The solvents influence in the continuous extraction to antioxidant and α-glucosidase inhibition of Cinnamomum burmannii bark

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Abstract

The proanthocyanidins, the polyphenolic compounds from Cinnamomum burmannii (Lauraceae), have been known for their insulin-like effects. The different use of solvents and extraction methods would influence the bioactive phytochemicals. This research examined the solvents used in the continuous extraction method to provide a higher yield of bioactive extracts. The use of ethanol only compared to the gradual polarity of n-hexane, ethyl acetate, and ethanol in the soxhletation to the standardized dried cinnamon bark. The extracts were then analyzed for their total phenolic content, antioxidant, and α-glucosidase inhibition. The extracts contained essential oils, tannins and flavonoids, triterpenoids, coumarins, and glycosides. The ethanolic solvent provides a higher yield and total phenolic content compared to other extracts. The ethanolic extracts phenolic content was highest, and its IC₅₀ DPPH antioxidant activity was no significant difference from rutin. The IC₅₀ α-glucosidase inhibition of most extracts was lower than acarbose, thus classified as vital to moderate activity. The correlation analysis preceded a negative result of total phenolic content to α-glucosidase inhibition activities. These results supported the beneficial use of C. burmannii bark ethanol extract as α-glucosidase inhibitor and oxidative stress relieving in diabetes dietary supplements.

1. Introduction

Type 2 Diabetes Mellitus (T2DM), a chronic endocrine metabolic disease, has dramatically increased in world statistics for the last decade (International Diabetes Federation (IDF), 2021). The main therapeutic goal for T2DM patients’ therapy is to maintain blood glucose at a normal level to prevent complications. This may be achieved by diet and lifestyle adjustments to the use of synthetic and natural medicine. This effort may improve the capacity production of pancreatic islet cells and insulin qualities. Among plants, the Cinnamons have been known for positive results in clinical improvement of glycemic control (Megadama, 2015).

The Cinnamomum genus has approximately 250 species, with different types and varieties. They spread across Southeast Asia, China, and Australia. They contain phytochemicals that are beneficial for human life. Cinnamon’s phytochemicals are used as spices in food, beverages, cosmetics industries, and traditional and modern medicine. Among the species, Cinnamomum burmannii is mainly found in Sumatra and Java islands in Indonesia (Sangal, 2011). Prior research has found the water extract of C. burmannii phenolics content and observed their antioxidant and α-glucosidase inhibitor activities (Ervina et al., 2019). These findings support the use of Cinnamon in preventing oxidative stress in diabetes insulin resistance pathogenesis and complications (Khan et al., 2015; Kanwugu et al., 2022). Moreover, Cinnamon’s phytochemicals showed α-glucosidase inhibition activity similar to acarbose, thus promising for T2DM therapy.

The extraction method and the solvents influenced the extract’s phytochemical and pharmacological properties. The polar solvents are frequently used to extract phenolic compounds from plants. Moreover, continuous extraction with ethanol is suitable for heat-stable phenolic compounds. Thus, this research examined the solvents used in continuous extraction (soxhletation) with ethanol only to gradient solvent extraction with n-hexane, ethyl acetate, and ethanol on
C. burmannii bark. The extracts were then evaluated on their phytochemicals, total phenolic content (TPC), in vitro DPPH (2,2-diphenyl-1-pyrlyhydradrazil) antioxidant activity (AA), and α-glucosidase inhibition (αGI). This work aimed to obtain a more efficient extraction method for the Cinnamomum (higher yield and activities) bark than the previous water extract (Ervin et al., 2019).

2. Materials and methods

2.1 Materials

A local representative provided the bark for medicinal plants (Materia Medica Batu, Indonesia). The solvents and reagents are obtained from local suppliers.

2.2 Sample preparation and continuous extraction

The bark was prepared as described by Ervin et al. (2019) and standardized its quality parameters according to the monograph (Ministry of Health Republic of Indonesia, 2008). The continuous extractions were divided into two parts, which were ethanolic and gradual polarity of n-hexane, ethyl acetate, to ethanol soxhletation. Both were carried out until the dripped filtrate from the siphon was colorless (90°C, approximately 4 hrs for each solvent). The extract was evaporated on the rotary vacuum evaporator until viscous or dry extracts. The four final extracts were coded as T (total extract) for the ethanolic extract), EA (ethyl acetate extract), and EE (ethanolic extract) for the latter products. All obtained extracts were examined for their identity, physical characteristics (color, odor), and water content.

2.3 Determination of total polyphenolic content

The total phenolic content (TPC) of extracts was determined with Folin Ciocalteau (FC) method based on Ervin et al. (2019). The 0.02 mL extracts or gallic acid (GA), mix thoroughly with 10% FC reagents (0.1 mL) and Na₂CO₃ 7.5% (0.08 mL). The microplate was incubated in a dark condition for 1 hr at room temperature. The absorbance was measured with a multiscan GO Microplate Reader UV/Vis Spectrophotometer at 765 nm (multiscan GO Microplate Reader UV/Vis Spectrophotometer). The absorbance was plotted to the linearity of enzyme concentration to the absorbance curve. The optimum enzyme concentration was chosen at 3 U/mL. This concentration was applied in the further α-GI determination. The IC₅₀ of the samples and acarbose were obtained by each sample’s concentration versus % α-glucosidase inhibition.

2.5 In vitro antioxidant activity assay

The DPPH scavenging method was performed according to Ervin et al. (2019). The antioxidant activity is expressed as IC₅₀. Rutin and gallic acid were used as the reference standard.

2.6 Statistical analysis

The results are presented as mean and standard deviation values from the triplicate experiments. The significant difference and the correlation among data were obtained using one-way ANOVA (p-value < 0.05) with SPSS version 24 software.

3. Results and discussion

T2DM is a metabolic disease directly related to insulin quality and production. Moreover, radicals and oxidative stress have recently played a role in diabetes pathogenesis. Some processes are increasing production of free radicals, which are glucose autoxidation, disproportions on cellular oxidation or reduction, reducing antioxidant defenses, increasing some prooxidants, and interaction of advanced glycation end products (AGEs) with AGE receptors. Furthermore, diabetes complications are assumed to be caused by oxidative stress (Kanwugu et al., 2022)

Cinnamon has been used as a supplement in managing T2DM therapy. Though their coumarins contents arose attention since their hepatotoxicity side effect on human health. The Cinnamomum zeylanicum and C. burmannii are considered safer than Cinnamomum cassia since their higher coumarin content in the latter species. The antidiabetic mechanism of cinnamon was proposed as glucose transporter-4 (GLUT -4) receptor synthesis and translocation. It acted as an
insulin receptor in the auto-, and de-phosphorylation, or via pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) in modulating hepatic glucose metabolism. It may also alter the expression of PPAR (γ) and inhibit intestinal glucosidas. In animal models, the cinnamon reduced fasting, postprandial plasma glucose, and HbA1c. Furthermore, the aqueous or powder form of C. cassia has been tested in clinical trials. The improvement in glycemic control was seen in patients who take Cinnamon only, in those with pre-diabetes and high pre-treatment HbA1c (Megadama, 2015). Anderson et al. (2016) found that aqueous extracts of C. cassia have insulin-like effects by increasing glucose uptake into adipocytes. Previous results also obtained that the water extract of C. burmannii was comparable to the acarbose reference (Ervina et al., 2019). Thus, this research aimed to improve the efficiency of the extraction method. The continuous extraction (soxhletation) with a different sequence of solvents was applied, which were ethanol only compared to polar gradient solvents from n-hexane, ethyl acetate, and ethanol regarding its yield contents and AA to α-GI activities.

The quality sample of the dried cinnamon was established and compared to the national standard. The results showed the model’s character and quality complied with the cinnamon characteristic standard and sequential to reported by Ervina et al. (2016). The pharmacognosy standardized qualities of C. burmannii bark are presented in Table 1.

The phytochemical screening of the extract was conducted with the TLC method (mobile phase toluene: ethyl acetate 7: 3) and sprayed with specific spotting reagents (Ervina et al., 2019). The results observed polyphenol, tannin and flavonoids, essential oils, saponins, quinone, triterpenoids, glycosides, and coumarin in the extracts. The TLC of T and EE showed differences in their chromatogram pattern. The EE content was less and simple than ET. The cinnamaldehyde’s purple spot at retention factor (Rf) 0.87 was presented on all extracts. These findings were consistent with previous research and added content information of glycoside, coumarin, and cinnamaldehyde which did not observe in Cinnamon’s water extract (Ervina et al., 2019). Moreover, the phenolics and flavonoid compounds were observed as brighter fluorescence spots with AlCl3 spray reagent, on the initial line at the plate of T, EA and EE. This highlighted the polarity of the compound with one or more aromatic rings or hydroxyl groups, which binds stronger on the silica gel polar stationary phase. The previous finding was also observed in the polyphenolics proanthocyanidin and quercetin from the Cinnamon’s water extract. Both have antioxidant and inhibited α-glucosidase. These findings were consistent with previous research (Ervina et al., 2016; Ervina et al., 2019) and added content information on glycoside and coumarin in C. burmannii (Ervina et al., 2019).

The quality of extracts was determined in Table 1. The yield of the extracts was in a range from 1.17±0.14% to 24.88±0.89% (Table 2). The yield of n-hexane extract was the lowest quantity of non-polar extracted substances. This included volatile oil and the nonpolar compound of the bark. The best yield was produced by ethanolic extract (T). This explains the T as the total extract, which contained most of Cinnamon’s contents. The EE was Cinnamon’s polar fractions, which were separated from non- and semi-polar substances in the prior solvents used. The statistical analysis showed a significant difference in the ethanolic extract (T) yields.

Table 1. Pharmacognosy quality parameters of dried and C. burmannii extract.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dried bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Cinnamomum burmanii bark</td>
</tr>
<tr>
<td>Appearance</td>
<td>roll shape with a length of 15-22 cm, typical appearance and specific odour of cinnamon, reddish-brown colour</td>
</tr>
<tr>
<td>Ethanol soluble extractive content (%)</td>
<td>27.02±0.42</td>
</tr>
<tr>
<td>Water-soluble extractive content (%)</td>
<td>9.95±0.32</td>
</tr>
<tr>
<td>Total ash content (%)</td>
<td>4.50±0.19</td>
</tr>
<tr>
<td>Drying shrinkage (%)</td>
<td>5.35±0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extracts</th>
<th>T</th>
<th>EH</th>
<th>EA</th>
<th>EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Cinnamomum burmanii extractum siccum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appearance</td>
<td>Reddish-brown dried extract, cinnamon typical smell.</td>
<td>Yellowish-brown viscous extract, cinnamon typically aroma.</td>
<td>A reddish-brown dried extract, cinnamon commonly smell.</td>
<td>A reddish-brown dried extract, cinnamon commonly smell.</td>
</tr>
<tr>
<td>Drying shrinkage</td>
<td>7.02±0.08%</td>
<td>-</td>
<td>-</td>
<td>6.69±0.14%</td>
</tr>
</tbody>
</table>

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The TPC with FC method was examined for all extracts, and rutin as gallic acid equivalent (Table 2). The TPC of the samples ranged from 151.11±8.15; to 328.54±6.45; while rutin of 369.62±9.80 µg GAE/mg samples; in which all showed significant different statistics groups. These results were higher than the previous Cinnamon water extract (Ervina et al., 2019). The ethanolic extracted most of plant’s contents. Akomolafe et al. (2014) observed that tannic and gallic acid suppressed ROS generation, lipid peroxidation, and oxidative stress in kidney tissues and exhibited the nephroprotective effect. Gallic acid is produced by tannic acid oxidizing or dilute sulphuric acid hydrolyzing. The statistical analysis of TPC obtained no significant difference between ethanolic extract. It showed that ethanolic solvent (T) was more efficient in extracting polyphenolic compounds and no significant difference in the use of gradient polar solvent. According to Huang et al. (2002), most phenolic compounds are hydrophilic. They are more soluble in the solvent with high index polarity, such as ethanol.

The DPPH free radical is a common chemical used in antioxidant screening for scavenging activity. The antioxidant activity of extracts was concentration-dependent (Table 2). The statistical analysis showed no significant difference between ethanolic, ethyl acetate extracts, gallic acid, and rutin. This result indicated that Cinnamon extracts have antioxidant potency compared to both reference compounds. The latter substances are glycoside flavonoids and polyphenolic compounds. The lowest activity of DPPH scavenging was observed on n-hexane extract, which was supposed to correlate to the low potency of Cinnamon essential oils as electron or proton donors. This result was also supported by the TLC autographic of the extracts and sequence to the previous conclusion (Ervina et al., 2016). The proanthocyanidins, another cinnamon’s contents, are naturally occurring compounds widely found in fruits, vegetables, nuts, seeds, flowers, and the bark. The molecular structure describes it as polyphenolic compounds, oligomers, or polymers of polyhydroxy-flavan-3-ol units, which is consisting of (+)-catechin and (−)-epicatechin (Figure 1). This compound has antioxidant activity and acts moreover as an inhibitor against some cancer cell line growth (Lin et al., 2016).

Table 2. The yields percentage, total phenolic content, DPPH scavenging activity, and α-GI of C. burmannii extracts.

<table>
<thead>
<tr>
<th>Extract or reference compound</th>
<th>Yield (%)</th>
<th>TPC (µg GAE equivalent/mg sample)</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>24.88±0.89</td>
<td>328.54±6.45</td>
<td>16.80±0.37</td>
</tr>
<tr>
<td>EH</td>
<td>1.17±0.15</td>
<td>151.11±8.15</td>
<td>5812.36±287.06</td>
</tr>
<tr>
<td>EA</td>
<td>3.05±0.09b</td>
<td>243.79±4.64</td>
<td>20.83±0.17</td>
</tr>
<tr>
<td>EE</td>
<td>22.71±0.96c</td>
<td>292.65±17.73</td>
<td>17.29±0.40</td>
</tr>
<tr>
<td>R</td>
<td>--</td>
<td>369.62±9.80</td>
<td>15.60±0.92</td>
</tr>
<tr>
<td>GA</td>
<td>--</td>
<td>--</td>
<td>4.47±0.94</td>
</tr>
<tr>
<td>A</td>
<td>--</td>
<td>--</td>
<td>104.40±1.30c</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different at α = 0.05.
activity of the α-glucosidase enzyme. The chosen level of the enzyme was 3 U/mL since it showed in the middle step the exponential rate of the enzyme. Table 2 shows the IC$_{50}$’s extract comparable to the acarbose. The IC$_{50}$ was in the range of 0.42±0.01 to 10.33±0.32; while acarbose was 104.40±1.30 µg/mL. The IC$_{50}$ α-GI of the extract was 10 – 300 higher than acarbose as an oral anti-diabetic reference drug (Figure 2). The IC$_{50}$ α-GI of total and ethanolic extracts was similar to the rounding calculation result. ANOVA analysis showed significant differences among all extracts and references, except for ethanolic extracts. The IC$_{50}$ α-GI indicates no difference between continuous ethanolic and gradual use of polar solvent on soxhletation to the potency of the cinnamon extract as α-glucosidase inhibitor agents. Salehi et al. (2013) determined that C. zeylanicum methanol extract was stronger than acarbose, and so did Shihabudeen et al. (2011).

This study found that α-glucosidase inhibition is supposed to be due to the higher total phenol content of ethanolic extracts compared to n-hexane and ethyl acetate extracts (Figure 2). The correlation analysis of TPC, AA, and α-GI exhibited negative (-0.873) results at a significance of α 0.01 level. This result was described in a 3D graph among the factors (Figure 3). The relationship among those three was also obtained by Ervina et al. (2019) and Miao et al. (2016), who found a correlation among Hawthorn fruit content of polyphenols and triterpenoids, protocatechuic acid and epicatechin to the α-glucosidase inhibition activity. Moreover, they observed the contribution of flavonoids, polyphenols, vanillic acid, gallic acid, catechin, and chlorogenic acid to its antioxidant activity. They also obtained the highest DPPH scavenge and ferric reducing power exhibited both in the deionized water extract. Some studies have shown that phytochemical compounds inhibit α-glucosidase, such as triterpenoid compounds (Lai et al., 2012), phenolics and flavonoids (Mugaranja and Kulal, 2020; Barber et al., 2021). Kim et al. (2016) proposed the role of dietary polyphenols in preventing and managing T2DM. Healthy polyphenols may improve glucose homeostasis in the small intestine by inhibiting α-amylase and α-glucosidase, inhibiting sodium-dependent glucose transporter 1 (SGLT1); thus, reducing digestion and intestinal glucose absorption of dietary carbohydrates in the muscle, adipocyte, stimulate pancreas insulin secretion and reduce hepatic glucose output in the liver. Polyphenols may also enhance insulin -dependent glucose uptake, activate 51-adenosine monophosphate-activated protein kinase (AMPK), modify the microbiome in the large intestine, and have anti-inflammatory effects. The mechanism of proanthocyanidin (PA) (Figure 1) binds islet cells was proposed by Jiao et al. (2013). They found anti-amyloidogenic PA by preventing human islet amyloid polypeptide (hIAPP) oligomers and aggregation. The misfolding of hIAPP was supposed to cause β-cell dysfunction in T2DM. Furthermore, the result revealed that Cinnamon’s PA as a primary compound effectively inhibited hIAPP. The PA with trihydroxy-phenyl rings or gallate esters have a similar structure to (–)-epigallocatechin-3-gallate, which previously exerts an inhibitor of hIAPP amyloid formation.

![Figure 2](https://doi.org/10.26656/fr.2017.7(4).1022)

**Figure 2.** TPC, IC$_{50}$ AA, and IC$_{50}$ αGI of C. burmannii extracts and reference compounds. T= total extract, EH: n-hexane extract, EA: ethyl acetate extract, EE: ethanolic extract, R: rutin, GA: gallic acid, A: acarbose

![Figure 3](https://doi.org/10.26656/fr.2017.7(4).1022)

**Figure 3.** The 3D negative correlations among TPC to IC50 αGI of extracts. The axis represents TPC’s (x), IC$_{50}$AA (y) and αGI (z)

### 4. Conclusion

The experiment showed that the ethanol solvent only with the soxhletation method produced a higher yield than the gradual polarity. The TPC of ethanolic extract was not significantly different from rutin. Moreover, the IC$_{50}$ α-glucosidase inhibition of the ethanolic extracts was more potent than acarbose. A negative correlation resulted between the total phenolic content, antioxidant activity, and α-GI of the extracts. Cinnamomum burmannii provides benefits as α-glucosidase inhibitor...
and oxidative stress relieving in diabetes dietary supplements.

Conflict of interest
The authors declare no conflict of interest.

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