (2015) was conducted for measuring the antioxidant activity of cinnamon oleoresins and white chocolates. Briefly, 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were

mixed at a ratio of 1:1:1 to create a reagent solution. Afterwards, 4.5 ml antioxidant reagent was mixed with 0.5 ml of the sample. The solution was kept in a water bath at 95 °C for 90 min. After reaching room temperature, the absorbance was measured at 695 nm. A standard plot of tannic acid was used to measure the total antioxidant content of the cinnamon extracts, and therefore, the total antioxidant content was expressed as milligrams of tannic acid equivalents per gram of the plant extract (mg TAE/g extract).

2.9.2 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) analysis of cinnamon oleoresins and white chocolates was based on the protocol of Udayaprakash et al. (2015). Phosphate buffer (0.2 M, pH 7) was prepared by mixing Na_2HPO_4 and NaH_2PO_4 in an appropriate ratio. The phosphate buffer (2.5 mL) was mixed with the sample (1 mL) and 1% potassium ferricyanide (2.5 mL) and incubated at 50 °C for 30 min. Afterwards, 2.5 mL of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 6500 rpm for 10 min. Furthermore, 2 ml of the supernatant was mixed with 2 ml distilled water and 0.4 ml of 0.1% $FeCl_3$. The absorbance of the solution was measured at 700 nm and the antioxidant power was expressed as mmol/L of ascorbic acid equivalents per gram of the plant extract.

2.9.3. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity analysis

The DPPH radical scavenging activity of the oleoresins was investigated by measuring the IC_{50} value of the samples (Udayaprakash et al., 2015). In brief, the sample was diluted to various concentrations with the appropriate solvent (10 – 100 µg/mL). Also, 100 µL of each sample was added into 4 mL DPPH 0.01 mM and its absorbance was read at 517 nm after incubated in dark

conditions and at room temperature for 30 min. BHA (butylated hydroxyl anisole) and gallic acid were used as the reference. The DPPH radical scavenging activity was calculated using Eq. 1.

$$\%\text{inhibiton} = \frac{(\text{Absorbance of the control (Ac)} - \text{Absorbance of the sample (As)})\text{x } 100}{\text{Absorbance of the control (Ac)}} \%\text{inhibiton} =$$

$$\frac{(\text{Absorbance of the control (Ac)} - \text{Absorbance of the sample (As)})\text{x } 100}{\text{Absorbance of the control (Ac)}} \quad \text{(Eq. 1)}$$

2.10. Statistical analysis

Statistical analyses were performed using SPSS Statistics 22. Also, analysis of variance (one-way ANOVA) was conducted to find the significant differences among the treatments. DMRT (Duncan's Multiple Range Test) was also adopted as a post-hoc test when significant differences were found. Also, the Independent Samples T-test was used to test the statistical differences between the antioxidant properties of the oleoresins obtained by the two different extraction methods (traditional and ultrasonic) and to further analyse the statistical differences between the antioxidant properties of the white chocolate with and without cinnamon oleoresin. The differences were considered significant at $p<0.05$. Also, the Pearson product-moment correlation test was conducted to determine the correlation coefficient between phenolic and flavonoid content and the antioxidant activity of the cinnamon oleoresins. GraphPad Prism 6 was adopted for statistical analysis of the UPLC-HRMS results. The paired Student's T-test was carried out and differences were considered significant at $p <0.05$.

3. Results and Discussions

3.1. Polyphenolic profile and antioxidant properties of *C. burmannii* oleoresins

The extraction of oleoresins from cinnamon powder using various solvents reported yields ranging from 14.4 to 29.8%, which is attributed to the different type of solvent and method. The yields of

292 the aqueous, acetonic, methanolic, and ethanolic oleoresins using the traditional method were
293 15.9±0.1% (w/w), 28.1±0.4% (w/w), 27.3±0.8 % (w/w) and 27.5±0.3 % (w/w), respectively;
294 while, those of the above-mentioned oleoresins using the ultrasonic method were 14.4%±0.1 %
295 (w/w), 28.4±1.0 % (w/w), 28.5±0.3 % (w/w) and 29.8±0.9 % (w/w), respectively.

296 The total phenolic and flavonoid contents of the cinnamon oleoresins are presented in Fig. 1. Each
297 gram of *C. burmannii* oleoresin contained phenols up to 310 mg ECE (epicatechin equivalents)
298 and possessed antioxidant activity up to 260 mg TAE (tannic acid equivalents) depending on the
299 mode of extraction. In the traditional method, methanol was the most efficient solvent to recover
300 phenolic constituents from cinnamon, followed by ethanol, acetone and water. The total phenolic
301 content of methanolic, ethanolic acetonic and aqueous cinnamon oleoresins were 309, 272, 215
302 and 126 310 mg ECE per gram oleoresin, respectively. Also, the total flavonoid content of
303 cinnamon oleoresin extracted using methanol, ethanol, acetone and water by traditional method
304 were cinnamon 30, 24, 26 and 17 mg quercetin equivalents (QE) per gram oleoresin. These results
305 are in agreement with the study of Dvorackova et al. (2015), who reported that methanol was better
306 than ethanol and water for the extraction of phenolic compounds from cinnamon.

307 Nevertheless, in the ultrasonic extraction, methanol was comparable with ethanol in extracting
308 phenolic constituents and flavonoids from cinnamon at a level of around 205 mg ECE and 26 mg
309 QE per gram oleoresin, respectively. The acetonic aqueous cinnamon oleoresins contained phenols
310 at a concentration of 195 and 190 mg ECE per gram oleoresin, respectively and flavonoids at a
311 level of 23 and 15 mg QE per gram oleoresin, respectively. Even though flavonoids are highly
312 soluble in water, the organic solvents in this study were more effective to extract it from cinnamon
313 than water. Zielinski and Kozlowska (2000) reported that phenol extraction with water was

inadequate because of its lower solvent specificity. As emphasised by Dvorackova et al. (2015), the recovery of polyphenols depends on the polarity of the solvent applied for the extraction.

Fig. 1 shows that the ultrasonic extraction resulted in a lower phenolic and flavonoid content than the traditional extraction. For instance, the total phenolic content of methanolic cinnamon oleoresins obtained by traditional and ultrasonication were 309 and 205 mg ECE per gram oleoresin, respectively. This finding is consistent with the results obtained by Dvorackova et al. (2015), who showed that an ultrasonic extraction was less effective to extract antioxidant compounds from cinnamon than a shaking method. However, in this study, the ultrasonic extraction is considered as a good process to recover phenolic compounds and flavonoids from cinnamon, as it recovered reasonably high amounts of phenolic and flavonoid compounds in a very short time (1 h). As explained by Duval et al. (2016), the benefit of ultrasonic extraction is the reduced removal time in addition to the possibility to use a wide range of solvents. Also, the traditional extraction technique is relatively long and time-consuming, since it is only based on the extracting power of different solvents using mixing. However, this method is simple and inexpensive. Therefore, this technique is still much in use to obtain bioactive compounds from many plant materials till date.

Furthermore, to investigate phenolic constituents of the cinnamon oleoresins prepared by the traditional and ultrasonic methods, a UPLC-HRMS analysis was employed. A total of 25 compounds were identified in the traditional and ultrasonicated methanolic oleoresins of *C. burmannii* (Table 1). Also, the chromatograms of the cinnamon oleoresins are shown in Supplementary Information (S3). Shan et al. (2007) investigated *C. burmannii* by GC-MS and LC-MS, and in addition to cinnamic acid, catechin and epicatechin, they tentatively identified a limited number of procyanidins. In this research, a more extended number of procyanidins was tentatively

337 identified, based on their specific m/z values. However, only procyanidin B_2 was identified with
338 certainty due to the unavailability of reference compounds. For the other procyanidins, only the
339 type and/or number of subunits were defined. Also, eight of the identified compounds were present
340 in levels that enable their quantification (Table 2). Klejdus and Kováčik (2016) reported the levels
341 of a number of phenolic compounds in *C. zeylanicum* and *C. cassia* after LC-MS/MS analysis with
342 cinnamic acid and protocatechuic acid as the major phenolic constituents. In this study,
343 protocatechuic acid was identified as one of the major phenolic acids of the C. burmannii , while
344 cinnamic acid was only detected in low levels.

345 In the UPLC-HRMS analysis, it was shown that ultrasonication of cinnamon extract resulted in a
346 higher catechin, epicatechin, procyanidin B2, 3,4-dihydroxybenzaldehyde, cinnamic acid and
347 salicylic acid content. Rao and Rathod (2015) reported that during ultrasonic extraction, ultrasound
348 promotes swelling and hydration of vegetal tissue, induces high diffusion rates across cell walls
349 and enhances mass transfer. The ultrasonic waves also cause a cavitation effect, which induce
350 particle disintegration and fast inter-particle collisions. Also, fractures and disruption of cell walls
351 facilitate the extracting solvent infiltration into the tissue and the diffusion of solute from the solid
352 phase to the solvent.

353 More so, the difference on surface morphologies of the cinnamon powder before and after
354 extraction is shown in Fig. 2. The raw powdered cinnamon bark had an irregular shape with a
355 rough surface (Fig. 2A). After extraction using the traditional method with methanol, visible
356 physical changes on the cinnamon powder was observed. As shown in Fig. 2B, the powder
357 appeared swollen and expanded, indicating that it absorbed the solvent during extraction for 48 h.
358 The absorption initiated a rupture of the tissue inside the cinnamon particle, and subsequently the
359 bioactive compounds in the cinnamon powder dissolved easily. Fig. 2C demonstrates the surface

morphology of the cinnamon powder after ultrasonication. In the ultrasonic treatment, a high-level disruption is observed. This enables the solvent to penetrate into the tissue, consequently, the cinnamon constituents quickly diffused from the solid phase to the solvent. In traditional extraction however, there was a mild disruption, while a more pronounced disruption was identified in the ultrasonic extraction. Furthermore, to verify the ultrasonic effect, the cinnamon powder collected after the traditional and the ultrasonic extraction using ethanol were observed. As shown, the phenomenon was similar to the cinnamon powder collected after the extraction using methanol (Fig. 2D and Fig. 2E). Remarkably, the rupture of the residue of cinnamon powder extracted with methanol were more pronounced compared to ethanol. This explains why the methanolic extract possessed a higher phenolic and flavonoid content than ethanolic extract.

It is important to note that the total phenolic content of the cinnamon extract obtained by the traditional method was higher than the ultrasonic method (Fig. 2). However, the result of UPLC-HRMS analysis shows that some phenolic constituents (catechin, epicatechin, procyanidin B2, cinnamic acid and salicylic acid) were more abundant in the cinnamon extract obtained by the ultrasonic method compared to the traditional method (Table 2). This discrepancy is due to the different methodologies used. The total phenolic content was measured by the Folin-ciocalteu method. In this method, polyphenol compounds are oxidised by the Folin-ciocalteu reagent (a basic medium consisting of phosphotungstic and phosphomolybdic acid). The Folin-ciocalteu reagent reacts with any phenolic group in the cinnamon, including monomeric and polymeric phenols. However, the quantification of phonelic compounds using the UPLC-HRMS was carried out on a limited set of compounds only. Therefore, even though some phenolic compounds were more abundant in the cinnamon extract obtained by the ultrasonic extraction compared to the traditional extraction, it does not imply that the total phenolic content in that sample was also higher.

Several articles described the significant role of phenolic compounds in the antioxidant capacity of a plant extract (Udayaprakash et al., 2015; Dvorackova et al., 2015; Przygodzka et al., 2014) this study, therefore, was continued by investigating the antioxidant activity of the concentrated extracts of *C. burmannii*. The possible mechanism of action of phenolic antioxidants of cinnamon oleoresins is that they stabilise radicals through hydrogen atom donation to form antioxidant radicals. The antioxidant radicals are more stable and less accessible to stimulate auto-oxidation (Muhammad et al., 2017). It was discovered that methanolic oleoresin exhibited the highest antioxidant activity among the oleoresins based on phosphomolybdenum method and FRAP assay as shown in Fig. 1C and Fig. 1D. Also, cinnamon oleoresins extracted by traditional method using methanol, ethanol acetone and water exhibited antioxidant activity at a level of 260, 207, 181 and 157 mg tannic acid equivalents (TAE) per gram oleoresin, respectively, and showed ferric reducing antioxidant power at a level of 2673, 2483, 2582 and 1007 mmol/l ascorbic acid equivalents (AAE) per g oleoresin.

To confirm its antioxidant power, a determination of the DPPH radical scavenging activity in terms of the IC_{50} value was carried out (Table 3). Similar to the result of the previous assays, the methanolic oleoresin was the best antioxidant, followed by the ethanolic, acetonic and aqueous oleoresins. The IC_{50} of cinnamon oleoresins were found at 31.5 and 33.3 µg/mL for the samples obtained by traditional and ultrasonication method, respectively using methanol. Furthermore, extraction using water resulted in oleoresins with the least power in scavenging DPPH radical. The IC_{50} value of cinnamon oleoresins obtained by traditional and ultrasonication method, respectively, using water were around 93 and 61 µg/mL. Also, the DPPH radical scavenging activity of the water concentrated extracts was lower than that of the other type of oleoresins, since it contained less phenolic compounds (Fig. 1). This outcome corresponds to the results obtained by Chua et al.

406 (2008) who reported that an aqueous extract of cinnamon contains less phenolic compounds and
407 was less effective in inhibiting radical activity than a methanolic extract of cinnamon. To
408 investigate the relationship of phenolic compounds and antioxidant activity of the cinnamon
409 oleoresins, Pearson product-moment correlation tests were performed and it was found that the
410 antioxidant activity of the concentrated extracts was positively and significantly related with the
411 total phenolic and flavonoid contents (Supplementary Information, S4). More so, the antioxidant
412 activity of the cinnamon oleoresins was lower than that of epicatechin. Interestingly, the oleoresins
413 obtained by the organic solvents possessed a higher antioxidant capacity than BHA, indicating that
414 the *C. burmannii* oleoresins can be potentially used as a natural antioxidant thereby substituting
415 synthetic antioxidants.

416

417 **3.2. Phenolic content and antioxidant activity of white chocolate enriched with cinnamon**
418 **oleoresins**

419 The cinnamon nanoparticles were added to white chocolate at the level of 2% (w/w) which equals
420 an addition of 1% (w/w) of cinnamon oleoresin. The addition of the cinnamon nanoparticles in
421 white chocolate was determined based on the information obtained from Hong et al (2012) that
422 the minimum extract containing polyphenol (8.8%) administered in the body to have anti-
423 inflammatory activity was 20 mg/kg body weight per day. Consider an average body weight of
424 70kg of a person and a total of 100mg/g cinnamon oleoresin, then white chocolate with 2%
425 cinnamon nanoparticles is likely sufficient to fulfil minimum polyphenol level in a piece of
426 chocolate (20 g) consumed 3 times per day. The incorporation of cinnamon significantly increased
427 the total phenolic content, antioxidant activity and FRAP activity of white chocolate as shown in
428 Table 4. The findings of the increased total polyphenolic content in the chocolates enriched with

cinnamon nanoparticles was supported by the results of the UPLC-HRMS analysis. In the chromatographic profiles of the samples, it was found that various compounds could be detected in the white chocolate supplemented with the cinnamon oleoresin, however, not in the white chocolate controls. Some compounds quantified in the cinnamon extract were found in significant levels in the white chocolate with cinnamon (Table 4). These compounds include various procyanidin dimers, trimers and tetramers, which were tentatively identified based on their *m/z* values, and were also found in the traditional and sonicated cinnamon extracts (see Table 1 for the *m/z* values).

Most compounds quantified in the cinnamon oleoresins were also found in significant levels in the white chocolate enriched with cinnamon. An exception is quercitrin, which was detected in the white chocolate enriched with cinnamon, however, it was not quantified due to its low concentration (<LOQ = <0.4 µg/g defatted chocolate). Cinnamic acid was tentatively identified in the white chocolate with cinnamon (*m/z* 147.0447, $[M-H]^-$), yet, not in the white chocolate controls. As the calibration curve of the standard compound was not satisfactory, therefore, no results of its quantification were shown. In addition to the compounds quantified in the cinnamon extracts, apigenin and *p*-hydroxybenzaldehyde were also quantified in the white chocolate control, and in the white chocolate with cinnamon, while they were not present in significant levels in the cinnamon extract. Therefore, the apigenin and *p*-hydroxybenzaldehyde most probably originate from the white chocolate itself. Chromatograms of the white chocolate with and without cinnamon nanoparticles are shown in Supplementary Information (S5).

Therefore, it is concluded that the addition of cinnamon extract to white chocolate result in an end-product rich in polyphenols, including catechin, epicatechin, procyanidin B_1, procyanidin B_2 and protocatechuic acid. However, it is important to note that the knowledge of phenolic content and

antioxidant activity in food are useful for a preliminary prediction, however, it is insufficient to assess the overall health effects of the chocolate enriched with cinnamon oleoresin. Therefore, further studies on the bioavailability of cinnamon oleoresins constituents incorporated in white chocolate matrix are required. In addition, as discussed in the previous work of Muhammad et al. (2018), the incorporation of cinnamon oleoresin resulted in the distinctive characteristics of the chocolates to some extent. Therefore, a consumer test would be desirable to investigate the consumer acceptance of the cinnamon-enriched chocolate on the market.

4. Conclusions

In the present study, bioactive compounds of *C. burmannii* were identified and the antioxidant activity of the *C. burmannii* extract was studied. The extraction technique had a significant influence on the phytochemical composition of the cinnamon extract. UPLC-HRMS analysis revealed the presence of various phenolic compounds, such as catechin, epicatechin, quercitrin and protocatechuic acid in the *C. burmannii* extract. Also, the antioxidant activity of cinnamon extract was positively correlated with its phenolic content, therefore, corresponding to the common theoretical recognition of the antioxidative properties of phenols. Incorporation of cinnamon extract significantly increased the catechin, procyanidin B1, procyanidin B2, and protocatechuic acid contents as well as antioxidant activity of white chocolate. This knowledge is useful for further application of cinnamon extract in different food and pharmacological formulations. Natural antioxidants derived from cinnamon may help in preventing oxidative stress in the human body. However, to challenge this hypothesis, further research regarding the absorption of the bioactive compounds of cinnamon in the gastro-intestinal tract is required. Furthermore, evaluation on the

consumer acceptance of white chocolate incorporated cinnamon oleoresins might also be interesting.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Phytochemicals identified in methanolic *C. burmannii* extracts by UPLC-HRMS. Identification was based on comparison to authentic reference compounds, or, in the case of procyanidin dimers, trimers, and tetramers, on their specific *m/z* values and comparison to literature data.

Compound number	Compound	Molecular formula	Retention time (min.)	*m/z*
1	**Protocatechuic acid**	$C_7H_6O_4$	3.66	153.0189 $[M-H]^-$
2	**3,4-dihydroxybenzaldehyde (protocatechualdehyde)**	$C_7H_6O_3$	4.08	137.0237 $[M-H]^-$
3	**Catechin**	$C_{15}H_{14}O_6$	6.64	289.0727 $[M-H]^-$
4	Procyanidin dimer, A type	$C_{30}H_{24}O_{12}$	7.48	575.1187 $[M-H]^-$
5	Syringic acid	$C_9H_{10}O_5$	7.68	197.0448 $[M-H]^-$
6	Procyanidin dimer, A type	$C_{30}H_{24}O_{12}$	8.74	575.1184 $[M-H]^-$
7	**Epicatechin**	$C_{15}H_{14}O_6$	8.96	289.0726 $[M-H]^-$
8	**Procyanidin B2**	$C_{30}H_{26}O_{12}$	9.05	577.1360 $[M-H]^-$
9	Procyanidin dimer, A type	$C_{30}H_{24}O_{12}$	11.13	575.1181 $[M-H]^-$
10	Procyanidin trimer, A type	$C_{45}H_{36}O_{18}$	11.74	863.1776 $[M-H]^-$
11	Procyanidin trimer, A type	$C_{45}H_{36}O_{18}$	11.99	863.1797 $[M-H]^-$
12	procyanidin dimer, A type	$C_{30}H_{24}O_{12}$	12.91	575.1187 $[M-H]^-$
13	procyanidin dimer, A type	$C_{30}H_{24}O_{12}$	13.50	575.1198 $[M-H]^-$
14	Procyanidin tetramer, A type	$C_{60}H_{48}O_{24}$	14.42	1151.2428 $[M-H]^-$
15	Procyanidin tetramer, A type	$C_{60}H_{48}O_{24}$	14.69	1151.2428 $[M-H]^-$
16	Procyanidin tetramer, A type	$C_{60}H_{48}O_{24}$	14.86	1151.2428 $[M-H]^-$
17	Procyanidin trimer, A type	$C_{45}H_{36}O_{18}$	14.99	863.1799 $[M-H]^-$
18	Procyanidin tetramer, A type	$C_{60}H_{48}O_{24}$	15.15	1151.2428 $[M-H]^-$
19	Procyanidin trimer, A type	$C_{45}H_{36}O_{18}$	15.95	863.1799 $[M-H]^-$
20	**Cinnamic acid**	$C_9H_8O_2$	17.00	147.0447 $[M-H]^-$
21	Rutin	$C_{27}H_{30}O_{16}$	19.73	609.1516 $[M-H]^-$
22	**Quercitrin (quercetin-3-rhamnoside)**	$C_{21}H_{20}O_{11}$	20.87	447.0966 $[M-H]^-$
23	Coumarin	$C_9H_6O_2$	14.69	147.0453 $[M+H]^+$

585 Note: Compounds in bold were present in levels that allowed for their quantification.

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Table 2. Levels of selected compounds (µg/g dry material) in methanolic extracts of *C. burmannii*, prepared by traditional and ultrasonic extraction

Compound	Traditional extraction	Ultrasonic extraction
Catechin	14.9 ± 3.7 *	51.1 ± 5.0 *
Epicatechin	15.6 ± 3.2*	52.5 ± 3.2*
Procyanidin B2	984.0 ± 62.4 *	1396.0 ± 94.6 *
Quercitrin	16.1 ± 1.3	13.23 ± 0.6
3,4-dihydroxybenzaldehyde	1078.0 ± 195.3	1138.0 ± 55.4
Protocatechuic acid	193.1 ± 13.7 *	227.8 ± 7.3 *
Cinnamic acid	828.3 ± 113.5 *	934.2 ± 477.9 *

Data are means ± SDs (n = 4). Notation (*) indicates significant differences between extraction methods at 0.05 level of student's t-test

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Table 3. The IC_{50} value of *C. burmannii* oleoresins prepared by different solvents and methods determined by DPPH assay

Type of oleoresin	IC_{50} (µg/mL)	
	Traditional extraction	Ultrasonic extraction
Water	92.9 ± 0.7^{dA}	60.9 ± 0.7^{cB}
Acetone	46.5 ± 0.3^{cA}	45.4 ± 1.3^{bA}
Methanol	31.5 ± 0.4^{aA}	33.3 ± 0.2^{aB}
Ethanol	35.9 ± 1.8^{bA}	33.0 ± 0.5^{aA}
Reference		
BHA	75.6 ± 0.2	
Epicatechin	21.6 ± 0.7	

Mean values with the same lowercase and uppercase letter do not differ significantly ($p>0.05$) in the same columns and the same rows, respectively.

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Table 4. Improvement of bioactive compound content and antioxidant properties of white chocolate by incorporation of cinnamon

	White chocolate control	**White chocolate with cinnamon**
*Amount of selected compounds (per g defatted chocolate)**		
Catechin (µg)	< LOQ (0.050)	0.481 ± 0.046
Epicatechin (µg)	1.343 ± 0.074	2.034 ± 0.119
Procyanidin B1(µg)	< LOQ (1.66)	6.509 ± 0.636
Procyanidin B2 (µg)	< LOQ (1.53)	12.959 ± 1.049
3,4-dihydroxybenzaldehyde (µg)	0.043 ± 0.002	1.355 ± 0.078
p-hydroxybenzaldehyde (µg)	0.147 ± 0.006	0.241 ± 0.023
Protocatechuic acid (µg)	< LOQ (0.100)	5.589 ± 0.392
Apigenin (µg)	0.153 ± 0.005	0.112 ± 0.008
*Antioxidant properties (per g defatted chocolate) **		
Total phenolic content (µg epicatechin equivalent)	47.6 ± 46.7	1060.6 ± 32.3
Total antioxidant activity (mg tannic acid equivalent)	0.4 ± 0.2	2.3 ± 0.6
FRAP activity (µmol l^{-1} ascorbic acid equivalent)	48.5 ± 11.7	2530.1± 86.5

*Data are means ± SDs (n = 6). **Data are means ± SDs (n = 3).
FRAP = Ferric Reducing Antioxidant Power

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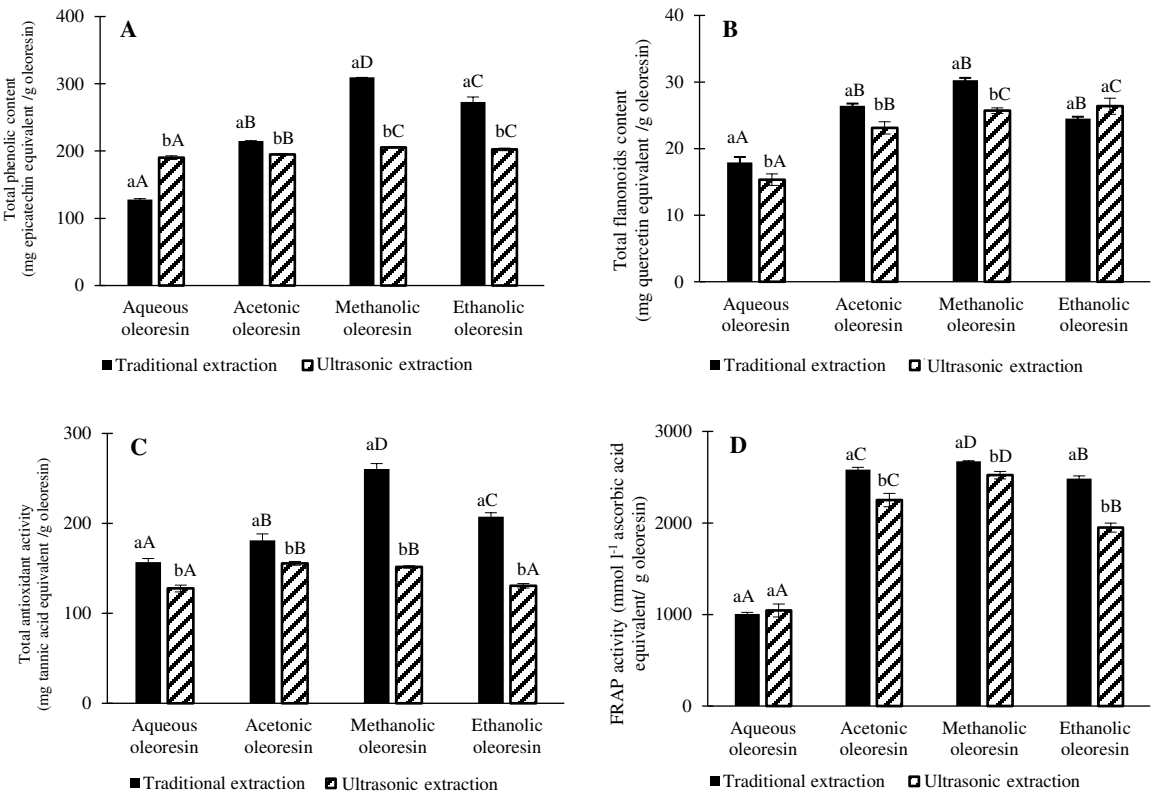

Fig. 1. Total phenolic content (A), total flavonoids content (B), total antioxidant activity (C) and Ferric Reducing Antioxidant Power (FRAP) activity (D) of *C. burmannii* oleoresins prepared by different solvent and extraction methods. Samples with different lowercase letters differ significantly in the same type of oleoresin. Samples with different uppercase letters differ significantly in the same extraction method ($p<0.05$).

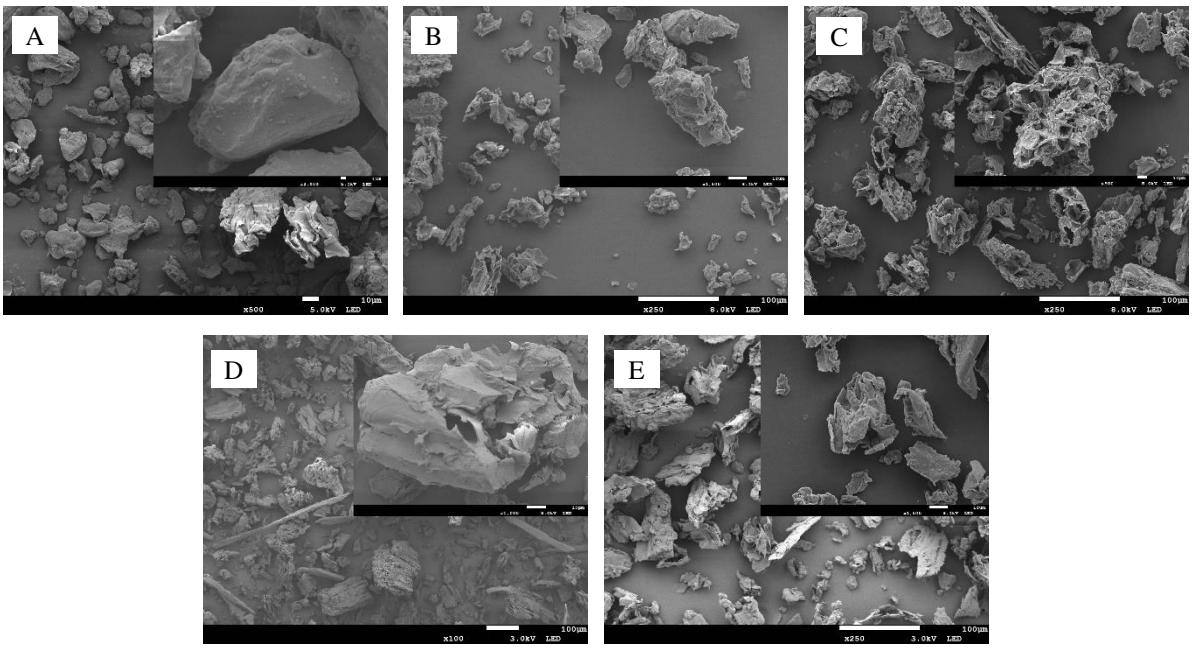

Fig. 2. Cryo-SEM images of the surface morphology of C. burmannii powder: before extraction (A); after traditional extraction using methanol (B); after ultrasonic extraction using methanol (C); after traditional extraction using ethanol (D); and after ultrasonic extraction using ethanol (E).