Optimization of water extract of Cinnamomum burmannii bark to ascertain its in vitro antidiabetic and antioxidant activities

by Martha Ervina

Submission date: 23-Feb-2023 12:38PM (UTC+0700)
Submission ID: 2021054453
File name: 5-Optimization_of_water_extract_.pdf (820.26K)
Word count: 5954
Character count: 31849

Biocatalysis and Agricultural Biotechnology 19 (2019) 101152

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Optimization of water extract of *Cinnamomum burmannii* bark to ascertain its in vitro antidiabetic and antioxidant activities

Martha Ervina[a,*], Han Sanjaya Lie[a], Jesslyn Diva[a], Caroline[b], Sundus Tewfik[c], Ihab Tewfik[d]

[a] Department of Pharmaceutical Biology, Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, Jl. Raya Kalisari Selatan 1, Laguna Pakuwon City, Surabaya, 60245, East Java, Indonesia

[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Widya Mandala Catholic University Surabaya, Jl. Raya Kalisari Selatan 1, Laguna Pakuwon City, Surabaya, 60245, East Java, Indonesia

[c] International Forum for Public Health, London, EN4 8EA, United Kingdom

[d] School of Life Sciences, University of Westminster, 115 New Cavendish Street, London, UK, W1W6UW, United Kingdom

ARTICLE INFO

Keywords:
Optimization
Factorial design
Water extraction
DPPH
Diabetes mellitus
Cinnamomum burmannii

ABSTRACT

The antidiabetic and antioxidant activity of water extract of *Cinnamomum burmannii* bark is well documented. This research aimed to optimize cinnamon water extraction process and verify active components instigating its in vitro antidiabetic activity. The study employed a Design Expert 7.0 program to derive factorial design and optimization conditions. The extraction step comprised of three factors (temperature, concentration and time of extraction) and two levels (low and high), with four responses observed (yield, total phenolic content, IC_{50}DPPH antioxidant activity, and IC_{50} α-glucosidase inhibition). The polynomial equations revealed influence and interaction among the selected factors to the responses and obtained overlay optimization of factors to responses. The results indicated that optimal temperature, concentration, and extraction time were 98 °C, 30% and 20 min, respectively. Corresponding DPPH, α-glucosidase, TPC, and yield values were 3.45 μg/mL, 0.50 μg/mL, 259.08 μg GAE/mg of sample, 6.28%, respectively. LCMS analysis of the optimum extract confirmed typical characteristic of *C. burmannii* contents (coumarins, polymers of proanthocyanidins A-type and protonated heterodimer of flavan-3-ol group). The optimized water extract of *C.burmannii* has the potency to assist in complementary therapy to modulate diabetes mellitus.

1. Introduction

World incidence of diabetes mellitus (DM) has shown a dramatic increase over the last decade (ADA, 2018; IDF, 2018). DM is described as the chronic endocrine metabolic disease that characterized by elevated blood glucose level and disturbances of carbohydrate, lipid and protein metabolism. The role of free radicals and oxidative stress has been reported in the pathogenesis of DM; in which it triggers insulin resistance to micro and macro-vascular of DM complications (Khan et al., 2015). While, α-glucosidase and α–amylase inhibition are useful methods for phytochemical screening in managing type 2 DM (DM-2), the phenolics raise the attention in DM-2 therapy among secondary metabolic compounds (Shahidi and Ambigaipalan, 2015). Phenolic hydroxyl groups [PHGs] would scavenge reactive oxygen or nitrogen species and produce more stable radical than the initial form. PHGs may also have an important role in preventing the onset and propagation of DM oxidative disease. The phenolic containing compounds such as flavonoid, tannins, proanthocyanidins, and coumarins were the majority of the natural-occurring antioxidant source (Asif, 2015). In vitro plants based, pre- and clinical trial research has documented phenolic antioxidant as a beneficial supplement in DM management and preventing its complication (Lin et al., 2016).

Cinnamomum burmannii (Indonesian Cinnamon, Lauraceae) is a cinnamon species used for daily needs (spice in food) and also as herbs in traditional medicine (Al-Dhuhiab, 2012). In the preclinical study of cinnamon, a reduction of fasting and postprandial plasma glucose and HbA1c has been documented; while its clinical trial on pre-diabetes patients (*with impaired fasting glucose or impaired glucose tolerance*) and pre-treatment HbA1C (Haemoglobin A1C) with aqueous or powder of *C cassia*, resulted in an improvement in glycemic control (Al-Dhuhiab, 2012; Hasanzade et al., 2013; Medagama, 2015). A large amount of bioactive compounds classes were determined by extraction technique as well as extraction solvent. Adaramola and Onigbinde (2017) reported that the soxhlet extraction of ginger oil with n-hexane resulted in

* Corresponding author.
E-mail addresses: martha.pharm@ukwms.ac.id (M. Ervina), hanxionglie96@gmail.com (H.S. Lie), jesslyndwa@gmail.com (J. Diva), catcarol_2000@yahoo.com (Caroline), sundus@ifph.org (S. Tewfik), I.Tewfik@westminster.ac.uk (I. Tewfik).

https://doi.org/10.1016/j.bcab.2019.101152
Received 7 February 2019; Received in revised form 6 May 2019; Accepted 6 May 2019
Available online 07 May 2019
1878-8181/ © 2019 Elsevier Ltd. All rights reserved.

higher antioxidant and TPC (total phenolic content) compared to water distilled-solvent extraction or cold maceration. Ingawale et al. (2018) obtained that TPC, antioxidant and α-glucosidase inhibition of *Xanthium strumarium* L. fruit were optimum on the ultra-sonication extraction with methanol, time and solid to solvent ratio were 60%, 30 min and 1 respectively. Previous results on solvents influence reported that DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant of the water extract (infusion) has shown the highest value compare to ethanolic and reference rutin (a flavonoid glycoside). The IC_{50} of *C. burmannii* water extract was 3.03 ± 0.22 μg/mL, while ethanolic extract was 8.36 ± 0.73 μg/mL and rutin was 15.27 ± 0.69 μg/mL) (Ervina et al., 2016). Hence, the objectives of this study were to optimize cinnamon water extraction process and verify compounds of instigating its in vitro antidiabetic activity from *C. burmannii* bark using factorial design.

2. Materials and methods

Factorial design (3 factors and 2 levels) has been used in this optimization process, these included: yield percentage (%), TPC (total phenolic content), IC_{50}AA (inhibition concentration DPPH antioxidant activity), IC_{50}αGI (inhibition concentration of α-glucosidase activity) as responses (Dejaegher and Heyden, 2011).

Table 1 demonstrates these factors and levels: Temperature (98 °C (X_1)as high and 90 °C as low levels); concentration (30% and 10%); time of extraction (20 min and 15 min). The polynomial equation was applied to interpret the results of each parameter-response and establish the optimized condition of water extract.

2.1. Chemical and reagents

C. burmannii (*Cb*) dried bark was obtained from local the region (UPT Materia Medica Batu, East Java, Indonesia; ± 875 m above sea levels, with average a temperature of ± 20–25 °C). The sample was then authenticated and deposited in Pharmacognosy and Phytochemistry Laboratory (document number C07-052-15), Faculty of Pharmacy Widya Mandala Catholic University. All solvent and chemicals used were pro-analytical grades. The employed reagents were ethanol, *n*-hexane, ethyl acetate, formic acid, methanol, toluene (Mallinckrodt Baker, USA); $FeCl_3$, $AlCl_3$, H_2SO_4, acetic acid anhydrate, phosphate buffer (67 mM, pH 6,8), aqua demineralisation, and sodium carbonate bismuth subnitrate, KI and HNO_3 (Dragendorff), $HgCl_2$ and KI (Mayer's), α-naphthol; cinnamaldehyde, rutin, Folin-Ciocalteu (Merck, KGaA, Darmstadt, Germany); gallic acid, α-glucosidase (from *S. cerevisiae*), *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), acarbose, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, Germany). These chemicals were purchased from local suppliers.

2.2. Sample preparation

The bark prepared as outlined in Ervina, Nawu & Esar, study (2016) and the determination of quality parameters was based on national quality standard (IFDA, 2000). The results of sample quality than compared to national herbal pharmacopeia (IHP, 2012). Phytochemical screening of the extract was detected by using spot reagents test (Trease and Evans, 2000).

Table 1
Optimization design of Indonesian cinnamon bark.

Code	Real value			Notation value						
	T (X_1) (°C)	C (X_2) (%)	t (X3) (min)	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$
1	98	30	20	+1	+1	+1	+1	+1	+1	+1
2	98	30	15	+1	+1	−1	+1	−1	−1	−1
3	98	10	20	+1	−1	+1	+1	+1	−1	−1
4	98	10	15	+1	−1	−1	−1	−1	+1	+1
5	90	30	20	−1	+1	+1	−1	−1	+1	−1
6	90	30	15	−1	+1	−1	−1	+1	−1	+1
7	90	10	20	−1	−1	+1	−1	−1	−1	+1
8	90	10	15	−1	−1	−1	+1	+1	+1	−1

2.3. Design optimization with factorial design method for extraction

The factorial design method obtained for optimization with 3 factors and 2 levels, that was (98 °C as high level (+1) and 90 °C as low level (−1)); concentration (30% as high level (+1) and 10% as low level (−1)); and extraction time (20 min as high level (+1) and 15 min as low level (−1)). The number of experiments performed was $2^3 = 8$ as presented in Table 1. The responses obtain %yield, DPPH IC_{50} AA, TPC (% w/w Gallic acid equivalent (GAE), and IC_{50} of αGI. A polynomial equation: $y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_{12} \times 1 \times {}_2 + B_{13} \times 1 \times {}_3 + B_{23} \times 2 \times {}_3 + - B_{123}X_1X_2X_3$ would obtain for each response and establish the most factor and or interaction influenced the response most. The collected filtrate was evaporated in a water bath. The final products were investigated of their identity, physical characteristics (color, odor), and water content.

2.4. Determination of total polyphenol content

Folin Ciocalteau (FC) reagent was employed for total phenolic content (TPC) determination of extracts; based on an earlier reported experiment (Stankovic et al., 2011) by mixing thoroughly extracts or gallic acid (0.02 mL), to 10% FC reagents (0.1 mL) and Na_2CO_3 7.5% (0.08 mL). The mixture was then incubated (for 1 h, at room temperature and in dark conditions), and the absorbance was measured (at 765 nm), using multiscan GO *Microplate Reader UV/Vis Spectrophotometer*. TPC was analyzed by plotting gallic acid calibration curves (12.5–500 μg/mL) and expressed as the percentage of milligram gallic acid equivalent per milligram of dry extract (% w/w GAE/sample). Rutin was used as the reference standard and blank was prepared to correct absorption.

2.5. In vitro antioxidant activity assay

DPPH scavenging method was used to obtain antioxidant activities of the extracts based on Ervina, Nawu, & Esar studied (2016). IC_{50} is the expression of antioxidant activity and resulted by sample linearity curve of % Inhibition versus sample concentration (% Inhibition = $[(A_{0DPPH} - A_{Sample})/A_{0DPPH}] \times 100\%$). Rutin was used as an antioxidant reference compound and the solvent blank was prepared to correct absorption.

2.6. Determination of α-glucosidase inhibition

The α-glucosidase activity inhibition (αGI) was determined based on Salehi et al. (2013), the method with minor modification. The enzyme used (3 U/mL, 0.02 mL) was resulted from the preliminary test. The αGI was obtained as follow: extract or acarbose was dissolved and diluted with phosphate buffer 67 mM, pH 6.8 at various concentrations (0.13 mL), the enzyme was added then shake (1 min). The mixture was pre-incubated (15 min at 37 °C), following the addition of substrate for the enzyme reaction 5 mM *p*-nitrophenyl-α-D-glucopyranoside (pNPG) (0.02 mL). The mixture was incubated (15 min at 37 °C) and 0.1 M sodium carbonate (0.08 mL) was added as a stopper of the enzyme reaction. The absorbance was measured at 405 nm, using multiscan GO *Microplate Reader UV/Vis Spectrophotometer*. IC_{50} (the concentration of the sample required to inhibit 50% of enzyme activity) of the samples and acarbose were obtained from the linearity curve of the % inhibition versus concentration of the sample. The reagent and solvent blanks also observed to correct absorption in the calculation.

2.7. Statistical analysis

All experiments were carried out in triplicates, the results presented as average values and standard deviations. The statistical mean comparison was performed with the SPSS version 24 program (one-way analysis of variance (p values < 0.05); and correlation analysis among dependent factors). Optimization of design analysis used Design-Expert version 7.0 program with the results obtained in the form of polynomials and contour plots.

2.8. Liquid chromatography-mass spectrometry of cinnamon extract

Cinnamon extract was pre-treated with solid phase extraction oasis® HLB Solvents (Waters). The extract was dissolved with methanol and filtered through 0.2 µm syringe filter; injected 5 µl to the column. The LC system operation conditions: UPLC (Ultra Performance Liquid Chromatography - ACQUITY UPLC®H-Class system (waters, USA)); C-18 (1.8 µm 2.1 × 100 mm) column HSS; temperature: 50 °C (column), 25 °C (room); mobile phase: water + 5 mM ammonium formic (A) and acetonitrile + 0.1% formic acid; flow rate: 9.2 mL/min (step gradient) running 23 min. Mass spectrometry system (Xevo G2-S QTof (waters, USA)): ES (Electrospray ionization); mode: positive mode; mass analysis range: 50 – 1300 m/z; source temperature: 100 °C; desolvation gas flow: 350 °C; cone gas flow: 0 L/hour; desolvation gas flow: 793 L/hour; collision energy: 4 V (low energy); ramp collision energy: 25–50 V (high energy).

3. Results and discussion

Oxidative stress reactions which are triggered by free radicals; are increased in diabetes pathogenesis complications (Penckofer et al., 2001; Rahimi et al., 2005). Cinnamon has been used as a supplement in managing type 2 diabetes therapy; though effectivity and safety data are needed for long-term trials. Kim et al. (2016) proposed the role of dietary polyphenols in the prevention and modulation of type 2 diabetes. It was supposed improving glucose homeostasis by inhibiting α-amylase and α-glucosidase, sodium-dependent glucose transporter 1 (SGLT1) in the small intestine. The inhibition would reduce digestion and intestinal glucose absorption of dietary carbohydrate. In the muscle and adipocyte, it would stimulate insulin-dependent glucose uptake, activate 51-adenosine-monophosphate protein kinase (AMPK), and modify micro-biome in the large intestine and reduce the inflammation.

Optimization of the process is one of two applications of experimental designs in pharmaceutical sciences. This research was on screening phase in which obtained factors (temperature, concentration and time of extraction) and interaction among factors influenced the response of interest (%yield, TPC, IC_{50}AA, IC_{50} αGI) (Dejaegher and Heyden, 2011).

The quality sample of the dried cinnamon (Table 2) was determined and compared to the standard guidance (IHP, 2012). The results obtained the character and quality of the sample accordance and fulfilled to the cinnamon characteristic (2008). The physicochemical of the extracts were consistent with previous research and added some information data on *C. burmannii* phytochemical content, which was glycoside, and coumarins content (Ervina et al., 2016; Shahidi and Naczk, 2013). The specific and nonspecific parametric result of *C. burmannii* are macroscopic, microscopic, secondary metabolite content, water content, drying shrinkage, ethanol, and water-soluble content. The phytochemical screening revealed alkaloid, polyphenol, tannin and flavonoids, essential oils, saponins, quinone, triterpenoids, glycosides, and coumarin content of the extracts. Physical appearances of the extracts were from red-brown to brown-black color, have dry consistency, and all have the cinnamon specific odor. The light red color extract might cause little content of phlobatannin (condensed tannin) extractive matter in which observed to all extracts. Though phlobatannin as a polymer of phenolic is insoluble in water, it can be filtered in water filtrate and add to the yield result weigh. The fact that water as the extractive solvent has a disadvantage compared to ethanol in which solubility of carbohydrate and protein occur, and difficulties to remove water from the extracts. On the other hand, water has multi-advantages in cinnamon extraction as it is safer, inexpensive and simpler to perform compared to others solvents (Bele et al., 2010).

The response of the optimization was determined as in Table 4. The equation of response optimization has obtained the influence of each factor and the interaction among them. The yield of the extracts was in a range from 3.22 ± 0.08 to 11.02 ± 0.41%. The lowest was for extract −1, +1, −1 (code 6); while the highest was shown by extract +1, −1, −1 (code 4). Statistical analysis showed a significant difference ($p = 0.05$ level) to all extracts (extract 1 to 7 and 8), except for extracts 5 to 6. The polynomial equation for the % yield response was $y = 6.274 + 1.539X_1 - 2.027X_2 - 0.059X_3 - 0.586 \times 1 \times {}_2 + 0.055 \times 1 \times {}_3 + 0.390 \times 2 \times {}_3 + 0.202X_1X_2X_3$. This equation established that temperature and interaction among temperature to extraction time and concentration to time extraction give the positive result; while concentration, extraction time and interaction between temperature and concentration revealed negative effect to the % yield. The yield response influenced more by the temperature (1.539) was also found by Jong et al. (2015), who extracted deer antler plants with hot water in hot water extraction of extract yield.

The TPC with FC method obtained of cinnamon extract and rutin with gallic acid equivalent as shown in Table 3. The TPC of extract was ranged from 105.71 ± 18.37 for extract 8 (−1,-1,-1); to 259.08 ± 15.46 µg GAE/mg extract for extract 1 (+1, +1, +1). The statistical analysis of TPC obtained significance difference among all extracts ($p = 0.05$ significance different level). TPC showed that condition 1 (high temperature, concentration and longer time of extraction) was more efficient in the extraction of polyphenol compounds

Table 2
Phytochemical characteristic of the *C. burmannii*.

Parameters							
Macroscopic	specific		Non-specific (content %)				
	Microscopic	Chemical screening (reagents test)	water	total ash	drying shrinkage	ethanol soluble	Water soluble
Rolls bark with a coarse surface, length 10–28 cm, cinnamon typical smell, reddish-brown color.	fragments of sclerenchyme fiber, oil cells, schlereids and schlerenchyme fibers, calcium oxalate crystals	Alkaloid + (Dragendorf & Mayer) Flavonoid + (Wilstater's test) Saponin + (foam test) Tannin + ($FeCl_3$, salt, gelatin) Quinone + (KOH) Triterpenoid + (Liebermann Burchard test) Glycoside + (Molish's test) Coumarin + (NaOH)	5.43 ± 0.26	4.11 ± 0.98	11.44 ± 1.11	28.13 ± 0.84	11.44 ± 1.11

Table 3
Responses results of the *C. burmannii* water extracts.

Experiment (code)	Moisture Content (%)	Yield (%)	TPC (μg GAE/mg sample)	IC_{50} (μg/mL)	
				DPPH AA	α-GI
(1)	4.72 ± 0.97	5.79 ± 0.59^{c}	259.08 ± 15.46^{d}	3.45 ± 0.04^{a}	0.485 ± 0.004^{a}
(2)	5.46 ± 1.19	4.61 ± 0.08^{b}	212.45 ± 11.38^{c,d}	9.46 ± 0.52^{b}	0.632 ± 0.003^{a}
(3)	6.16 ± 1.41	9.83 ± 0.13^{d}	203.95 ± 30.70^{b,c}	9.19 ± 0.13^{b}	0.608 ± 0.008^{a}
(4)	4.50 ± 1.53	11.02 ± 0.41^{e}	197.51 ± 13.26^{b,c}	13.07 ± 0.15^{c}	0.705 ± 0.003^{a}
(5)	6.32 ± 1.15	3.46 ± 0.10^{a}	181.23 ± 8.45^{b,c}	15.85 ± 0.18^{d}	0.760 ± 0.007^{a}
(6)	5.39 ± 0,.59	3.22 ± 0.08^{a}	157.82 ± 19.63^{b}	18.46 ± 0.09^{e}	0.822 ± 0.007^{a}
(7)	5.36 ± 1.19	5.85 ± 0.30^{c}	193.16 ± 21.12^{b,c}	19.32 ± 0.29^{f}	0.900 ± 0.002^{a}
(8)	4.22 ± 0.97	6.48 ± 0.32^{c}	105.71 ± 18.37^{a}	20.53 ± 0.30^{g}	1.044 ± 0.012^{a}
R	–	–	–	18.10 ± 0.33^{e}	–
A	–	–	–		103.35 ± 1.440^{a}

TPC = total phenolic content, GAE = gallic acid equivalent, IC_{50} = inhibition concentration, DPPH = 2,2-diphenyl-1-picrylhydrazyl antioxidant activity, α-GI = α-glucosidase inhibition, R = rutin, A = acarbose, different superscriptions in the same column represent for significant difference (α = 0.05).

compared to others. The equation was derived as $y = 188.86145 + 29.38359X_1 + 13.781X_2 + 20.489X_3 + 3.736X_1X_2 - 7.224X_1X_3 - 2.981 \times 2 \times {}_3 + 13.029 \times 1 \times {}_2X_3$; which showed all positive influence of all factors and all factors interaction; while negative interaction was observed on the interaction of temperature (X_1) to concentration (X_2) and extraction time (X_3) (Table 4). The temperature was observed as the most factor influence on TPC (29.384).

Scavenging DPPH activities of the extracts were in a concentration-dependent manner (Table 3). IC_{50}AA extracts 1–5 were lower than rutin reference, while statistical analysis obtained no significant difference among all extracts, except extracts 2 to 3 and extract 6 to rutin. The lowest activity of AA was observed on extract 8; which mean low potency of Cinnamon extract with extraction condition -1 -1 -1. The highest potency showed to extract 1 with IC_{50} AA 3.45 ± 0.04 μg/mL. The IC_{50}AA showed the potency of the extracts were comparable to rutin as flavonoid glycoside compound. Antioxidants, which can neutralize free radicals by donating hydrogen, may inhibit or quench the reactive oxygen or nitrogen species. Among these antioxidants are tannins, flavonoid, and coumarins. Tannin, the polyphenolic compounds which are able to scavenge free radicals, chelates trace metals and bind proteins of oxidative enzymatic activity. The existence of galloyl groups, *ortho*-dihydroxy, and hydroxyl groups structure are among the main determinants of scavenging activity, while the chelating mechanism depends on hydroxyl groups only (Yokozawa et al., 1998).

Tannin was described to enhance glucose uptake and inhibit adipogenesis, thus have benefit for the treatment of non-insulin dependent diabetes mellitus (Muthusamy et al., 2008). Other compounds that showed some advantages in managing non-communicable diseases e.g phenolic of flavan-3-ols which exerted antioxidative, anti-thrombogenic, and anti-inflammatory in the pathogenesis of the cardiovascular disease. Additionally, proanthocyanidins and flavan-3-ol monomers enhance lowering plasma cholesterol levels, inhibit LDL oxidation, and activate endothelial nitric oxide synthase to prevent platelet adhesion and aggregation that contributes to blood clot formation (Bagchi et al., 2003). The equation of IC_{50} AA determined as $y = 13.699–4.911X_1 - 1.845X_2 - 1.682X_3 - 0.493X_1X_2 - 0.776X_1X_3 - 0.421X_2X_3 - 0.125X_1X_2$ (Table 4). The equation showed all factors (temperature, concentration and extraction time) and obtained negative interaction among all factors to IC_{50} AA of extracts.

Table 3 showed IC_{50}αGI was in the range of 0.485 ± 0.004 (extract 1) to 1.044 ± 0.012 (extract 8) correspond to acarbose 103.35 ± 1.440 μg/mL. ANOVA analysis revealed a significant difference among all extracts and reference. The equation of the IC_{50}αGI was $y = 0.773–0.127X_1 - 0.084X_2 - 0.043X_3 + 0.026X_1X_2 - 0.028 \times 1 \times {}_3 + 0.014X_2X_3 - 0.027X_1X_2X_3$; which showed the slight positive influence of interaction among temperature and concentration, concentration to extraction time; and negative factors to IC_{50}αGI response (Table 4). The IC_{50}αGI of extract observed 'so potent' compare to that of the 'reference acarbose' (oral antidiabetic drug). The IC_{50}α-GI of the extract was 100–200 higher than acarbose. Salehi et al. (2013) determined IC_{50} of *Cinnamomum zeylanicum* from maceration with methanol solvent. It was 20.8 stronger compared to acarbose; while Shihabudeen, Priscilla and Thirumurugan (2011) obtained IC_{50} of methanol soxhletation of *C. zeylanicum* was 6.32 higher than acarbose. The distinct in findings may have resulted from different employed species of cinnamon, and types of solvents used in extraction which influence the composition (quality and quantity) of the extracted compounds and also different αGI activities of the extracts.

The results of this study found that the high activity of α-glucosidase inhibition is supposed due to TPC containing (Table 3). Correlation analysis of TPC, IC_{50}AA, and IC_{50}αGI exhibited negative correlation among TPC to IC_{50}AA (−0.808), and to IC_{50} α-GI (−0.754); while a positive correlation of IC_{50}AA to IC_{50}α-GI (0.892); at significance level (p = 0.01). The negative correlation means the higher TPC value, the lower concentration of α-glucosidase enzyme (or the stronger activity to scavenge DPPH radical and inhibit α-glucosidase enzyme activity); in which sequence with IC_{50} of the extracts (Fig. 1). The correlation among those three was also obtained by Mi et al. (2012) who found correlation among hawthorn fruit content of polyphenols, triterpenoids, protocatechuic acid and epicatechin to the alpha-glucosidase inhibitory activity. It was also found the contribution of polyphenols (flavonoid,

Table 4
Equation analysis results for each response.

Response (y)	Equation analysis of components
Yield	$y = 6.274 + 1.539X_1 - 2.027X_2 - 0.059X_3 - 0.586 \times 1 \times {}_2 + 0.055 \times 1 \times {}_3 + 0.390 \times 2 \times {}_3 + 0.202X_1X_2X_3$
TPC	$y = 188.86145 + 29.38359X_1 + 13.781X_2 + 20.489X_3 + 3.736X_1X_2 - 7.224X_1X_3 - 2.981 \times 2 \times {}_3 + 13.029X_1X_2X_3$
IC_{50}AA	$y = 13.699–4.911X_1 - 1.845X_2 - 1.682X_3 - 0.493X_1X_2 - 0.776X_1X_3 - 0.421X_2X_3 - 0.125X_1X_2X_3$
IC_{50}αGI	$y = 0.773–0.127X_1 - 0.084X_2 - 0.043X_3 + 0.026X_1X_2 - 0.028 \times 1 \times {}_3 + 0.014X_2X_3 - 0.027X_1X_2X_3$

X_1 = temperature, X_2 = concentration, X_3 = extraction time.

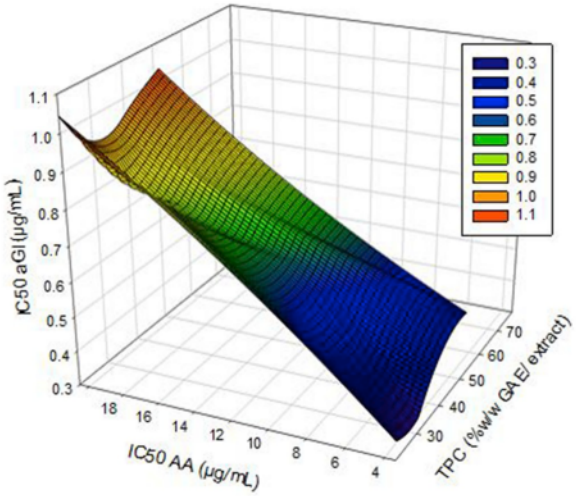

Fig. 1. 3D Correlation graph among responses of total phenolic content (x), IC_{50} antioxidant activity (y) and IC_{50} α-glucosidase inhibition (z) of the water extraction of *C burmannii* bark.
The color legend shows the value. The **blue area** of the curve showed the minimum concentration of DPPH IC_{50} antioxidant and α-glucosidase inhibition of the extracts; while **yellow to a red area** obtained of maximum concentrations. The negative correlation of TPC to IC_{50} α-GI (−0.754) and positive relationship IC_{50} AA to IC_{50} α-GI (0.892) at significance 0.01 level. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

gallic acid, catechin), vanillic acid, and chlorogenic acid to the antioxidant activity. The research also observed that 80% of acetone extract has the highest alpha-glucosidase inhibitory, while deionized water extract has the highest DPPH scavenge capacity and ferric reducing power. Furthermore, some researchers reported that triterpenoid (Lai et al., 2012), flavonoids (Wang et al., 2010) and flavonols, luteolin, myricetin and quercetin (Tadera et al., 2006) inhibit α-glucosidase.

Fig. 2 portrays contour plot of all optimized factor responses determined (%yield 5–11, TPC 40–50, IC_{50} AA 3.5–20, and IC_{50} αGI 0.5–0.7). The yellow area showed the optimum process with the alternative solution to 6.28% of yield, 3.44 µg/mL of IC_{50}AA, 0.50 µg/mL of IC_{50} αGI, and TPC 259.08 µg GAE/mg extract; on temperature 98 °C, concentration 30% and 20 min of time extraction, respectively. This theoretical condition pointed to extract number 1 (+1, +1, +1). No significant difference was detected in values between theoretical and factual parameters in the validated equation of result.

LCMS chromatogram of the optimized extract revealed 19 peaks (Fig.3A). Among these peaks, 11 peaks have a percentage above 1%. Two highest peaks were 57.12% and 12.05% on Rt 11.96 and 15.18 min respectively. Interestingly both peaks showed similar fragments pattern at m/z 621 (Fig. 3B). M/z 620 proposed to be a protonated heterodimer with one monohydroxy-dimethoxylated flavan-3-ol group and one trimethoxylated flavan-3-ol group (Mouls et al., 2011). Other specified m/z is 147 (Fig. 3C) in which observed at Rt. 7.7 (1.34%) and 865 at Rt 1.33 (1.62%), and 4.06 (4.61%) (Fig. 3D). These two fragments are characterized by fragments of *C. burmannii*. Chen, Sun and Ford (2014) found that at m/z 147, 865 are dominant in *Cb* and differentiate to other Cinnamon species (*C. cassia, C. verum* and *C. laureiroi*). The m/z 147 and 865 proposed as coumarins and polymers of A-type proanthocyanidins. Though m/z 865 was observed on its highest abundance, it had m/z 1153.2629 that was identified as A-type tetramers respectively. Another Cinnamon's specific fragment at m/z 133 (cinnamaldehyde) was not found. The compound might not dissolve in water base extraction sequentially. The type-A proanthocyanidins isolated from *Cb* were proposed to have insulin-like biological activity (Anderson et al., 2004) thus verifying its capacity to modulate DM-2 in human studies or biological assays.

4. Conclusion

Water extraction of *C. burmannii* has been optimized with 3 factors (temperature, concentration and time of extraction), 2 levels (high and low) and 4 responses (%yield, TPC, IC_{50}AA, and IC_{50} α-GI) by design experiment method. The theoretical optimization equation

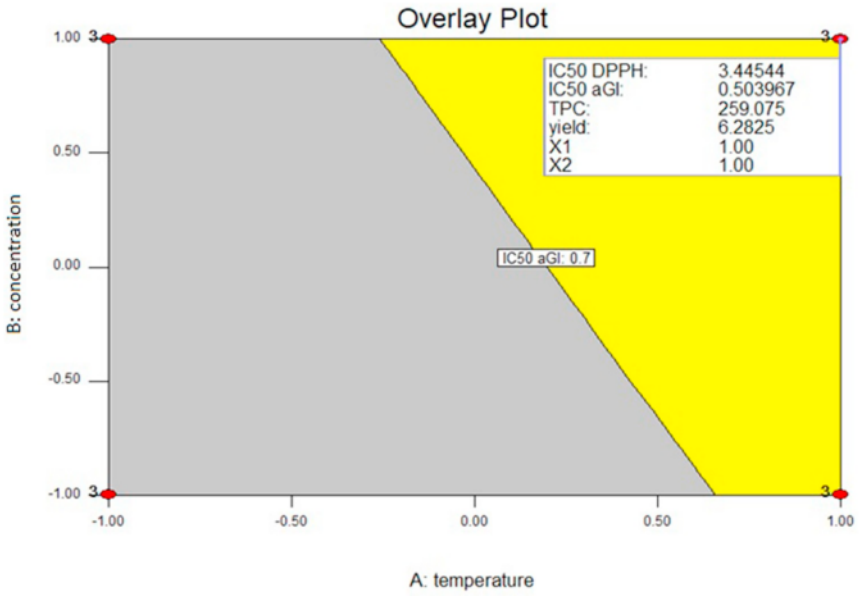

Fig. 2. Solution 1 of overlay graph among all parameters (yield, TPC, DPPH IC_{50}, and IC_{50} α-glucosidase inhibition) for optimization purposes.
The graph designed point in a range of temperature (x) and concentration (y), TPC and yield; while the minimum value of IC_{50}'s DPPH and α-glucosidase inhibition of Cinnamon water extracts. This graph was resulted by factorial design (3 factors: temperature, concentration and extraction time with 2 level low and high). The yellow area obtained optimum condition area of *C. burmannii* water extraction. TPC = total phenolic content, IC_{50}aGI = α-glucosidase inhibition, temp = temperature, cons = concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

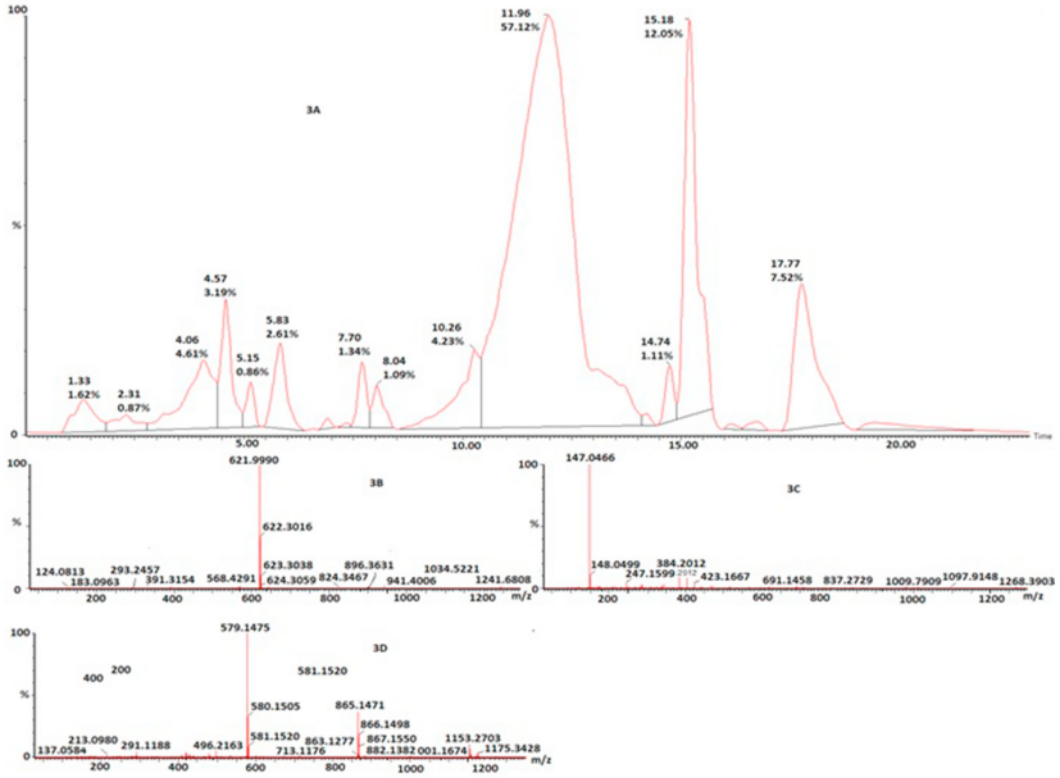

Fig. 3. LCMS of *C. burmannii* extract (**3A**) chromatogram peak of UPLC, mass fragments ES positive mode at Rt; (**3B**) 11.96 min (57.12%) with m/z 621.2999; (3C) 7.7 min (1.34%) with m/z 147.0466; (**3D**) 4.06 min (4.61%) with m/z 579.1475.

underpinned optimized of extract 1 (98 °C, 30% and 20 min). LCMS analysis of the optimum extract verified the typical characteristic of *C. burmannii* contents which are coumarins, polymers of proanthocyanidins A-type and protonated heterodimer of flavan-3-ol group content. The active components can assist in complementary therapy of DM as they possess antidiabetic activity.

Conflicts of interest

All authors declare that there is no conflict of interest.

Acknowledgments

We thank Widya Mandala Catholic University Surabaya for Faculty Pharmacy financial support through Faculty of Pharmacy research project (2016); and Institute for Research and Community Services for writing publication program. We also thank DR. Ignatius Srianta for the critical and proofread of this paper.

References

Adaramola, B., Onigbinde, A., 2017. Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome. Chem. Int. 3 (1), 1–7. http://isci.

Al-Dhuhiab, B.E., 2012. Pharmaceutical application and phytochemical profile of *Cinnamomum burmannii*. Phcog. Rev. 6, 125–131.

American Diabetes Association (ADA), 2018. Management of Diabetes. Clinical Practice Guidelines. accessed on 2018 August. https://diabetesed.net/wp-content/uploads/2017/12/2018-ADA-Standards-of-Care.pdf/.

Anderson, R.A., Broadhurst, C.L., Polansky, M.M., Schmidt, W.F., Khan, A., Flanagan, V.P., Schoene, N.W., Graves, D.J., 2004. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J. Agric. Food Chem. 52, 65–70.

Asif, M., 2015. Chemistry and antioxidant activity of plants containing some phenolic compounds. Chem. Int. 1 (1), 35–52.

Bagchi, D.B., Sen, C.K., Ray, S.D., Das, D.K., Bagchi, M., Preuss, H.G., Vinson, J.A., 2003. Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. Mutation Res 523, 87–97.

Bele, A.A., Jadhav, V.M., Kadam, V.J., 2010. Potential of tannnins: a review. Asian J. Plant Sci. 9 (4), 209–214.

Chen, P., Sun, J., Ford, P., 2014. Differentiation of the four major species of Cinnamons (*C. burmannii, C. verum, C. cassia*, and *C. loureiroi*) using a flow injection mass spectrometric (FIMS) fingerprinting method. J. Agric. Food Chem. 62, 2516–2521.

Dejaegher, B., Heyden, Y.V., 2011. Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. J. Pharm. Biomed. Anal. 56, 141–158.

Ervina, M., Nawu, Y.E., Esar, S.Y., 2016. Comparison of in vitro antioxidant activity of infusion, extract and fractions of Indonesian Cinnamon (*Cinnamomum burmannii*) bark. Int. Food Res. J. 23, 1346–1350. http://www.ifrj.upm.edu.my.

Hasanzade, F., Toliat, M., Emami, S.A., Emamimoghaadam, Z., 2013. The effect of cinnamon on glucose of type 2 diabetes patients. J. Trad. Complement. Med. 3, 171–174.

IDF (International Diabetes Federation), 2018. Atlas. https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf./, Accessed date: September 2018.

IFDA (Indonesian Food and Drug Administration, Indonesian language), 2000. The General Standard Parameter of Herbal Drug Extract. vols. 3–5. Ministry of Health The Republic of Indonesia, Jakarta, pp. 30–37 10–21.

IHP (Indonesian Herbal Pharmacopoeia, Indonesian language), 2012. Ministry of Health of the Republic of Indonesia, Jakarta. pp. 41–44.

Ingawale, A.S., Sadiq, M.B., Nguyen, L.T., Ngan, T.B., 2018. Optimization of extraction conditions and assessment of antioxidant, α-glucosidase inhibitory and antimicrobial activities of *Xanthium strumarium* L. fruits. Biocat. Agri. Biotech. 14, 40–47. https://doi.org/10.1016/j.bcab.2018.02.004.

Jong, H.J., Ei, H.C., Ju, H.H., Sung, W.C., Seung, T.S., Wooki, K., Dae, O.K., Byung, Y.K., Moo, Y.B., 2015. Optimization of hot water extraction and ultrahigh pressure extraction for deer antler. Food Sci. Biotech. 24, 507–512.

Khan, A.N., Khan, R.A., Ahmad, M., Mushtaq, M., 2015. Role of antioxidant in oxidative stress and diabetes Mellitus. J. Phcogn. Phytochem. 3, 217–220.

Kim, Y., Keogh, J.B., Clifton, P.M., 2016. Review polyphenols and glycemic control. Nutrients 8 27 pages.

Lai, Y.C., Chen, C.K., Tsai, S.F., Lee, S.S., 2012. Triterpenes as α-glucosidase inhibitors from *Fagus hayatae*. Phytochemistry 74, 206–211.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., Chen, S., 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21, 1374–1393.

Medagama, A.B., 2015. The glycaemic outcomes of cinnamon, a review of the experimental evidence and clinical trials. Nutr. J. 14, 108–120.

Miao, J., Li, X., Fan, Y., Zhao, C., Mao, X., Chen, X., Huang, H., Gao, W., 2012. Effect of different solvents on the chemical composition, antioxidant activity and alpha-glucosidase inhibitory activity of hawthorn extracts. Int. J. Food Sci. Technol. 51, 1244–1251.

Mouls, L., Mazauric, J.P., Sommerer, N., Fulcrand, H., Mazerolles, G., 2011. Comprehensive study of condensed tannins by ESI mass spectrometry: average degree of polymerisation and polymer distribution determination from mass spectra. Anal. Bioanal. Chem. 400, 613–623.

Muthusamy, V.S., Anand, S., Sangeetha, K.N., Sujatha, S., Lakshmi, B.A.B.S., 2008. Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chem. Biol. Interact. 174, 69–78.

Penckofer, S., Schwertz, D., Florczak, K., 2001. Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and prooxidants. J. Cardiovasc. Nurs. 16, 68–85.

Rahimi, R., Nikfar, S., Larijani, B., Abdollahi, M., 2005. A review on the role of antioxidants in the management of diabetes and its complications. Biomed. Pharmacother. 59, 365–373.

Salehi, P., Asghari, B., Esmaeili, M.A., Dehghan, H., Iraj, G., 2013. α-Glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes. J. Med. Plants Res. 7, 257–266.

Shahidi, F., Naczk, M., 2013. Extraction and analysis of phenolics in food. J. Chromatogr. A 1054, 95–111.

Shahidi, F., Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects – a review. J. Funct. Foods. 18, 820–897.

Shihabudeen, H.M.S., Priscilla, D.H., Thirumurugan, K., 2011. Cinnamon extract inhibits a-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. Nutr. Metab. 8, 46–57.

Stankovic, M.S., Niciforovic, N., Topuzovic, M., Solujic, S., 2011. Total phenolic content, flavonoid concentrations and antioxidant activity of the whole plant and plant parts extracts from *Teucrium montanum* L. var. Montanum, F. Supinum (L.) Reichenb. Biotechnol. Biotechnol. Equip. 25, 2222–2227.

Tadera, K., Minami, Y., Takamatsu, K., Matsuoka, T., 2006. Inhibition of α-glucosidase and α-amylase by flavonoids. J. Nutr. Sci. Vitaminol. 52, 149–153.

Trease, G.E., Evans, W.C., 2000. Pharmacognosy, fourteenth ed. W.B. Sanders Company Ltd, London.

Wang, H., Cui, Y., Zhao, C., 2010. Flavonoids of the genus iridaceae: mini-review. Med. Chem. 10, 643–661.

Yokozawa, T., Chen, C.P., Dong, E., Tanaka, T., Nonaka, G.-I., Nishioka, I., 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. Biochem. Pharmacol. 56, 213–222.

Optimization of water extract of Cinnamomum burmannii bark to ascertain its in vitro antidiabetic and antioxidant activities

ORIGINALITY REPORT

7%	5%	7%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Made Dira Swantara, Wiwik Susanah Rita, Made Asmarani Dira, Kadek Karang Agustina. "Cervical anticancer activities of Annona squamosa Linn. leaf isolate", Veterinary World, 2022 Publication	2%
2	bosaljournals.com Internet Source	1%
3	Wuryanto Hadinugroho, Suwaldi Martodihardjo, Achmad Fudholi, Sugeng Riyanto. "Esterification of citric acid with locust bean gum", Heliyon, 2019 Publication	1%
4	Amol Subhash Ingawale, Muhammad Bilal Sadiq, Loc Thai Nguyen, Tu Bao Ngan. "Optimization of extraction conditions and assessment of antioxidant, α-glucosidase inhibitory and antimicrobial activities of	1%

Xanthium strumarium L. fruits", Biocatalysis and Agricultural Biotechnology, 2018
Publication

5	Serpil Kılıç Depren, Bilge Coşkuner Filiz, Büşra Nur Kulaklı, Aysel Kantürk Figen. "Ultra-layered sheet Cu Co nanoparticles for optimized application in catalytic reduction of organic dye", Materials Characterization, 2020 Publication	1 %
6	onlinelibrary.wiley.com Internet Source	1 %
7	mdpi-res.com Internet Source	1 %
8	www.jmbfs.org Internet Source	1 %
9	Submitted to University of Strathclyde Student Paper	1 %
10	Madhav Poudel, Meena Rajbhandari. "Phytochemical Analysis of Ampelopteris Prolifera (Retzius) Copeland", Nepal Journal of Science and Technology, 2020 Publication	1 %
11	Submitted to Universitas Jenderal Achmad Yani Student Paper	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		