In vitro anti-diabetic effects and phytochemical profiling of novel varieties of *Cinnamomum zeylanicum* (L.) extracts (#48032)

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In vitro anti-diabetic effects and phytochemical profiling of novel varieties of *Cinnamomum zeylanicum* (L.) extracts

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Background: Diabetes mellitus type 2 (DMT2) is a metabolic disorder that is common in the world. Anti-diabetic actions of phytochemicals from various medicinal herbs are considered as an alternative to therapeutic drugs for the management of DMT2 due to adverse side effects of synthetic drugs. α-Amylase and α-glucosidase inhibitory potential and phytochemical profiling was investigated in aqueous extracts from two new accessions of *Cinnamomum zeylanicum*. *C. zeylanicum* (Sri Wijaya, SW), *C. zeylanicum* (Sri Gemunu, SG) and commercially available *C. zeylanicum* (CC).

Methods: Microwave Digestion (MD), Pressurized Water Extraction (PWE), Steam Distillation (SD), Solvent Extraction (SE), Decoction Water Extraction (DWE) and Infusion Water Extraction (IWE) methods were used to prepare Cinnamon extracts. The total phenolic content (TPC, Folin-Ciocalteu method) and the Proanthocyanidin Content (PC, vanillin assay), α-amylase and α-glucosidase inhibition of Cinnamon extracts were determined spectrophotometrically. The results of α-amylase and α-glucosidase inhibition were reported in terms of IC_{50} value. The phytochemical profiling was accomplished by GC-MS technique.

Results and discussion: Lowest IC_{50} values were observed in pressured water extracts and extracts prepared as decoctions of SW. Highest PC and TPC was also observed in PWE and DWE of SW. Pressured water and decoctions are promising methods for the extraction of antidiabetic constituents from Cinnamon. Benzoic acid, Cinnamyl alcohol, Benzyl alcohol and 4-Allyl-2,6-dimethoxyphenol were identified as major compounds in extracts. These compounds were believed to be responsible for the strong enzyme inhibitory activity of the extract.

Conclusions: This is the first study to explore the use of pressured water and decoctions to extract anti diabetic phytochemicals from Cinnamon. The extensive metabolite profiling of novel SW and SG quills extracts and comparison with commercially available CC were carried out the first time and has never been reported previously. The *C. zeylanicum,* SW accession hold some promise in the management of diabetes. Toxicity evaluation of the extracts has to be mitigated for nutraceutical development for the control of diabetic in the future.

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Abstract

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Introduction

Diabetes is a leading metabolic disorder with multiple side effects and complications (*Schwartz et al., 2016*). More than 90% diabetic patients are suffering from diabetes mellitus type 2 (DMT2). Modern lifestyles, unhealthy food habits and obesity are the key factors responsible for the progression of DMT2 (*William et al., 2019*). These factors may also generate the state of oxidative stress by producing reactive oxygen species. The stress condition may generate hyperglycemia by affecting the insulin secretion and the action *(Akash et al., 2011*). There is emphasis to use antioxidant based therapies in treating the DMT2 and its complications (*Rahimi-Madiseh et al., 2016*). Furthermore, there is growing interest to develop herbal drugs and their bioactive compounds as an alternate treatment to DMT2 due to the serious side effects of synthetic therapeutic agents (*William et al., 2019*). A treatment strategy adopted for this pathological condition is to inhibit enzymes involved in hydrolyzing carbohydrates. Bioactive ingredients in the herbal plants are associated with the α-amylase and α-glucosidase inhibition (*Funke & Melzig, 2006*). α-Amylase breaks down the large and insoluble starch molecules into absorbable disaccharides. α-Glucosidase helps to convert oligosaccharides and disaccharides into monosaccharaides *(Kazeem, Ogunbiyi & Ashafa, 2013*). The metabolic actions of these enzymes may result hyperglycemia. The inhibitors of these enzymes restrict the carbohydrate absorption through competitive inhibition, thus subsequently inhibit the hydrolysis of disaccharides, and

hence limit the postprandial glucose level. Plant phytochemicals are also rich in natural antioxidants and it may help to reduce the risks for the progression of DMT2 (*Zaid et al., 2015*).

Cinnamomum zeylanicum (Family Lauraceae) which is also known as Ceylon Cinnamon or true Cinnamon, is an indigenous plant in Sri Lanka. Recent studies have shown many potential beneficial health effects of Cinnamon such as anti-inflammatory properties, anti-microbial activity, blood glucose control, reducing cardiovascular disease and reducing risk of colonic cancer (*Ouattara et al.*, *1997; Khan, et al., 2003; Shen et al., 2010)*. Eight Cinnamon species, categorized on the basis of the taste of the bark, have been identified in Sri Lanka. Among them only *Cinnamomum zeylanicum* is grown commercially. Two accessions of *Cinnamomum zeylanicum* named as "Sri Wijaya" and "Sri Gemunu" have been developed for commercial cultivation with improved chemical profiles and yields (https://www.lankabusinessonline.com/sri-lanka-develops-new-Cinnamon-varieties/). Scientific evidences on biological activities and the anti-diabetic properties of these novel varieties have not been explored. This is the first study to evaluate six types of aqueous extracts of SW and SG Cinnamon quill varieties and commercially available CC quills for *in vitro* anti-diabetic properties and compare with standard hypoglycemic drug Acarbose. The study was extended to explore the phytochemical profiles of Cinnamon extracts.

Materials & Methods

Collection of plant materials and preparation of extracts

Commercial *Cinnamomum zeylanicum* dried quills (1 kg) were collected from Dassanayake Walauwa Cinnamon plantation, Nape, Kosgoda, Southern Province in Sri Lanka. Sri Wijaya and Sri Gemunu Cinnamon quills (2 kg per each) were collected from Cinnamon Research Station, Palolpitiya, Thihagoda, Southern Province, Sri Lanka. The collected samples were transported to

the laboratory at University of Kelaniya, Sri Lanka in side sealed, sterilized polythene bags. The samples were stored in the refrigerator (2-8 ºC) until its use.

Cinnamon accessions were authenticated by the botanist at the Department of Botany, University of Kelaniya. The voucher specimen of "Sri Wijaya" and "Sri Gemunu" accessions were deposited at the publicly available herbarium, Department of Botany, the University of Kelaniya under the family Lauraceae (Deposition numbers are CIN-SW-001 and CIN-SG-002 respectively for "Sri Wijaya" and "Sri Gemunu" accessions).

Cinnamon quills were pulverized (10 g, 35 mesh) and for the MD, the sample (10 g) was digested with distilled water (80 mL for 30 minutes) using a microwave digester (mass 6 instrument, vessel type Mars Xpress). Pulverized Cinnamon quills (10 g) were extracted with pressurized water (0.098 MPa, 200 mL for 10 minutes) for the preparation of PWE. Quills of Cinnamon were extracted by traditional SD (40 g) and SE (10 g) *(Wong, 2014; Lee et al., 2018)*. Ethanol (75%) was used for the SE. The volatile compounds from SD were separated from the aqueous layer three times using hexane (30 mL). The volatiles were concentrated by using rotary evaporator (IKA® RV 10 basic, Germany). The percentage yield of the resultant oleoresin was calculated and stored at -20 °C. For the preparation of DWE Cinnamon quills (10 g) were boiled with water (200 mL) until the volume was reduced to 1/8. Ten grams of powder was mixed with boiled water (200 mL) and was allowed to stand for five minutes to obtain IWE. The MD, PWE, DWE, and IWE were filtered through Whatman 1 filter paper and concentrated under vacuum at 45 ^{0}C and further dried by passing a stream of N_2 air. The % yield was calculated and stored at -20 ^{0}C.

α- Glucosidase inhibitory activity

The α-glucosidase inhibitory assay *(Apostolidis & Lee, 2010)* was used to determine the i*n vitro* anti-diabetic properties of Cinnamon extracts. Varying concentrations (400 µg/mL- 12.5 µg/mL)

of Cinnamon quills extract (100 µL) and phosphate buffer (100 µL, 0.1 M, pH 6.8) with α-glucosidase enzyme solution (1 Unit/ml) was incubated in 96 well plates at 37 ºC for 10 minutes. After pre-incubation, 4-Nitrophenyl β-D-glucopyranoside (pNPG) solution (20 µL of 2.5 mM) in phosphate buffer (0.1 M, pH 6.8) was added to each well. The reaction mixture was incubated at 37 ºC for 20 minutes. The absorbance was recorded at 405 nm by the micro plate reader (Spectra Max M5, Molecular Devices, CA, USA) followed by the 20 minutes incubation. Acarbose was used as the positive control (12.5 µg/mL - 400 µg/mL). The blank was done without the extract. The IC_{50} was calculated as follows;

$$\text{Inhibition (\%)} = 1 - \left\{\frac{A_{sample}}{A_{control}}\right\} \times 100$$

Where, A_{sample} and $A_{control}$ were defined as absorbance of the sample and the control (blank) respectively.

α- Amylase inhibitory activity

The α-amylase inhibitory assay *(Ranilla et al., 2010)* was used to evaluate the anti-diabetic properties of Cinnamon extracts. Various concentrations (400 µg/mL- 12.5 µg/mL) of the extract (100 µL) and the sodium phosphate buffer (100 µL, 0.02 M, pH 6.9), amylase enzyme solution (0.5 mg/ml, 10 µL) was incubated at room temperature (28 ± 2 ºC) for 10 minutes in a test tube. After pre-incubation, starch solution (100 µL, 1% in 0.02 M sodium phosphate buffer, pH 6.9) was added to each tube. The reaction mixtures were incubated at room temperature (28 ± 2 ºC) for 10 minutes. The reaction was quenched by adding Dinitrosalicylic acid color reagent (100 µl). The test tubes were incubated in a boiling water bath until the yellowish orange color was developed and the tubes were allowed to cool. The reaction mixture was diluted with distilled water (5.00 ml), and a 250 µl aliquot of the reaction mixture was transferred into a 96 well micro titer plate

and the absorbance was measured at 540 nm using a micro plate reader (Spectra Max M5, Molecular Devices, CA, USA). Acarbose was used as the positive control (12.5 µg/mL - 400 µg/mL).

The α-amylase inhibitory activities of the extracts are expressed as inhibition percent which was calculated as follows:

$$\text{Inhibition (\%)} = 1 - \left\{\frac{A_{sample}}{A_{control}}\right\} \times 100$$

Where, A_{sample} and $A_{control}$ are defined as absorbance of the sample and the control respectively. Control was conducted without adding the extract.

Total phenolic content determination assay

The Folin-Ciocalteu method *(Wang et al., 2012)* was used to determine the TPC. The plant extract (3.00 mg extract dry weight) was mixed with Folin-Ciocalteu reagent (5.00 mL, 10%) and the mixture was incubated at room temperature (28 ± 2 ºC) for five minutes. Sodium Carbonate (4.00 mL, 7.5% v/w) was added and the mixture was allowed to stand for one hour at room temperature (28 ± 2 ºC). The absorbance was measured at 765 nm using a UV-visible spectrophotometer. A calibration curve for Gallic acid in concentrations from 0.02 mg/mL to 1.00 mg/mL ($R^2 = 0.99$) was used to interpolate results of the TPC and results were expressed as gallic acid equivalents (GAE) mg/g dried extract.

Proanthocyanidine determination assay

Vanillin assay was used to determine the PC. Plant extract (3.00 mg extract dry weight) of Cinnamon quills was mixed with 4.00 mL of vanillin solution (1% w/v in 7 M H_2SO_4) and samples were incubated at room temperature (28 ± 2 ºC) for 15 minutes. After the incubation, the absorbance was measured at 500 nm. Catechine was used as the standard. A calibration curve from

153 catechin in concentrations from 0.05 mg/mL to 0.25 mg/mL ($R^2 = 0.99$) was used to interpolate
154 the results of the PC and results were expressed as catechin equivalents *(Toda, 2005)*.

155 **GC-MS Analysis**

156 Crude Extract (4.00 mg), was dissolved in 1.00 ml of hexane and the samples were filtered using
157 a nylon filter having 0.45 mm pore size. A 0.7 µL aliquot of the above was injected in the split
158 less mode into a GC/MS 7890B Gas Chromatograph (Agilent, American) equipped with a 5977B
159 mass spectrometer (Agilent, American). A fused silica capillary Agilent Technology HP-5 (5%
160 phenyl-methyl polysiloxane) column (30 m x 0.25 mm x 0.25 µm) was used for the separation.
161 The injector temperature was 250 °C. The initial temperature was kept at 40 °C, for 3 minutes and
162 the temperature was gradually increased to 220 °C at the rate of 4 °C min^{-1} and was then held for
163 2 minutes at 220 °C. Again the temperature was gradually increased to 230°C at the rate of 8 °C
164 min^{-1} and held for 3 minutes at 230°C. The Post run was at 235°C held for 3 minutes. The Total
165 GC running time was 54.25 minutes. Helium was used as the carrier gas at a constant flow rate of
166 1.0 mL min^{-1} at the split-less mode. EI was used as the ion source, and the ion source temperature
167 was 230 °C. The sector mass analyzer was set to scan from 40 to 650 amu The volatile components
168 of the extracts were identified using mass spectral data, with computer assisted matching with
169 WILEY 275 and National Institute of Standards and Technology (NIST6.0) libraries.

170 **Statistical analysis**

171 All the results are expressed as mean ± SD of triplicate assay. Statistical analyses of data were
172 performed using Microsoft Office Excel (2013) and Graph Pad Prism 7 statistical package
173 (GraphPad Software, USA). Significant differences among the data were analyzed by the SPSS
174 Statistical computer package (IBM® SPSS® Statistics Version 23, USA). The results are analyzed
175 using one-way ANOVA test followed by Dunnett/Tukey test for multiple comparisons, paired

sample t-test and determination of significance level. Group means were considered to be significantly different at $P < 0.05$.

Results

Extract yield analysis

The yield of extracts ranged from 0.42% ± 0.03% - 2.19% ± 0.25% for SW; 0.39% ± 0.02% - 1.90% ± 0.05% for SG and 0.15% ± 0.00% - 1.26% ± 0.08% for CC (*Fig*. 1). The results indicated that there was significant differences ($p < 0.05$) in the yield of the extracts depending on the Cinnamon accessions and the extraction methods. The maximum yield was obtained from SW, PWE (2.19% ± 0.25%). Microwave digestion gave the lowest yield in all Cinnamon varieties (0.42% ± 0.03% for SW; 0.39% ± 0.02% for SG and 0.15% ± 0.00% for CC).

α-glucosidase, α-amylase enzyme inhibition activity

The α-glucosidase and α-amylase enzyme inhibitory activities of sample extracts are expressed in terms of IC_{50} values, and the lower IC_{50} indicated a stronger effect of inhibition. SW, PWE exhibited the significantly highest ($P < 0.05$) inhibitory activity for α -glucosidase and α-amylase (42 ± 8 µg mL^{-1} and 78 ± 7 µg mL^{-1} respectively) followed by SW, DWE (116 ± 17 µg mL^{-1} and 132 ± 11 µg mL^{-1} respectively). The inhibition of these extracts were comparable with the positive control, Acarbose (173 ± 7 µg mL^{-1} and 95 ± 4 µg mL^{-1} respectively) as in *Table 1*.

TPC and PC analysis

Total phenolic content was expressed as mg GAE/g and PC was expressed as mg of catechin equivalent g^{-1}. In comparing the TPC and the PC of extracts, the highest TPC and the highest PC were obtained for the DWE (2.24 ± 0.00 mg GAE/g and 40.05 ± 0.10 mg of catechin equivalent

197 g^{-1} respectively) for SW. The extracts obtained by using SD had the lowest TPC and PC for SW
198 (0.21 ± 0.01 mg GAE/g and 1 ± 0.01 mg of catechin equivalent g^{-1}**)** as in *Table 2*.

199 **GC-MS analysis**

200 (*E*)- Cinnamaldehyde, was the dominant compound in CC, SW and SG extracts prepared using
201 steam distillation, Infusion and solvent extraction respectively (*Table 3*). In pressured water
202 extracts of SW and SG, Benzoic acid (1.6%, 15.49%); (*E*)-cinnamaldehyde (5.8%, 5.46%);
203 Cinnamyl alcohol (32.44%, 40.08%); 4-Allyl-2,6-dimethoxyphenol (10.2%, 12.71%) were the
204 major compounds present (*Table 3*).

205 Benzoic acid (2.58%, 22.51%); (*E*)-Cinnamaldehyde (34.6%, 5.58%); Trans-Cinnamic acid
206 (16.86%, 4.51%); O-Methoxy-Cinnamaldehyde (2.04%, 2.14%); Cinamyl alcohol (21.3%,
207 42.48%); 4-Allyl-2,6-dimethoxyphenol (3.24%, 8.79%) were the major compounds present in SG
208 and SW accession bark prepared as a decoction. Benzoic acid and Cinnamyl alcohol were not
209 detected in CC, PWE and DWE. 4-Allyl-2,6-dimethoxyphenol (1.79%, 1.05%) was seen as a
210 minor constituent in CC extracted using pressured water and as a decoction (*Table 3*).

211 Twenty seven chemical components were identified for the first time in SW; PWE. Benzcatechin
212 (1.14%); 1-Methoxy-7-methyl-3,4-dihydrobenzo[c]pyran (1.31%); (+)-(1S,3R,4S)-4(a)-
213 Methyladamantane (2.14%); 1-(2'-hydroxyphenyl)prop-2-en-1-ol (0.71%); 1,3,5-benzenetriol
214 (1.11%); 4-propyl-1,2-Benzenediol (1.615%); 3,4,5-trimethoxyphenol (0.54%); 4-((1E)-3-
215 Hydroxy-1-propenyl)-2-methoxyphenol (1.00%); Vanillylmandelic acid (0.78%) were present
216 only PWE of SW. These compounds and 4-Allyl-2,6-dimethoxyphenol were detected for the first
217 time in any Cinnamon accessions (*Table 3*).

Discussion

Cinnamon incorporated foods, nutraceuticals are popular choice in Sri Lanka due to the belief of its anti-diabetic properties. However, the anti-diabetic effect of two selected cinnamon accessions, *Cinnamomum zeylanicum* (Sri Wijaya) and *Cinnamomum zeylanicum* (Sri Gemunu) have not been scientifically verified and not well understood. In the current study, water extracts were prepared from selected Ceylon Cinnamon accessions to determine the anti-diabetic activity. The α-Glucosidase/α-amylase inhibitory activities, total phenolic compounds, and proanthocyanidin content were evaluated in aqueous extracts of *Cinnamomum zeylanicum* and the chemical profiles were identified using GC-MS.

The yield of extracts prepared using different methods from Cinnamon quill samples are given in *figure 1*. The yield of the bark essential oil of novel Cinnamon accessions prepared using hydro distillation have been reported previously in literature for SW (1.25%), SG (1.49) *(Ariyarathne, Weerasuriya & Senarath, 2018;)*, and CC (1.2%) (*Jayawardena & Smith,* 2010). Higher yield was reported for Sri Gemunu Cinnamon accessions (3.4%. v/w) than the Sri Wijaya by the same distillation method in another study *(Lokuge et al. 2018)*. In the current study, the percent yield obtained for extracts prepared using SD is comparable to the reported values for SW SG and CC. *Sihoglu Tepe & Ozaslan, (2020)* reported 2.6% yield for the commercially available *C. zeylanicum* in Turkey. Another finding showed that 352 mg/g yield for the C. *zeylanicum* freeze dried aqueous extract and that was higher than the yield in the current study *(Takács et al., 2017)*. Species of Cinnamon, climate, growth condition, cultivation site, age of the bark, thickness of the bark and the density of the oil cells are positively correlated with the yield of the Cinnamon essential oils *(Li, Kong & Wu, 2013)*. Color of the bark, peeling ability, texture of the bark, odor and the morphology of the leaves ware examined in SW and SG varieties *(Ariyarathne, Weerasuriya &*

241 *Senarath, 2018)*. However, scientific evidences regarding the bark thickness, density of the oil
242 cells of SW and SG have not been explored. Hence further investigations must be conducted to
243 evaluate the properties of the bark of these accessions.
244 The inhibitory activity for α-glucosidase and α-amylase varied depending on the sample and the
245 type of extraction. By comparing the α-Glucosidase and α-amylase inhibitory activities of the
246 samples tested, the IC_{50} value, of SW- PWE exhibited the strongest inhibitory activity compared
247 with the positive control Acarbose. The methanolic extracts of the Ceylon Cinnamon are reported
248 to have the potential to control hyperglycemia *(Nair, Kavrekar & Mishra, 2013)*. The IC_{50} values
249 for the α-amylase and α-glucosidase inhibition were 130.55 ± 10.50 μg ml^{-1} and 140.01 ± 10.08
250 μg ml^{-1} respectively for the *C. zeylanicum* methanol extracts. Another comparable α-amylase
251 inhibition is reported for methanol extract of Cinnamon bark (IC_{50}: 86.84 μg mL^{-1}), Cinnamon
252 stick (IC_{50}: 54.69 μg mL^{-1}) and the clinical drug, Methformin hydrochloride (53.03 μg mL^{-1})
253 *(Wickramasinghe, 2018)*. According to the findings of Wickramasinghe (2018), the poor α-
254 amylase inhibition was shown in Cinnamon drink (3207.01 μg mL^{-1}), Cinnamon capsule (2537.49
255 μg mL^{-1}) and Cinnamon powder (771.67 μg mL^{-1}). The enzyme inhibitory activities of SW
256 prepared using pressured water and as a decoction gives better inhibition (IC_{50} for the α-
257 glucosidase inhibition, 42 ± 8 μg mL^{-1}; 116 ± 17 μg mL^{-1} and for the α-amylase inhibition, 78 ± 7
258 μg mL^{-1} 132 ± 11 μg mL^{-1} respectively) when compared with previous reported values for
259 methanolic extracts. The enzyme inhibition mechanism of the Acarbose is well established.
260 Acarbose inhibit the α-glucosidase activity as a competitive inhibitor *(Van de Laar, 2008)*.
261 However, phytochemicals in plant extracts mainly phenolics, act as a non-competitive inhibitors
262 against dietary enzymes. Non-competitive mode is better form of inhibition due to the multiple

side interactions of phenolic compounds with dietary enzyme and also it does not depend on the substrate concentration.

Polyphenols and Proanthocyanidin oligomers are a large groups of phytochemicals that can be extracted from plants such as tea, coffee, wine, cocoa, grains, legumes, fruits and berries *(Chen et al., 2012)*. Several findings have confirmed that there is positive correlation between the antioxidant such as polyphenols present and the inhibition of α-glucosidase and α-amylase. *(Cai et al., 2004; Peng et al., 2010; Abeysekera, Premakumara & Ratnasooriya, 2013; Premakumara et al., 2013)* SW; DWE and PWE had high contents of PC and TPC, which can be correlated with the observed better anti-diabetic activities

The effectiveness of phenolic compounds in inhibiting α-amylase depends on the number and the position of hydroxyl group *(Funke & Melzig 2006)*. In the current study, a high amount of phenolic compounds were extracted as water was used as a solvent and hence the extracts had potent anti-diabetic properties.

GC–MS technique is a powerful and suitable tool for the determination of volatile compounds because of its high separation efficiency and sensitive detection *(Li, Kong & Wu, 2013)*. Cinnamaldehyde and the Cinnamic acid are the major compounds of Cinnamon aqueous extracts *(Hafizur et al., 2015)*. The chemical composition in the aqueous extracts in our study, slightly deviated from their findings. Benzoic acid, Cinnamyl alcohol, Trans-Cinnamic acid and 4-Allyl-2,6-dimethoxyphenol were the major compounds present in SW and SG aqueous extracts. According to the findings of *Hafizur et al., 2015*, it is desirable to have high concentrations of Cinnamic acid as it has the potential to decrease the blood glucose levels, improve glucose tolerance and stimulate insulin secretion in diabetic rats in a time and dose dependent manner. Hence, it is favorable to have extracts with high concentrations of Cinnamic acid for better diabetic

286 control. Cinnamaldehyde is identified as the major compound present in Cinnamon extracted
287 using hydro distillation in several studies *(Paranagama et al., 2001; Kaskoos, 2019; Mota,*
288 *Campelo & Frota, 2019)*. The α-Amylase inhibition evaluated using a combination of (*E*)-
289 Cinnamaldehyde and (*E*)-Cinnamyl acetate has shown better potency than Cinnamaldehyde
290 *(Sihoglu Tepe & Ozaslan, 2020)*. The findings of the study indicated the synergistic effect on each
291 other in considering enzyme inhibition. Poor enzyme inhibition observed in SD and SE are
292 consistent with the above findings. Higher amount of (*E*)-Cinnamaldehyde alone does not improve
293 the enzyme inhibitory potential. The inhibition in PWE and LW accessions was higher due to the
294 synergistic effect of the compounds present in the extract.

295 Preparations of decoction is a common method practiced in the preparation of Ayurveda drugs in
296 therapeutic regimen *(Daswani et al., 2011)*. Pressurize water is an environmentally friendly non-
297 toxic novel method to effectively extracts plant metabolites without using organic solvents.
298 However, the most common method practiced for the extraction of Cinnamon is steam distillation
299 or hydro distillation. The loss of volatile compounds and long extraction times are some of the
300 drawbacks in the use of steam distillation.
301 Super critical Carbon dioxide is an alternative used for the extraction of thermo sensitive
302 phytochemicals. However, in some studies supercritical carbon dioxide extracts have exhibited
303 low antioxidant activity compared to the ethanol extracts. The reason is due to the low polarity of
304 super critical carbon dioxide as a solvent compared with ethanol *(Singh et al., 2007)*.
305 Hence, there is interest to develop new methods of extraction which gives better yields and
306 biological activity. Pressurized water extraction has the advantage of extracting polar compounds
307 from plant extracts hence can impart better biological activity *(Jayawardena & Smith, 2010)*. In
308 the current study the extract prepared using pressured water had the most potent anti-diabetic

activities. Further, low cost, safe for human consumption, short extraction times compared to steam distillation are added advantages of pressurized water extractions.

Conclusions

The current study has identified the potential antidiabetic properties of the Sri Wijaya Cinnamon accessions, which exhibited significant α-amylase and α-glucosidase inhibitory activities. The extracts prepared using pressured water and as a decoctions were more potent in inhibiting α-amylase and α-glucosidase activity, and had the potential to extract higher content of proanthocyanidin than other methods of extraction. Hence, pressured water extraction and decoction preparation can be recommended as an effective method to extract antidiabetic constituents from plants. Based on the results obtained in this study, Sri Wijaya Cinnamon water extract prepared using high pressure and the decoction method had the highest anti-diabetic potential. The extracts have to be further purified and developed for the treatment of diabetes mellitus.

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Table 1(on next page)

IC_{50} for α-glucosidase and α-amylase of SW, SG and CC, extracted by different methods.

Each data point represents the mean of three replicates ± SEM. Mean values in each column, in each cinnamon accessions followed by the same letter, are not significantly different ($p \geq 0.05$) by Tukey's multiple range comparison tests.

SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction

Table 1:

IC_{50} for α-glucosidase and α-amylase of SW, SG and CC, extracted by different methods.

Extraction methods	IC_{50} for α-glucosidase (μg mL^{-1})	IC_{50} for α-amylase (μg mL^{-1})
	"SW"	
SE	650 ± 121^{c}	615 ± 99^{c}
SD	150 ± 5^{ab}	172 ± 9^{b}
MD	160 ± 14^{b}	192 ± 7^{b}
DWE	116 ± 17^{ab}	132 ± 11^{ab}
IWE	144 ± 31^{ab}	171 ± 14^{b}
PWE	42 ± 13^{a}	78 ± 7^{a}
Acarbose	173 ± 7^{b}	95 ± 4^{a}
	"SG"	
SE	785 ± 124^{c}	708 ± 77^{d}
SD	165 ± 5^{ab}	180 ± 7^{c}
MD	182 ± 6^{b}	215 ± 12^{c}
DWE	121 ± 5^{a}	143 ± 16^{bc}
IWE	149 ± 2^{a}	169 ± 17^{c}
PWE	72 ± 3^{ab}	186 ± 10^{b}
Acarbose	173 ± 7^{b}	95 ± 4^{a}
	CC	
SE	606 ± 139^{e}	201 ± 5^{c}
SD	296 ± 25^{d}	120 ± 5^{b}
MD	159 ± 6^{b}	111 ± 2^{a}
DWE	162 ± 8^{b}	260 ± 13^{d}
IWE	129 ± 9^{a}	246 ± 17^{d}
PWE	132 ± 5^{a}	88 ± 7^{a}
Acarbose	173 ± 7^{b}	95 ± 4^{a}

16 Each data point represents the mean of three replicates ± SEM. Mean values in each column, in
17 each cinnamon varieties followed by the same letter, are not significantly different ($p \geq 0.05$) by
18 Tukey's multiple range comparison tests.[1]

19

[1] SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction

Table 2(on next page)

TPC and PC from quills of CC, SW and SG, with different extraction methods.

Each data point represents the mean of three replicates ± SD. Mean values in each column, in each cinnamon accessions followed by the same letter, are not significantly different ($p \geq 0.05$) by Tukey's multiple range comparison tests.

SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction

Table 2:

TPC and PC from quills of CC, SW and SG, with different extraction methods.

Extraction methods	TPC (mg GAE/g)			PC (mg of catechin equivalent g^{-1})		
	"SW"	"SG"	CC	"SW"	"SG"	CC
SE	0.90 ± 0.01^{e}	0.65 ± 0.01^{d}	0.90 ± 0.01^{b}	5.12 ± 0.01^{b}	3.56 ± 0.01^{b}	7.34 ± 0.01^{c}
SD	0.21 ± 0.01^{a}	0.15 ± 0.07^{b}	0.68 ± 0.01^{a}	3.00 ± 0.01^{a}	1.00 ± 0.01^{a}	5.65 ± 0.01^{a}
MD	0.67 ± 0.01^{c}	0.12 ± 0.01^{a}	1.73 ± 0.02^{d}	15.00 ± 0.01^{c}	12.00 ± 0.01^{c}	6.14 ± 0.01^{b}
DWE	2.24 ± 0.00^{f}	1.00 ± 0.00^{f}	0.91 ± 0.01^{b}	40.05 ± 0.10^{f}	26.00 ± 0.09^{f}	17.5 ± 0.01^{d}
IWE	0.87 ± 0.01^{d}	0.83 ± 0.01^{e}	1.51 ± 0.05^{c}	27.21 ± 0.07^{d}	21.78 ± 0.09^{e}	32.77 ± 0.01^{e}
PWE	1.53 ± 0.01^{b}	0.18 ± 0.01^{c}	2.90 ± 0.08^{e}	36.08 ± 0.01^{e}	17.21 ± 0.01^{d}	7.39 ± 0.03^{c}

Each data point represents the mean of three replicates ± SD. Mean values in each column, in each cinnamon accessions followed by the same letter, are not significantly different ($p \geq 0.05$) by Tukey's multiple range comparison tests.[1]

[1] SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction

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Table 3(on next page)

Chemical compositions of extracts from CC. SW and SG, by various extraction methods.

Each data point represents the mean of three replicates.

SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; 1, Solvent Extraction; 2, Steam Distillation; 3, Pressurized Water Extraction; 4, Decoction Water Extraction; 5, Infusion Water Extraction

1 **Table 3:**

2 **Chemical compositions of extracts from CC. SW and SG, by various extraction methods.**

Compounds	SG					CC					SW				
	Area percentage (%)														
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
3-carene	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-
Cymol	-	-	-	0.89	-	-	-	-	-	-	-	-	0.16	-	-
Benzoic acid	-	-	1.6	2.58	-	-	-	-	-	-	-	-	15.49	22.51	0.81
Benzyl alcohol	-	-	0.17	-	-	-	-	0.42	-	-	-	-	3.8	3.98	-
Undecane	-	-	0.46	-	-							-	1.1	-	-
Methyl salicylate	-	-	0.26	-	2.34		-	13.52	10.35	-		-	0.42	-	5.24
Terpinen-4-ol	-	1.36	-	-	-	0.48	0.41	-	-	-	0.78	0.44	-	-	-
Naphthalene	-	-	-	-	-	0.19	-	-	-	-	-	-	-	-	-
Linalool	-	5.17	-	-	-	2.6	2.07	-	-	0.31	1.07	2.21	-	-	-
Benzcatechin	-											-	1.14	-	-
β-Fenchyl alcohol	-						0.77	-		0.31		0.81	-	-	-
Benzenepropanal	-	1.68	-	-	-	0.14	0.41	-	0.35	0.24	0.14	0.43	-	-	-
Benzenepropanol	-	-	1.2	2	0.74		-	0.29		0.46	0.19	-	0.54	-	0.34
3-cyclohexene-1-methanol	-	-	-	-	-	0.69	-	-	-	-	-	-	-	-	-
Salicylic acid	-	-	-	-	-	-	-	12.93	4.05	-	-	-	-	-	-
Acetic acid, cinnamyl ester	-	-	-	-	-	-	6.05	1.74	1.85	-	-	2.85	-	-	-
(E)-cinnamaldehyde	43	65.21	5.8	34.6	55.72	48.77	79.06	43.41	57.7	73.25	39.24	38.85	5.46	5.58	40.48
Benzaldehyde	-	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-
1-phellandrene	-	-	-	-	-	1.28	-	-	-	-	-	0.09	-	-	-
(E)- 2,3-epoxycarane	-	-	-	0.83	-	-	-	-	-	-	-	-	-	-	-
Dimethylsulfonium dicyanomethylide	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-
2,4-Dimethyl-2,4-pentadien-1-ol	-	-	-	1.58	-	-	-	-	-	-	-	-	-	-	-
Eugenol	8	2.47	0.33	0.59	1.82	1.94	6.75	7.85	7.67	8.83	14.67	12.51	1.74	1.27	6.55
Cinnamaldehyde dimethyl acetal	-	-	11.84	5.3	18.53	6.2	-	0.54	0.32	2.06	0.49	-	0.39	-	5.74
Vanillylmandelic acid	-	-	0.78	-	-	-	-	-	-	-	-	-	0.78	-	-
Trans-Cinnamic acid	-	-	0.14	16.86	2.07	-	-	-	-	-	-	-	1.74	4.51	0.92
O-methoxy-Cinnamaldehyde	-	0.65	0.95	2.04	1.78	1.97	1.79	-	1.52	2	2.62	2.18	2.04	2.14	3.57

Para methoxy cinnamic aldehyde	-						-	1.66	1.85	0.36	-	-	-	-	-
Propanoic acid, phenylmethyl ester	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-
1,2-Dihydroxy-4-(1-propyl)benzene	-	-	-	0.86	-	-	-	-	-	-	-	-	-	-	-
Benzyl benzoate	20	1.17	-	0.6	0.39	3.42	2.69	0.33	1.52	0.62	5.78	6.29	-	1.15	1.74
Sabinene	-	0.97	-	-	-	-	-	-	-	-	-	0.1	-	-	-
γ-terpinene	-	-	-	0.42	-	0.24	-	-	-	-	-	0.11	-	-	-
Cinnamyl alcohol	1	0.72	32.44	21.3	10.87	0.7	-	-	-	6.15	5.55	0.66	40.08	42.28	14.32
Phenylpropyl acetate,	-	-	-	-	-	-	-	-	-	-	0.61	-	-	-	-
Copaene	-	-	-	-	-	0.46	-	-	-	-	-	-	-	-	-
Caryophyllenyl alcohol	-	-	-	-	-	0.33	-	-	-	-	-	-	-	-	-
(+) Spathulenol	-	-	-	-	-	0.24	-	-	-	-	-	-	-	-	-
Caryophyllene	3	-	-	0.51	-	4.33	-	-	-	-	0.26	-	-	-	-
trans-Cinnamyl acetate	23	19.85	-	-	3.52	3.85	-	-		2.09	25.39	28.59	0.13	-	9.73
(+)-(1S,3R,4S)-4(a)-Methyladamantane	-	-	-	-	-	-	-	-	-	-	-	-	2.14	-	-
1-(2'-hydroxyphenyl)prop-2-en-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	0.71	-	-
(1S,2S,6S,8S)-11-(Hydroxymethyl)-6-methyl-3 methylenetricyclo[6.3.0.0(2,6)]undec	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
1,3,5-benzenetriol	-	-	-	-	-	-	-	-	-	-	-	-	1.11	-	-
4-propyl-1,2-Benzenediol	-	-	-	-	-	-	-	-	-	-	-	-	1.61	-	-
Benzenemethanol	-	-	-	-	-		-	-		0.18	0.17	-	-	-	0.08
Caryophyllene oxide	-	-	-	-	-	2.18	-	-	-	-	-	0.11	-	-	-
Allylbenzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.18
3-Phenylprop-2-yn-1-ol	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-
1-(Trideuteriosylanyl)-benzene	-	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-
Methoxy-phenyl-Oxime	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-
Cineole	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-
3,7-dimethyl- 1,6-octadien-3-ol	-	-	-	-	-	-	-	-	-	-	-	0.26	-	-	-
α-terpinolene	-	-	-	-	-	-	-	-	-	-	-	0.41	-	-	0.07
O-Methoxyphenol	-	-	-	-	-	-	-	-	-	-		0.1	-	-	-
Vinyl phenyl carbinol	-	-	-	-	-	-	-	-	-	-	0.08	0.16	-	-	-
Ethyl benzenecarboxylate	-	-	-	-	-	-	-	-	-	-		0.07	-	-	-
2H-1-benzopyran	-	-	-	-	-	-	-	-	-	-		0.06	-	-	-
2-methoxy-4-propyl-Phenol	-	-	-	-	-	-	-	-	-	-		0.12	-	-	-
Benzenepropyl acetate	-	-	-	-	-	-	-	-	-	-		0.79	-	-	-
Homo - syringaldehyde	-	-	-	-	-	-	-	-	-	-		0.04	-	-	-

Ortho methoxy cinnamyl acetate	-	-	-	-	-	-	-	-	-	-	0.36	0.43	-	-	0.68
Oxalic acid, 2-phenylethyl propyl ester	-	-	-	-	-	-	-	-	-	-		0.21	-	-	-
Benzyl salicylate	-	-	-	-	-	-	-	-	-	-		0.03	-	-	-
Methyl palmitate	-	-	-	-	-	-	-	-	-	-		0.06	-	-	-
Linoleic acid	1	-	-	-	-	0.83	-	-	-	-	0.13	0.03	-	-	-
3,4,5-trimethoxyphenol	-	-	-	-	-	-	-	-	-	-		-	0.54	-	-
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	-	-	-	-	-	-	-	-	-	-		-	0.28	-	-
3-Methoxy-4-hydroxycinnamaldehyde	-	-	-	-	-	-	-	-	-	-		-	0.43	-	-
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	-	-	-	-	-	-	-	-	-	-		-	1.00	-	-
3-Phenylprop-2-yn-1-ol	-	-	-	-	-	-	-	-	-	-		-	-	-	0.22
Salicylic acid	-	-	-	-	-	-	-	-	-	-		-	-	-	2.95
Formic acid, 3-phenylpropyl ester	-	-	-	-	-	-	-	-	-	-		-	-	-	0.08
4,2,8-Ethanylylidene-2H-1-benzopyran, octahydro-2-methyl-	-	-	-	-	-	-	-	-	-	-		-	-	-	0.13
1-Methoxy-7-methyl-3,4-dihydrobenzo[c]pyran	-	-	-	-	-	-	-	-	-	-		-	-	-	0.4
2,6-dimethoxy-4-(2-propenyl)-Phenol,	-	-	-	-	-	-	-	-	-	-	0.98				
Butylated hydroxytoluene	-	-	-	-	-	-	-	-	-	0.4		-	-	1.85	0.21
Trans-3-Pinen-2-ol [2,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol]	-	-	-	-	-	-	-	-	-	0.49					
4-Allyl-2,6-dimethoxyphenol	-	-	10.2	3.24	0.92	-	-	1.79	1.05	1.7		0.77	12.71	8.79	2.97
4,7-Dihydro-4,7-methano-2H-indole	-	-	-	-	-	-	-	0.5		-					
Palmitic acid	-	-	-	0.44	-	1.53	-	-	-	-	0.28	0.05	-	-	-
4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	-	-	-	0.8	-	-	-	-	-	-	-	-	0.27	-	-
3-Methoxy-4-hydroxycinnamaldehyde	-	-	-	0.31	-	-	-	-	-	-	-	-	-	-	-
Styrene	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-
Camphene	-	-	0.08	-	-	-	-	-	-	-	-	-	-	-	-
1-Methyl-2-isopropylbenzene	-	-	0.81	-	-	-	-	-	-	-	-	-	-	-	-
Dodecane	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-
3,4,4-Trimethyl-2-pentenal	-	-	0.38	-	-	-	-	-	-	-	-	-	-	-	-
2-methylene-Cyclohexanol	-	-	1.29	-	-	-	-	-	-	-	-	-	-	-	-
1,5,9,9-tetramethyl-, Z,Z,Z-1,4,7,-	-	-	-	-	-	1.15	-	-	-	-	-	-	-	-	-

Cycloundecatriene,															
Δ-Cadinene	-	-	-	-	-	0.23	-	-	-	-	-	-	-	-	-
Hydrocinnamic acid	-	-	0.2	-	-	0.42	-	-	-	-	-	-	-	-	-
Phenol, 2,6-bis(1,1-dimethylethyl)-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-
(S)-(+)-5-sec-Butyl-2-pyrimidinol	-	-	0.56	-	-	-	-	14.08	10.25	-	-	-	-	-	-
Spiro[2-ethylidene-3-methylcyclohexane]oxirane	-	-	1.96	-	-	-	-	-	-	-	-	-	-	-	-
1-Methoxy-7-methyl-3,4-dihydrobenzo[c]pyran	-	-	-	-	0.28	-	-	-	-	-	-	-	1.31	-	1.3
Aspirin methyl ester	-	-	-	-	0.39	-	-	-	-	-	-	-	-	-	0.09
1H-Pyrrole-2,4-dicarboxylic acid, 3,5-dimethyl-, diethyl ester	-	-	-	-	0.4		-	0.94	0.85	-	-	-	-	-	1.31
Hexadecanoic acid	1														
α-Thujene						0.27									

Each data point represents the mean of three replicates.[1]

[1] SW, Sri Wijaya cinnamon accession; SG, Sri Gemunu cinnamon accession; CC, commercially available *Cinnamomum zeylanicum*; 1, Solvent Extraction; 2, Steam Distillation; 3, Pressurized Water Extraction; 4, Decoction Water Extraction; 5, Infusion Water Extraction

Figure 1(on next page)

Yields (%, w/w, dry basis) of compounds in extracts from different cinnamon quills with different extraction methods

Series 1: SW, Sri Wijaya cinnamon accession; Series 2: SG, Sri Gemunu cinnamon accession; Series 3: CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction

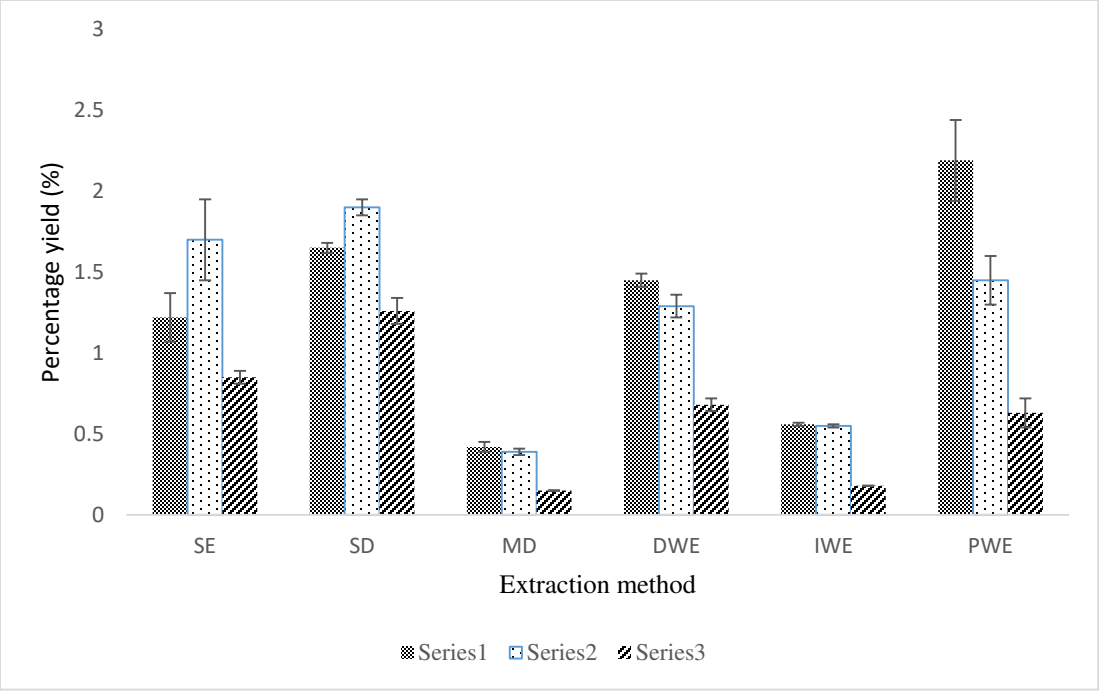

Figure 1: Yields (%, w/w, dry basis) of compounds in extracts from different cinnamon quills with different extraction methods (n=3). Series 1: SW, Sri Wijaya cinnamon accession; Series 2: SG, Sri Gemunu cinnamon accession; Series 3: CC, commercially available *Cinnamomum zeylanicum*; SE, Solvent Extraction; SD, Steam Distillation; MD, Microwave Digestion; DWE, Decoction Water Extraction; IWE, Infusion Water Extraction; PWE, Pressurized Water Extraction