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Process optimization to high gymnemic acid content from Gymnema inodorum (Lour.) Decne. leaves

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Abstract

Gymnema inodorum or Phak Chiang Da (PCD) vegetable is an indigenous medicinal plant used in Northern Thailand for diabetic treatment since ancient times. However, the low concentration of an active molecule, gymnemic acid (GA) in the leaves limit its applications among the large population. Therefore, the present study aimed to maximize the extraction of GA from PCD leaves using the solvent extraction method. The effect of concentration of ethanol and extraction temperature were investigated for the determination of optimum extraction conditions. A process was developed for the production of GA-enriched PCD extract powder and characterized. Results showed that a concentration of 50% (*v/v*) ethanol at 70°C for 2 h was appropriate to extract a higher GA concentration of 8,307 mg/kg from dried PCD leaves. During the drying process, maltodextrin with a concentration of 0.5% (*w/v*) was appropriate to produce PCD extract powder with maximum GA concentration. The color analysis (L*, a* and b*) revealed that the overall appearance of the PCD extract powder was a dark greenish tint mixed with yellow. The antioxidant activity assay showed that PCD extract powder at 0.1 g was able to neutralize 75.8% of DPPH free radicals. The results suggested that PCD extract powder rich in GA could be used as a possible source of nutraceuticals or as a functional food ingredient.

Introduction

Gymnemic acid is a group of complex molecules found in the leaves of *Gymnema sp.*, used in traditional Ayurvedic medicine[1]. It is believed that gymnemic acid is known for its hypoglycemic effects, making it a popular natural remedy for diabetes[2,3]. Gymnemic acid works by blocking the taste receptors on the tongue responsible for the sensation of sweetness. This effect can help reduce sugar cravings and may help control blood sugar levels in people with diabetes[4]. In addition to its potential anti-diabetic effects, gymnemic acid has also been studied for its anti-inflammatory, anti-obesity, and anti-cancer properties[3,5].

In this regard, *Gymnema inodorum*, indigenous to Thailand, one of the local Thai vegetables, found everywhere in the northern region and locally named “Phak Chin Da” or “Phak Chiang Da” (PCD) have been used as a traditional medicine for the management of diabetes[6–8]. While the leaves of the *G. inodorum* plant have been used for centuries in traditional medicine to treat various ailments, using whole leaf extract has some disadvantages that should be considered. For instance, Gymnema extract can contain varying amounts of gymnemic acid, which can make it difficult to achieve consistent dosing. The extract can contain other compounds that may interact with medications or have other side effects. Moreover, some compounds in whole leaf extracts may hinder the absorption of gymnemic acid by the body, reducing its bioavailability and effectiveness[9].

To overcome this, specific extraction methods can be optimized to improve the concentration of gymnemic acid and make a powdered formulation. The steps typically involve procuring *G. inodorum* leaves followed by drying, grinding into a fine powder, extraction with suitable solvents, concentration, mixing with a carrier and drying. This dry powdered extract not only helps to improve the solubility, stability and shelf life but also can increase the bioavailability of the gymnemic acid. Furthermore,

powder formulation rich in gymnemic acid can be used in a wider range of applications. For example, the powder can be mixed with other ingredients to create capsules or tablets, or it can be added to foods or beverages.

There have been several research articles that have investigated the extraction and quantification of gymnemic acid from *Gymnema* leaves using various extraction methods and solvents. Few studies found that ultrasound assisted extraction of *G. sylvestre* leaves was efficient and faster technique in extracting gymnemic acids and other bioactives[10–14] when compared to other conventional methods. In another study, Mandal et al.[15], compared different extraction methods such as heat flux extraction, maceration and stirring extraction and microwave-assisted extraction methods for the recovery of gymnemic acids from *G. sylvestre*. The authors found that microwave-assisted extraction was superior than conventional extraction methods in terms of time and energy consumption[15]. Although several extraction methods have been described in the literature, solvents-based extraction is the most widely used method where the solvent penetrates the solid matrix solubilizing the solute that diffuses out of the matrix. While some researchers have demonstrated that solvent-based Soxhlet extraction yielded highest gymnemic acids from *G. inodorum* leaves[16,17], others have stated that solvent maceration-based extraction was suitable for the recovery of gymnemic acids[18–23]. In general, solvent-assisted extraction methods have several advantages over other methods in terms of higher extraction efficiency, selectivity and reduced energy consumption.

The extraction of gymnemic acid from *Gymnema* leaves can be achieved using different solvents, including water, methanol, ethanol, and acetone. The choice of solvent may depend on various factors, including the solubility of gymnemic acid in the solvent, the extraction efficiency, and the safety and environmental impact of the solvent. Generally, polar to medium polar solvents are used to extract phenolic acids, alkaloids, flavonoids and tannins which are polar in nature. In this study, ethanol, an organic polar solvent was employed in the extraction of GA due its polarity[24]. It was also reported that a combination of polar solvents such as ethanol and water at different proportions can be ideal to extract phenolic acids since both the solvents have different polarity values (ethanol: 0.654, water: 1.0) and their acceptability for human consumption[25]. In addition to the choice of the solvent, several parameters such as solvent concentration, extraction temperature, solvent-to-solid ratio and extraction duration may significantly influence the extraction efficiency and concentration of bioactive compounds[26].

Although several reports have been published on the preparation of extracts from PCD leaves[27–29], there is scarce information on the preparation of powder formulation utilizing the extracts. The idea of preparing a powder formulation from the extracts of PCD leaves will more likely to gain popularity in the food and nutraceutical industry due to added benefits. Generally, to prepare a powder from the extracts, carriers such as maltodextrin[30,31], starch[31,32], and cellulose derivatives[33,34] are used during drying process. The selection of a carrier may depend on several factors such as the physical and chemical properties of the extract, solubility and stability of the active compounds, the compatibility of the carrier with the active compounds in the plant extract and the intended application[35–37]. Maltodextrin is

preferred over other carriers for their excellent flowability and dispersibility. It can reduce moisture reabsorption and reduce powder particle agglutination, make the extract powder retain its shape for a longer time and extend the shelf life[30]. Moreover, the addition of maltodextrin can have the advantages over other drying agents for its cost-efficient, highly soluble and poorly hygroscopic characteristics[30,38,39], thus chosen in the present study.

The aim of the present study is to optimize the extraction conditions for higher recovery of GA from *G. inodorum* leaves and to develop a process for the production of PCD dried extract powder.

Materials And Methods

Plant material and Chemicals. The medium-matured leaves of *G. inodorum* (Lour.) Decne. were procured from Wor Kaew Subdistrict, Hang Chat District, Lampang Province, which is a well-known area for commercial farms growing *G. inodorum* in Northern Thailand. The leaves were collected between January and February 2022 using species No. 6 from certified Agricultural Land Plot No. DOA-52120700-9058-0006. The use of this economic plant, which was not endangered or protected species, complied with international and national guidelines of Good Agricultural Practices (GAP) for Food Crops / Thai Agricultural Standard (TAS) 9001(G)-2021. All records of specimens and Agricultural Land Plot usage were kept at Plant Standard and Certification Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. The ethanol used for extraction in this study was food grade with 99.8% (v/v) purity (Liquor Distillery Organization, CAS No. 64-17-5, Chachoengsao, Thailand). All other chemicals utilized were either Analytical or HPLC grades.

Preparation of dried PCD leaves powder. PCD leaves were washed thoroughly, drained, and dried in a hot air dryer (Owner Foods Machinery Co., Ltd., Model SO 6A, Bangkok, Thailand) until a constant weight was obtained. Dried leaves were ground with a grinder mill (Huang Cheng, Model 4500A, China) and sieved through a sieve shaker (Endecotts, Model Octagon 200, London, United Kingdom) using a 100-mesh sieve size to yield the particle size not more than 150 µ.

Preparation of PCD extract powder. Figure 1 shows the step-by-step process of PCD extract powder production. Briefly, the PCD leaves were dried at optimized drying temperature (Section ‘Effect of PCD leaves drying temperature’) prior to attrition process with industrial high-speed grinder (Huang Cheng, Model 1500A, China) with screen size of 100 mesh (Endecotts, Model Octagon 200, London, United Kingdom) to attain PCD leaves powder. Further, 1 kg leaves powder was suspended in 20 L ethanol at the optimized concentration (Section ‘Effect of ethanol concentration during GA extraction from dried PCD leaves powder’) in a 50 L double jacketed continuous stirring bioreactor (FEBIX International, Model ST-50L, Chiang Mai, Thailand) and stirred at a speed of 50 rpm for 2 h. The extraction was carried out at optimized temperature (Section ‘Effect of temperature during GA extraction from dried PCD leaves powder’). The resultant slurry was filtered with double layer muslin cloth and the ethanol fraction was concentrated using a rotary evaporator (FEBIX International, Model REvap001, Chiang Mai, Thailand) at 50°C and 10 rpm. When 50% (*v/v*) of the ethanol volume was evaporated, maltodextrin was added at the

optimized concentration (Section 'Effect of maltodextrin concentration during production of PCD extract powder') to the concentrated extract. The extract was then evaporated to dryness at 65°C using a laboratory-scale vacuum dryer equipped with a diaphragm vacuum pump (Vacuubrand, Model MZ 2C NT + AK + EK, Wertheim, Germany) for creating the necessary vacuum (76 ± 5 cm Hg) inside the drying chamber. The dried material was pulverized into powder using an electric food chopper (Kenwood, Model CH550, Hampshire, UK).

Experimental design. In this study, firstly, a single factor experiment was used to optimize the extraction of GA from PCD leaves by studying the effects of three factors, namely drying temperature, ethanol concentration and extraction temperature. In this experiment, one of the parameters is changed at a time while the other parameters are kept constant. Secondly, the PCD extract obtained using optimized conditions was processed further into PCD extract powder and the maltodextrin concentration was optimized during the preparation. Finally, the PCD extract powder prepared with optimized concentration of maltodextrin was evaluated for its GA, chlorophylls content, water activity (a_w), moisture content, color (L*, a*, b*) and antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The details are described in the analytical section.

Effect of PCD leaves drying temperature. Three different temperature levels, 40, 50 and 60°C were applied during the drying process of PCD leaves while the concentration of ethanol and extraction temperature were kept constant at 50% (*v/v*) and 70°C, respectively during extraction of GA from dried leaves. The levels of drying temperature used in this study were selected based on the literature where the authors have reported that drying at above 70°C was detrimental to the bioactive properties and temperature up to 60°C retained most of the bioactive compounds[40,41]. The best drying temperature was chosen based on the color L*a*b* and GA content of dried leaves. For GA extraction, PCD leaves powder obtained from each drying temperature level was extracted with 50% (*v/v*) ethanol at a 1:20 (*w/v*) ratio in a water bath (Memmert, Model WNB 7–45, Germany) at 70°C for 2 h under static conditions. The concentration of ethanol, extraction temperature and duration were selected from our previous preliminary research where solvent concentration above 50%, extraction temperature above 70°C and duration beyond 2 h resulted in decreased GA content of *G. inodorum* cultivated in different region (unpublished data). The slurry after extraction was filtered through a double layered muslin cloth to separate the residue and extract. The extract was filtered using an Agilent PTFE syringe filter (0.45 µm) and aliquot was transferred to an HPLC vial. The content of GA in the extracts was quantified with HPLC as described in the analytical section.

Effect of ethanol concentration during GA extraction from dried PCD leaves powder. Ethanol was chosen for the extraction of GA as it considered to be a food grade solvent[42,43] and moreover it was found to be the appropriate solvent by virtue of safety and efficiency[44]. The effect of ethanol concentration on GA extraction was studied using the PCD leaves dried at best drying temperature chosen from the previous step and pulverized. Six levels of ethanol concentration, namely 0, 20, 30, 40, 50 and 60% (*v/v*) prepared in distilled water were used for the extraction of GA from PCD leaves powder while the extraction temperature was maintained at 70°C. The slurry after extraction was processed as stated above and

quantified for GA content. The minimum ethanol concentration that resulted in the optimized GA content and extraction yield was utilized for further experiments.

Effect of temperature during GA extraction from dried PCD leaves powder. The PCD leaves dried at best drying temperature determined in the first step and powdered was used to select the suitable extraction temperature. PCD leaves powder was extracted by holding at different extraction temperature namely, 40, 50, 60, 70 and 80°C for 2 h under stationary condition while the concentration of ethanol was kept constant at the best concentration chosen from the second step. The extracts were then filtered through muslin cloth and GA content in the filtrate was quantitated as mentioned earlier. The best extraction temperature was chosen according to the optimized GA content and extraction yield.

Effect of maltodextrin concentration during production of PCD extract powder. The PCD extract powder was produced by drying the PCD leaves at optimum drying temperature followed by powdering and extracting with optimum ethanol concentration at optimum extraction temperature. Different concentrations of maltodextrin ranging from 0–5.0% (*w/v*) were used for the study and added to the concentrated slurry which was concentrated by two times after rotary evaporation stage. The upper concentration of maltodextrin was restricted to 5% considering its high glycemic index. For the extraction of GA, PCD extract powder prepared with or without maltodextrin was suspended in 50% *(v/v)* ethanol, centrifuged twice at 2,822 × g (Nuve, Model NF 200, Ankara, Turkey) for 5 min followed by 6,742 × g (Eppendorf, Model Mini spin, Hamburg, Germany) for 1 min and filtered through Agilent PTFE syringe filter (0.45 µm) before HPLC analysis. The suitable maltodextrin concentration was chosen based on the higher GA content and extraction yield.

Analytical methods.

Color analysis. Color parameters of samples were measured using a color meter (Minolta, Model CR-300 chroma meter, Osaka, Japan) and reported as L*, a*, b* values. L* indicates lightness (100) or darkness (0), a* means red (+) or green (-) and b* represents chromaticity yellow (+) or blue (-)[45,46].

Determination of moisture content. The moisture content on mass basis was determined as per the 17th edition of Association of Official Analytical Chemists (AOAC)[47]. Briefly, a known amount of sample was weighed and dried in a hot air oven (Daihan Lab Co., Ltd., Gangwon, Korea) at 105°C for 4–5 h until constant weight to determine the moisture content and calculated according to the Eq. (1)[48].

% Moisture content = [(Initial weight - Final weight)/ Initial weight] × 100 (1)

Determination of water activity (a_w). The a_w of the samples was performed using a water activity meter (AQUA Lab, Model CX3TX, Washington, USA). The a_w was determined from the triplicate samples (2 g) held at 25 ± 0.1°C until equilibrium was reached[48].

Quantification of chlorophylls content. For the extraction of chlorophylls, 0.5 g of PCD extract powder was mixed with 10 mL of ice-cold acetone and shaked in an orbital shaker (LabTech, Model LSI 3016R,

Korea) at 250 rpm for 1 h at 30°C. Later, 5 mL of ice-cold ethanol was added to it and shaked again at the similar condition. The samples were filtered and the extraction process were repeated until the sample residue become colorless. The extracted samples were pooled together and concentrated under vacuum at 65°C[49]. The residue was re-dissolved in HPLC-grade absolute methanol (1 mL) before analyzed by HPLC. Chlorophylls content were determined using a reversed-phase HPLC system equipped with a degasser, autosampler and diode array detector (Agilent Technologies, Model 1260 Infinity series, Santa Clara, California, USA). HPLC analysis of chlorophylls was performed according to the method described by Varzakas and Kiokias[50] with some modifications. The column used for chlorophylls detection was an Agilent Poroshell 120 SB-C18 with a specification of 2.1 × 100 mm, 2.7 µ which was maintained at 30°C. The isocratic mobile phase consisted of ethyl acetate: methanol (20:80, *v/v*) at the flow rate of 0.60 mL/min with a run time of 15 min and the injection volume was 5 µL. The spectra were recorded and the chromatograms were obtained at 430 nm for chlorophyll a and 474 nm for chlorophyll b. The identification of chlorophylls was based on the respective standards and their retention times. The identified compounds were quantitated from the peak area using respective calibration curve prepared in the range from 0 to 200 ppm (1 ppm = 1 mg/L). The chlorophylls content calculated in ppm was converted into mg/L of extract and expressed as mg/kg on dry weight basis by considering the initial dried weight of powder used for the extraction.

Quantification of GA content. GA content was determined using a reversed-phase HPLC system equipped with a degasser, autosampler and diode array detector (Agilent Technologies, Model 1260 Infinity series, Santa Clara, California, USA). HPLC analysis of GA was performed according to the method described by Salunkhe and Magdum[51] with some modifications. The column used for GA detection was an Agilent Zorbax Eclipse Plus C18 with a specification of 4.6 × 100 mm, 3.5 µ which was maintained at 40°C. The gradient mobile phase consisted of solvent A as 1% (*v/v*) phosphoric acid and solvent B as absolute methanol (HPLC grade). The flow rate was fixed at 0.65 mL/min and the injection volume was 5 µL. The gradient elution program was started with 90% (*v/v*) A and 10% (*v/v*) B (0 min). At 4 min, the gradient reached was 30% A (*v/v*) and 70% B (*v/v*) and maintained for 12 min. The gradient then finally reached 90:10% (*v/v*) (A : B) at 16 min and maintained for 4 min with a total run time of 20 min. The detection was carried out at 210 nm[51]. The identification of GA was based on the standard and its retention time. The identified compounds were quantitated from the peak area using respective calibration curve prepared in the range from 0 to 100 ppm. The GA content calculated in ppm was converted into mg/L of extract and expressed as mg/kg on dry weight basis by considering the initial dried weight of powder used for the extraction. Mass yield percentage of extracted GA ($\%Y_{GA}$) was calculated as shown in Eq. (2).

$$\%Y_{GA} = [\text{GA content (mg/kg)} / (1000\ \text{g} \times 1000\ \text{g}) \times 100\%] \quad (2)$$

Improved purity percentage of GA content in PCD extract powder relative to dried PCD leaves powder ($\%P_{Ext/Leaves}$) was calculated as shown in Eq. (3).

$\%P_{Ext/Leaves}$ = [(GA content of PCD extract powder – GA content of PCD leaves powder) / GA content of PCD leaves powder] × 100% (3)

DPPH scavenging activity. DPPH free radical scavenging capacity of PCD leaves extract powder was analyzed as previously described by Meza et al.[52]. Briefly, a 0.1 mM ethanolic stock solution of DPPH was prepared and diluted ten times to obtain the working solution. A 300 µL of sample solution containing PCD extract powder suspended in absolute ethanol at 10 mg/mL concentration was mixed with DPPH working solution (1,600 µL). The mixture was incubated in the absence of light for 1 h and absorbance was measured at 515 nm using a UV-vis spectrophotometer (Agilent Technologies, Model Cary 60 UV-Vis, Santa Clara, California, USA). Ascorbic acid and gallic acid dissolved in absolute ethanol at 10 mg/mL were used as positive controls for comparing the antioxidant activity. The percentage of radical scavenging activity (%RSA) was calculated according to the Eq. (4).

$$\% RSA = [(A_0 - A_{60})/ A_0] \times 100 \quad (4)$$

where A_0 is the absorbance of DPPH radical solution at 515 nm without extract or standard at 0 min; A_{60} is the absorbance of DPPH radical solution at 515 nm in the presence of extract or standard at 60 min.

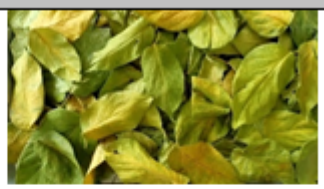
Statistical analysis. Three replications were carried out in each experiment and the results were expressed as mean ± standard error. Statistical analyses were carried out with Statistical Packages for the Social Sciences (SPSS, version 17.0) applying one-way analysis of variance (ANOVA). Statistical differences between means were measured using Duncan's multiple range test (DMRT) and considered significant at $p \leq 0.05$[54–56].

Results And Discussion

Effect of PCD leaves drying temperature. The GA content presented in PCD leaves when dried at 40, 50 and 60°C is shown in Table 1. The dried leaves at 40 and 50°C had a moisture content of no more than 5.0 ± 0.5% (*w/w*). The GA content of PCD leaves was significantly ($p \leq 0.05$) influenced by drying temperature beyond 40–50°C range and tended to decrease with increased temperature. For instance, when the drying temperature increased to 60°C, the GA content significantly ($p \leq 0.05$) decreased by 5% (*w/w*) when compared to drying at 50°C. However, the GA content (8,307 ± 39 mg/kg) presented in PCD leaves when dried at 50°C was not significant ($p > 0.05$) when compared to drying at 40°C. The decrease in GA content observed at 60°C could be attributed to the oxidation of compounds at relatively high temperatures in the presence of air[57]. This was also consistent with the reports of Peries et al.[58] and Sagrin and Chong[59] who reported that drying of leaves at the high temperature could destroy the heat-labile phytochemicals. Moreover, drying temperature above 60°C could also mitigate antioxidant content of the leaves significantly[59,60].

Table 1. Appearance and GA content of dried PCD leaves at different temperatures. Numbers with the same alphabet indicated no significant difference ($p > 0.05$) for comparison of the same column. Bold

and underlined data indicated the best average value(s) within each column. PCD, Phak Chiang Da; GA, gymnemic acid.

Drying temperature (°C)	Duration (h)	GA content (mg/kg dried leaves)	Appearance
40	24	**8,224^{A} ± 40**	
50	12	**8,307^{A} ± 39**	
60	8	7,882^{B} ± 21	

Color is an important parameter in which L* indicates a light–dark spectrum with a range from 0 (black) to 100 (white), a* denotes the green (-60) to red (+ 60) spectrum, and b* means the blue (-60) to yellow (+ 60) spectrum dimensions[45]. The different temperatures in the drying method had a significant effect ($p \leq 0.05$) on the lightness (L*), redness (a*), and yellowness (b*) of PCD leaves. As illustrated in Fig. 2, L* values decreased significantly ($p \leq 0.05$) from 63.8 to 54.8 when the drying temperature increased from 40 to 60°C. The lesser the L* value, the darker the leaves became. The decrease in L* value could be caused by the degradation of chlorophylls during the drying process[61]. It was also observed that the color of the leaves gradually shifted in green and yellow directions. This was evident from a decrease in greenness (a*) and an increase in yellowness (b*). However, the leaves dried at 40°C possessed less green color when compared to 50 or 60°C. This indicates that the degradation of chlorophylls depends not only on the drying temperature but also on the drying duration. The long drying duration of 24 h at 40°C in contrast to 12 and 8 h at 50 and 60°C respectively, might have caused the reduction in greenness. This is consistent with the results of Rudra et al.[62] who reported that drying at higher temperatures and long drying durations are the two important factors resulting in reduced lightness of samples during dehydration process. The increase in positive a* value in samples dried at 60°C might be due to exposure of chlorophylls to the high temperature that changed chlorophylls into pheophytin and pyropheophytin[63]. A similar trend was observed in a study conducted by Adhamatika et al.[61] who reported that drying of pandan leaves at high temperatures caused an increase in redness (a*). Similarly, the increase in b* value upon increasing temperature could explain the degradation of chlorophylls by high temperature into its derivatives and cause the brownish color of PCD leaves dried at 60°C[64]. Overall, the drying temperature and drying duration plays a crucial role in the appearance and color of leaves during the drying process.

As the drying temperature of 60°C resulted in the darker color of the leaves when compared to using a lower temperature, using the temperature between 40 and 50°C is thus suggested for drying. However, considering the energy consumption rate, drying at 40°C consumes more energy in terms of duration as it requires 24 h for complete drying. On the other hand, drying at 50°C is considered energy efficient as the drying process is completed within 12 h. The leaves were dried at 50°C for 12 h with a mass yield of 11.7 ± 1.1% (*w/w*) on a fresh weight basis further ground to powder, and sieved through a siever to yield powder particles of not more than 150 µm in size.

Effect of ethanol concentration during GA extraction from dried PCD leaves powder. The dried PCD powder obtained from the previous step was extracted with different concentrations of ethanol and the results are presented in Fig. 3. From the results, an increase in GA content was observed with the elevated concentration of ethanol used for the extraction. Distilled water as a solvent (0% ethanol) could extract the least amount of GA with only 3,894 ± 87 mg/kg of dried PCD leaves powder. Extraction of PCD leaves powder with 20 to 50% (*v/v*) ethanol resulted in 4,912 ± 71 to 8,414 ± 82 mg/kg. The Y_{GA} at 50% (*v/v*) ethanol was 2.2-fold higher when compared to GA extracted with distilled water. The $\%Y_{GA}$ was found to be 0.84% on dried weight basis of PCD leaves powder. In contrast, Ahamad et al.[13] reported that $\%Y_{GA}$ from *G. sylvestre* was 12.2% on dried weight basis of leaves. This difference in the yield might be due to the method of extraction where the authors used ultrasonic sound in coupled with Soxhlet extraction and usage of different species for the study[13]. Similarly, Suwan et al.[16] stated that PCD leaves extract using ethanol as an extractant produced about 40,000 mg/kg dried weight of GA which was about 5 times higher compared to the present data with the $\%Y_{GA}$ of 4%. This might be due to the prolonged extraction of 3 days used in the above study in contrast to the 2 h applied in the present study. Moreover, the authors reported that cultivated PCD from different region could result in different GA content[16] and this might be the case for the PCD leaves used in the current study with relatively less GA. From the results, it was observed that when the concentration of ethanol was increased to 60% (*v/v*), a significant ($p \leq 0.05$) drop in GA content was noticed when compared to 50% (*v/v*). Similarly, the research carried out by Srinuanchai et al.[27] reported that the leaves of *G. inodorum* which was extracted using different concentrations of ethanol exhibited variation in GA content and the authors also found that using 75% (*v/v*) ethanol resulted in a relatively higher GA content when compared to 95% (*v/v*) ethanol. This could be because the addition of water to the ethanol increases the mass transfer process, which enhances the solubilizing capacity and improves the surface area for GA-ethanol interaction. This can facilitate the extraction of GA from the plant material and lead to a higher GA content in the extract[65]. Moreover, the extraction of glycoside molecules such as GA is greater in the aqueous solvent than in the alcoholic solvent[14]. From the results, it is suggested that a concentration of 50% (*v/v*) ethanol was the optimum level for extracting 8,414 ± 82 mg/kg GA from dried PCD leaves.

Effect of temperature during GA extraction from dried PCD leaves powder. In this experiment, PCD leaves powder was extracted with 50% ethanol as optimized from the previous experiment and extraction temperature was studied ranging from 40 to 80°C maintained for 2 h. The results of the GA content in the extracts are depicted in Fig. 4. The study found that the concentration of GA tended to increase

significantly ($p \leq 0.05$) from 7,006 ± 78 to 8,416 ± 80 mg/kg dried weight with increasing extraction temperature from 40 to 70°C respectively. The $\%Y_{GA}$ increased from 0.7% at 40°C to 0.84% at 70°C on dried weight basis of PCD leaves powder. This was consistence with other research where the recovery of phenolics was increased with the increase in extraction temperature up to 60°C[66]. High extraction temperatures could cause increased solubility, diffusion rate, mass transfer rate and surface tension to improve the phenolics extractability[67]. However, when the extraction temperature was set at 80°C, the GA significantly ($p \leq 0.05$) mitigated by 6.14% when compared to that of 70°C. Decreased GA content at the extraction temperature of 80°C might be explained by the degradation of heat-sensitive GA as temperature increases. For instance, an observation made by Manika et al.[65] that drying of Gymnema leaves using sun drying drastically reduced GA content by 53% when compared to shade drying could rationalize the effect of temperature on GA degradation. The authors also suggested that exposure to light in addition to temperature might have deleterious effect on GA concentration[65]. Similar phenomena occurred when phenolics were extracted at varying extraction temperatures that resulted in decreased total phenolic content as the increase in extraction temperature was observed[68,69]. From the results, it is suggested that the extraction of leaves powder with 50% (*v/v*) ethanol at 70°C for 2 h is appropriate to yield a higher GA content of 8,414 ± 82 mg/kg from dried PCD leaves.

Effect of maltodextrin content during production of PCD extract powder.

GA content of PCD extract powder prepared with maltodextrin. Overall, there was an increase in purity percentage of GA in PCD extract powder relative to that of PCD leaves powder. A 252% (*w/w*) of $\%P_{Ext/Leaves}$ was seen in purity percentage on mass basis when PCD extract powder prepared without maltodextrin. However, the value gradually decreased from 223% (*w/w*) at the addition of 0.5% (*w/v*) maltodextrin to 116% (*w/w*) with 5% (*w/v*) maltodextrin. The results showed that the amount of GA found in PCD extract powder was inversely related to the amount of maltodextrin used for the preparation. The increased amount of maltodextrin resulted in decreased GA content as shown in Fig. 5. The extract powder prepared without maltodextrin contained a GA content of 20,900 ± 70 mg/kg. From Fig. 5, it was observed that the concentration of maltodextrin added between 0.5 and 5% (*w/v*) had a significant ($p \leq 0.05$) effect on the mitigation of the GA content by 53.8% (*w/w*) (9,660 mg/kg at 5.0% / 20,900 mg/kg at 0% × 100–100%). when compared to the powder prepared without maltodextrin. This is in line with the results of other researchers who demonstrated that increase in maltodextrin concentration would result in the reduction of polyphenols, flavonoids and antioxidant capacity in end products[70–72]. The decreasing trend in GA content in PCD powder with an increase in maltodextrin concentration might be due to the dilution effect caused by maltodextrin, which could have reduced the GA concentration in the extract[71]. Although the extract powder prepared with 0.5% (*w/v*) maltodextrin affected the GA content, the physical stability of the powder prepared with 0.5% (*w/v*) maltodextrin was retained after 30 days of storage while the powder prepared without maltodextrin caused solid coagulation. In general, the physicochemical state of the dried product has to be maintained for as long as possible to maintain the powder quality during storage. It has to be noted that powder prepared with a relatively low maltodextrin content will also

have an improved solubility[73]. Thus, low concentration, 0.5% (*w/v*) maltodextrin could be chosen as an anti-caking agent in the drying process to retain the powder quality during storage.

Characterization of PCD extract powder prepared with maltodextrin. The comparison of color parameters, moisture content, a_w, GA and chlorophylls content between current study and another research[74] has been depicted in Table 2. From the color analysis, PCD extract powder obtained in the current study had a brightness (L*) value of 47.5 ± 0.21, which is lower when compared to leaves dried at 50°C indicating that the final product had a darker appearance than starting material. This might be due to the addition of maltodextrin that contributed to the darker appearance when combined with PCD leaves extract at a high temperature (65°C). This was evident from the results of Binnisha et al.[74], who reported that Gymnema leaves powder prepared by drying using compound parabolic solar dryer at low temperature resulted in increased brightness, greenness and yellowness. Similarly, the mitigation in negative value of a* (-1.49 ± 0.01) and a positive sign of the b* (14.6 ± 0.3) are indicative of the destruction in green and yellow color of the sample respectively when compared to starting material. Thus, the overall appearance of the PCD extract powder was a dark greenish tint mixed with yellow. It is also essential to note that the degree of greenness depends on the content of chlorophylls which serves as an indicator of quality during drying of leaves as they are susceptible to heat and light[75]. Among the chlorophylls, chlorophyll a is more sensitive to pheophytization than chlorophyll b. In the present study, the PCD extract powder contained 356 ± 20 and 270 ± 14 mg/kg of chlorophyll a and b respectively. This is relatively low when compared to the results of Binnisha et al.[74] who demonstrated that usage of solar dryer could minimize the destruction of chlorophylls during drying. However, the GA concentration found in their study was much lower when compared to the current study (18,500 ± 60 mg/kg) and this might be due the variation in the plant species used for the study. Similarly, Jeytawan et al.[76] reported lowest concentrations of gymnemic acid in roasted *G. inodorum* leaves and the authors speculated that the variation in gymnemic acid content may be due to differences in the regions where the samples were obtained. The decrease in greenness in the PCD extract powder might be due to conversion of chlorophylls into pheophytin and pyropheophytin when exposed to high temperature during drying in presence of maltodextrin[63]. However, as far as nutritional benefits are concerned, chlorophyll degradation products also could exert similar health effects as chlorophylls[75]. The moisture content and a_w of the powder sample were found to be 4.76% ± 0.10 (*w/w*) and 0.246 ± 0.001, respectively (Table 2). In general, powders with a moisture content of less than 10% (*w/w*) can be considered microbiologically safe. The data obtained in the present study was well correlated with the results of others[30,77] who reported the moisture content of extract powder was less than 10% (*w/w*). Similarly, the powders with low a_w value (0.20–0.40) are considered stable for browning, lipid oxidation, microbial growth, hydrolysis and enzymatic reactions[78]. Moreover, a_w is a measure of available free water that is responsible for any chemical, biochemical changes and microbiological growth[78]. Thus, the lower a_w is indicative of the lower chemical potential of water and the driving force in interactions involving water, which is independent of the moisture content[79]. For instance, in the present study, the PCD powder with 4.76% ± 0.10 moisture had an a_w of only 0.246 ± 0.001. This

level of a_w and moisture content are sufficient enough to prevent the growth of various microorganisms and are ideal for dry or powdered products.

Table 2. Color parameters, moisture content, a_w, GA and chlorophylls content of PCD extract powder. PCD, Phak Chiang Da; GA, gymnemic acid; N/A, not available.

Parameter	Current study	Binnisha et al.[74]	Jeytawan et al.[76]
L* (no unit)	47.5 ± 0.21	71.18	-
a* (no unit)	-1.49 ± 0.01	-6.59	-
b* (no unit)	14.6 ± 0.30	17.72	-
Moisture content (%, *w/w*)	4.76 ± 0.10	7.00	-
Water activity (a_w) (no unit)	0.246 ± 0.001	N/A	-
GA (mg/kg)	18,500 ± 60	290	102
Chlorophyll a (mg/kg)	356 ± 20	1,150	-
Chlorophyll b (mg/kg)	270 ± 14	562	-

Although antioxidant activity can be tested by several methods such as DPPH, 2,2'-azino-bis-2- ethybenz-thiazoline-6-sulfonic acid (ABTS), reducing power method and Ferric ion Reducing Antioxidant Power (FRAP) method, DPPH scavenging activity assay is one of the most widely used methods in recent years. This is because the DPPH assay is relatively simple, inexpensive, and fast, and it can be used to test a wide range of samples, including natural extracts, synthetic compounds, and food products[80,81]. Table 3 compares the antioxidant potential of PCD extract powder prepared with 0.5% (*w/v*) maltodextrin with that of Gymnema extracts by others research[23,82,83]. From the results of present study, it was found that PCD extract powder was able to neutralize 75.8% ± 2.6 of DPPH free radicals at 1.58 mg/mL concentration. Results also showed that the standards (gallic acid and ascorbic acid) had stronger scavenging activities against the radical than the PCD extract powder at the same concentration tested. On the other hand, Subramanian et al.[23], Fazal et al.[82], Ghous et al.[83] and Jeytawan et al.[76] obtained radical scavenging activity of 73.4, 54.4, 83.0 and 62.6% respectively (Table 3). Jeytawan et al.[76] stated that antioxidant capacity of processed leaves was higher when compared to fresh leaves. The authors suggested that lower antioxidant activity in fresh leaves might be due to the absence of dry and wet weight adjustment[76]. It is also interesting to note that the difference in scavenging activity between different studies might be due to variation in plant species, source of plant collection, type of solvent used for extraction and the extraction parameters.

Table 3. Antioxidant capacity of PCD extract powder prepared with 0.5% (*w/v*) maltodextrin by DPPH radical scavenging activity. PCD, Phak Chiang Da; RSA, radical scavenging activity.

Solvent and conditions	% RSA	Reference
Ethanol (2 h, 70 °C)	75.8 ± 2.6	Current study
Ascorbic acid	93.7 ± 0.4	Current study
Gallic acid	96.8 ± 0.7	Current study
Ethanol (cold maceration method for 72 h at room temperature)	73.4	Subramanian et al.[23]
Methanol (periodic shaking for 5 days, at room temperature)	54.4	Fazal et al.[82]
Methanol (periodic shaking for 1 week at room temperature)	83.0	Ghous et al.[83]
Boiling water (15 min, 80 °C)	62.6	Jeytawan et al.[76]

Conclusion

Although the use of herbal extracts to manage diabetes and other ailments has been long practiced, its utilization in its native form hinders its popularity due to low concentration of active substances, poor absorption, lack of standardization and other characteristics. Thus, preparing a formulation in a form that is acceptable with enriched gymnemic acid will open up interesting applications in the nutraceutical, functional food and therapeutical sectors. In this connection, the present study evaluated the conditions for the preparation of GA-enriched extracts from PCD leaves and their formulation into a powder form with maltodextrin as an anti-caking agent. The results also showed that the PCD extract powder had high radical scavenging activity which is very crucial to destroy the deleterious free radicals in diabetes. Yet, further investigation to assess other specific antidiabetic assays, combine with other herbs for synergistic effects, determine efficient extraction, and apply alternative drying / extraction techniques could be considered in the future. It can be anticipated that when the outcome of the study realized in the future in numerous applications, farmers could prefer the Gymnema cultivation as an alternative cropping choice against sugarcane, rice and corn as disposal of their residues through open burning causes fine PM 2.5 air pollution.

Declarations

Data Availability: The data sets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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References

1. Tiwari, P., Mishra, B. & Sangwan, N. S. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *BioMed research international* **2014** (2014).
2. Alam, O. *et al.* Recent developments made in the assessment of the antidiabetic potential of gymnema species-From 2016 to 2020. *Journal of Ethnopharmacology* **286**, 114908 (2022).
3. Khan, F. *et al.* Comprehensive review on phytochemicals, pharmacological and clinical potentials of *Gymnema sylvestre*. *Frontiers in pharmacology* **10**, 1223 (2019).
4. Turner, S. *et al.* The Effect of a 14-Day *Gymnema sylvestre* Intervention to Reduce Sugar Cravings in Adults. *Nutrients* **14**, 5287 (2022).
5. Pamuru, R. R., Yarrapalle, A. & Lakshmi, D. V. in *Antidiabetic Plants for Drug Discovery* 23-53 (Apple Academic Press, 2023).
6. Bespinyowong, R., Pongthananikorn, S. & Chiabchalard, A. Efficacy and safety of *Gymnema inodorum* tea consumption in type 2 diabetic patients. *Chulalongkorn Medical Journal* **57**, 587-599 (2013).
7. Abbas, Q., Al-Thawadi, S. & Perna, S. Phytochemicals in the Management of Diabetes. *Nanotechnology for Diabetes Management* **59**, 128 (2022).
8. Soumyanath, A. *Traditional medicines for modern times: antidiabetic plants*. (CRC press, 2005).
9. Oboh, M., Govender, L., Siwela, M. & Mkhwanazi, B. N. Anti-diabetic potential of plant-based pentacyclic triterpene derivatives: Progress made to improve efficacy and bioavailability. *Molecules* **26**, 7243 (2021).
10. Waman, A. A., Dhanani, T. & Manivel, P. Gymnemagenin from *Gymnema sylvestre* (Retz.) R. Br. ex Sm.
11. Saeed, R., Ahmed, D. & Mushtaq, M. Ultrasound-aided enzyme-assisted efficient extraction of bioactive compounds from *Gymnema sylvestre* and optimization as per response surface

methodology. *Sustainable Chemistry and Pharmacy* **29**, 100818 (2022).

12. Ramalingam, R. *et al.* Antimicrobial properties and biocompatibility of electrospun poly-ε-caprolactone fibrous mats containing *Gymnema sylvestre* leaf extract. *Materials Science and Engineering: C* **98**, 503-514 (2019).
13. Ahamad, J., Amin, S. & Mir, S. R. Optimization of ultrasonic-assisted extraction of gymnemic acid from *Gymnema sylvestre* leaves using response surface methodology. *Analytical Chemistry Letters* **4**, 104-112 (2014).
14. Sheoran, S., Panda, B. P., Admane, P. S., Panda, A. K. & Wajid, S. Ultrasound-assisted Extraction of Gymnemic Acids from *Gymnema sylvestre* Leaves and its Effect on Insulin-producing RINm-5 F β Cell Lines. *Phytochemical Analysis* **26**, 97-104 (2015).
15. Mandal, V., Dewanjee, S. & Mandal, S. C. Microwave-assisted extraction of total bioactive saponin fraction from *Gymnema sylvestre* with reference to gymnemagenin: a potential biomarker. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques* **20**, 491-497 (2009).
16. Suwan, N., Baison, W. & Chuajedton, A. Purification of *Gymnema inodorum* Leaf Extract and Its Antifungal Potential Against *Colletotrichum gloeosporioides*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **92**, 667-677 (2022).
17. Kameswaran, S. & Perumal, K. Extraction and Purification of Gymnemic Acid from *Gymnema sylvestre* R. Br. *Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation*, 521-529 (2020).
18. Saiki, P. *et al.* Purified gymnemic acids from *Gymnema inodorum* tea inhibit 3T3-L1 cell differentiation into adipocytes. *Nutrients* **12**, 2851 (2020).
19. Srinuanchai, W., Nooin, R., Jarussophon, S., Kasemwong, K. & Nuchuchua, O. Determination of gymnemic acid level in *Gymnema inodorum* leaves using multiple reaction monitoring mass spectrometry. *J. Chem. Metrol* **13**, 75-79 (2019).
20. Shimizu, K. *et al.* Suppression of glucose absorption by some fractions extracted from *Gymnema sylvestre* leaves. *Journal of veterinary medical science* **59**, 245-251 (1997).
21. Youl, E. N. *et al.* Hypoglycemic and antihyperglycemic activities of the aqueous ethanolic extracts of *Gymnema sylvestre* (RETZ) R. Br. Ex SCHULT and Sclerocarya birrea (A RICH) HOCHST. *African Journal of Pharmacy and Pharmacology* **14**, 339-346 (2020).
22. Vaghela, M., Iyer, K. & Pandita, N. In vitro inhibitory effect of *Gymnema sylvestre* extracts and total gymnemic acids fraction on select cytochrome P450 activities in rat liver microsomes. *European Journal of Drug Metabolism and Pharmacokinetics* **43**, 227-237 (2018).
23. Subramanian, S., Dowlath, M. J. H., Karuppannan, S. K., Saravanan, M. & Arunachalam, K. D. Effect of Solvent on the Phytochemical Extraction and GC-MS Analysis of *Gymnema sylvestre*. *Pharmacognosy Journal* **12** (2020).
24. Sarker, S. D., Latif, Z. & Gray, A. I. Natural product isolation. *Natural products isolation*, 1-25 (2006).

25. Waszkowiak, K. & Gliszczyńska-Świgło, A. Binary ethanol–water solvents affect phenolic profile and antioxidant capacity of flaxseed extracts. *European Food Research and Technology* **242**, 777-786 (2016).
26. Gan, C. Y. & Latiff, A. A. Optimisation of the solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology. *Food chemistry* **124**, 1277-1283 (2011).
27. Srinuanchai, W. *et al.* Inhibitory effects of *Gymnema inodorum* (Lour.) Decne leaf extracts and its triterpene saponin on carbohydrate digestion and intestinal glucose absorption. *Journal of Ethnopharmacology* **266**, 113398 (2021).
28. Suwan, N., Baison, W. & Chuajedton, A. Purification of *Gymnema inodorum* Leaf Extract and Its Antifungal Potential Against *Colletotrichum gloeosporioides*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 1-11 (2022).
29. An, J.-P. *et al.* Oleanane triterpenoids from the leaves of *Gymnema inodorum* and their insulin mimetic activities. *Journal of natural products* **83**, 1265-1274 (2020).
30. Caliskan, G. & Dirim, S. N. The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technology* **287**, 308-314 (2016).
31. Feng, Y., Zhang, M., Fan, K. & Mujumdar, A. S. Effects of drying methods on quality of fermented plant extract powder. *Drying Technology* **36**, 1913-1919 (2018).
32. Lee, S. Y., Ferdinand, V. & Siow, L. F. Effect of drying methods on yield, physicochemical properties, and total polyphenol content of chamomile extract powder. *Frontiers in Pharmacology* **13** (2022).
33. Shang, F., Kryzhska, T. & Duan, Z. Study on the optimization of spray drying process for Areca taro powder with microcystalline cellulose. *Technology audit and production reserves* **6**, 62 (2021).
34. Cheng, H. *et al.* Optimization of Spray-Drying Process Parameters to Study Anti-Sticking Effect of Hydroxypropyl Methyl Cellulose-VLV on Corni fructus Extracts. *AAPS PharmSciTech* **23**, 58 (2022).
35. Stavra, K., Plati, F., Pavlidou, E. & Paraskevopoulou, A. Characterization of lemon juice powders produced by different drying techniques and carrier materials. *Drying Technology* **40**, 1923-1934 (2022).
36. Michalska-Ciechanowska, A., Hendrysiak, A., Brzezowska, J., Wojdyło, A. & Gajewicz-Skretna, A. How Do the Different Types of Carrier and Drying Techniques Affect the Changes in Physico-Chemical Properties of Powders from Chokeberry Pomace Extracts? *Foods* **10**, 1864 (2021).
37. Mutlu, C. & Erbas, M. Evaluating the effects of different drying methods, carrier materials and their ratios to produce bioactive honey-like powder. *Journal of Apicultural Research* **62**, 364-373 (2023).
38. Nadeem, H. Ş., Torun, M. & Özdemir, F. Spray drying of the mountain tea (*Sideritis stricta*) water extract by using different hydrocolloid carriers. *LWT-Food Science and Technology* **44**, 1626-1635 (2011).
39. Martinelli, L., Gabas, A. L. & Telis-Romero, J. Thermodynamic and quality properties of lemon juice powder as affected by maltodextrin and arabic gum. *Drying Technology* **25**, 2035-2045 (2007).

40. Oliveira, S. M., Brandao, T. R. & Silva, C. L. Influence of drying processes and pretreatments on nutritional and bioactive characteristics of dried vegetables: A review. *Food Engineering Reviews* **8**, 134-163 (2016).
41. Vimercati, W. C. *et al.* Influence of drying temperature on drying kinetics, energy consumption, bioactive compounds and cooking quality of pasta enriched with spinach. *Journal of Food Process Engineering* **43**, e13571 (2020).
42. Roopchand, D. E. *et al.* Food-compatible method for the efficient extraction and stabilization of cranberry pomace polyphenols. *Food Chemistry* **141**, 3664-3669 (2013).
43. Tavakoli, S. *et al.* Ultrasonic-assisted food-grade solvent extraction of high-value added compounds from microalgae *Spirulina platensis* and evaluation of their antioxidant and antibacterial properties. *Algal Research* **60**, 102493 (2021).
44. Wang, H. J., Pan, M. C., Chang, C. K., Chang, S. W. & Hsieh, C. W. Optimization of ultrasonic-assisted extraction of cordycepin from *Cordyceps militaris* using orthogonal experimental design. *Molecules* **19**, 20808-20820 (2014).
45. Youssef, K. M. & Mokhtar, S. M. Effect of drying methods on the antioxidant capacity, color and phytochemicals of *Portulaca oleracea* L. leaves. *Journal of Nutrition & Food Sciences* **4**, 1 (2014).
46. Phimolsiripol, Y. *et al.* Shelf life extension of chilled pork by optimal ultrasonicated Ceylon Spinach (*Basella alba*) extracts: Physicochemical and microbial properties. *Foods* **10**, 1241 (2021).
47. AOAC. *Official methods of analysis*. 17 edn, (Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC. USA, 2000).
48. Khemacheewakul, J. *et al.* Production and storage stability of antioxidant fiber from pigeon pea (*Cajanus cajan*) pod. *Journal of Microbiology, Biotechnology and Food Sciences* **2021**, 293-297 (2021).
49. Zeb, A. & Ullah, F. Reversed phase HPLC-DAD profiling of carotenoids, chlorophylls and phenolic compounds in *Adiantum capillus-veneris* leaves. *Frontiers in Chemistry* **5**, 29 (2017).
50. Varzakas, T. & Kiokias, S. HPLC analysis and determination of carotenoid pigments in commercially available plant extracts. *Current Research in Nutrition and Food Science Journal* **4**, 01-14 (2016).
51. Salunkhe, V. R. & Magdum, C. S. UV and HPLC method development of azadirachtin and gymnemic acid in polyherbal churna and its validation. *European Journal of Pharmaceutical and Medical Research* **3**, 324-335 (2016).
52. Meza, A., Rojas, P., Cely-Veloza, W., Guerrero-Perilla, C. & Coy-Barrera, E. Variation of isoflavone content and DPPH• scavenging capacity of phytohormone-treated seedlings after in vitro germination of cape broom (*Genista monspessulana*). *South African Journal of Botany* **130**, 64-74 (2020).
53. Rachtanapun, P. *et al.* Characterization of chitosan film incorporated with curcumin extract. *Polymers* **13**, 963 (2021).
54. Nunta, R. *et al.* Batch and continuous cultivation processes of *Candida tropicalis* TISTR 5306 for ethanol and pyruvate decarboxylase production in fresh longan juice with optimal carbon to nitrogen

molar ratio. *Journal of Food Process Engineering* **42**, e13227 (2019).

55. Khemacheewakul, J. *et al.* Validation of mathematical model with phosphate activation effect by batch (R)-phenylacetylcarbinol biotransformation process utilizing *Candida tropicalis* pyruvate decarboxylase in phosphate buffer. *Scientific Reports* **11**, 1-11 (2021).
56. Nunta, R. *et al.* Valorization of rice straw, sugarcane bagasse and sweet sorghum bagasse for the production of bioethanol and phenylacetylcarbinol. *Scientific Reports* **13**, 727 (2023).
57. Bahloul, N., Boudhrioua, N., Kouhila, M. & Kechaou, N. Effect of convective solar drying on colour, total phenols and radical scavenging activity of olive leaves (*Olea europaea* L.). *International journal of food science & technology* **44**, 2561-2567 (2009).
58. Peries, C. M., Navarthne, S., Wijesinghe, J., Henagamage, A. P. & Coorey, R. Effect of Different Drying Methods on Antioxidant Activity and Availability of Phytochemicals in Leaves of *Costus speciosus*, *Coccinia grandis* and *Gymnema sylvestre*. *Asian Journal of Engineering and Applied Technology* **11**, 29-35 (2022).
59. Sagrin, M. S. & Chong, G. H. Effects of drying temperature on the chemical and physical properties of *Musa acuminata* Colla (AAA Group) leaves. *Industrial Crops and Products* **45**, 430-434 (2013).
60. Duvivier, P., Hsieh, P., Lai, P. & Charle, A. Retention of phenolics, carotenoids, and antioxidant activity in the Taiwanese sweet potato (*Ipomoea batatas* lam.) CV tainong 66 subjected to different drying conditions. *African Journal of Food, Agriculture, Nutrition and Development* **10** (2010).
61. Adhamatika, A., Murtini, E. & Sunarharum, W. in *IOP Conference Series: Earth and Environmental Science.* 012073 (IOP Publishing).
62. Rudra, S. G., Singh, H., Basu, S. & Shivhare, U. Enthalpy entropy compensation during thermal degradation of chlorophyll in mint and coriander puree. *Journal of Food Engineering* **86**, 379-387 (2008).
63. Schwartz, S. J., Cooperstone, J. L., Cichon, M. J., Joachim, H. & Giusti, M. M. in *Fennema's food chemistry* 681-752 (CRC press, 2017).
64. Ferruzzi, M. G. & Blakeslee, J. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition research* **27**, 1-12 (2007).
65. Manika, N., Singh, S., Verma, R. & Bagchi, G. Extraction efficacy, stability assessment and seasonal variation of bioactive 'gymnemagenin'in *Gymnema sylvestre*. *Industrial Crops and Products* **44**, 572-576 (2013).
66. Gunathilake, K., Ranaweera, K., Rupasinghe, H. & RM TRK, R. Optimizing the Extraction of Bioactive Compounds from Leaves of *Gymnema lactiferum*, an Edible Green Leafy Vegetable. *South Asian Research Journal of Natural Products* **1**, 1-9 (2018).
67. Richter, B. E. *et al.* Accelerated solvent extraction: a technique for sample preparation. *Analytical chemistry* **68**, 1033-1039 (1996).
68. Bamba, B. S. B. *et al.* Influence of extraction conditions on ultrasound-assisted recovery of bioactive phenolics from blueberry pomace and their antioxidant activity. *Molecules* **23**, 1685 (2018).

69. Leksawasdi, N. *et al.* Ultrasonic extraction of bioactive compounds from green soybean pods and application in green soybean milk antioxidants fortification. *Foods* **11**, 588 (2022).
70. Vidović, S. S., Vladić, J. Z., Vaštag, Ž. G., Zeković, Z. P. & Popović, L. M. Maltodextrin as a carrier of health benefit compounds in *Satureja montana* dry powder extract obtained by spray drying technique. *Powder technology* **258**, 209-215 (2014).
71. Hamid, Thakur, N. & Thakur, A. Microencapsulation of wild pomegranate flavedo phenolics by lyophilization: Effect of maltodextrin concentration, structural morphology, functional properties, elemental composition and ingredient for development of functional beverage. *LWT* **133**, 110077 (2020).
72. Mishra, P., Mishra, S. & Mahanta, C. L. Effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (*Emblica officinalis*) juice powder. *Food and Bioproducts Processing* **92**, 252-258 (2014).
73. Mahendran, T. Physico-chemical properties and sensory characteristics of dehydrated guava concentrate: effect of drying method and maltodextrin concentration. *Tropical Agricultural Research and Extension* **13** (2011).
74. Binnisha, N. V., Nalina, L., Mahendiran, R., Rajamani, K. & Uma, D. Effect of different drying methods on quality parameters of Gymnema (*Gymnema sylvestre* R. Br). (2021).
75. Turkmen, N., Poyrazoglu, E. S., Sari, F. & Sedat Velioglu, Y. Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. *International journal of food science & technology* **41**, 281-288 (2006).
76. Jeytawan, N. *et al.* Antioxidant and Phytochemical Potential and Phytochemicals in *Gymnema inodorum* (Lour.) Decne in Northern Thailand. *Plants* **11**, 3498 (2022).
77. Chong, S. Y. & Wong, C. W. Production of Spray-Dried Sapodilla (*Manilkara zapota*) Powder from Enzyme-Aided Liquefied Puree. *Journal of Food Processing and Preservation* **39**, 2604-2611 (2015).
78. Quek, S. Y., Chok, N. K. & Swedlund, P. The physicochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing: Process Intensification* **46**, 386-392 (2007).
79. Chaul, L. T., Conceição, E. C., Bara, M. T. F., Paula, J. R. & Couto, R. O. Engineering spray-dried rosemary extracts with improved physicomechanical properties: a design of experiments issue. *Revista Brasileira de Farmacognosia* **27**, 236-244 (2017).
80. Gurning, K., Lumbangaol, S., Situmorang, R. F. & Silaban, S. Determination of phenolic contents and antioxidant activity test of ethanol extract of *Sirih merah* (*Piper crocatum* Ruiz & Pav.) leaves using the DPPH method. *J. Pendidik. Kim* **13**, 137-142 (2021).
81. Hasanaha, M. & Hilmaa, P. S. Antioxidant activity of extract and fractions from *Coffee arabica* L. leaves by DPPH radical scavenging method. *Science and Engineering* **3** (2016).
82. Fazal, H., Ahmad, N. & Khan, M. A. Physicochemical, phytochemical evaluation and DPPH-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. *Pak J Bot* **43**, 63-67 (2011).

83. Ghous, T. *et al.* Evaluation of α-glucosidase inhibition, antioxidant and antibacterial effects of *Gymnema sylvestre* R. Br. *Bangladesh Journal of Botany* **50**, 61-68 (2021).

Figures

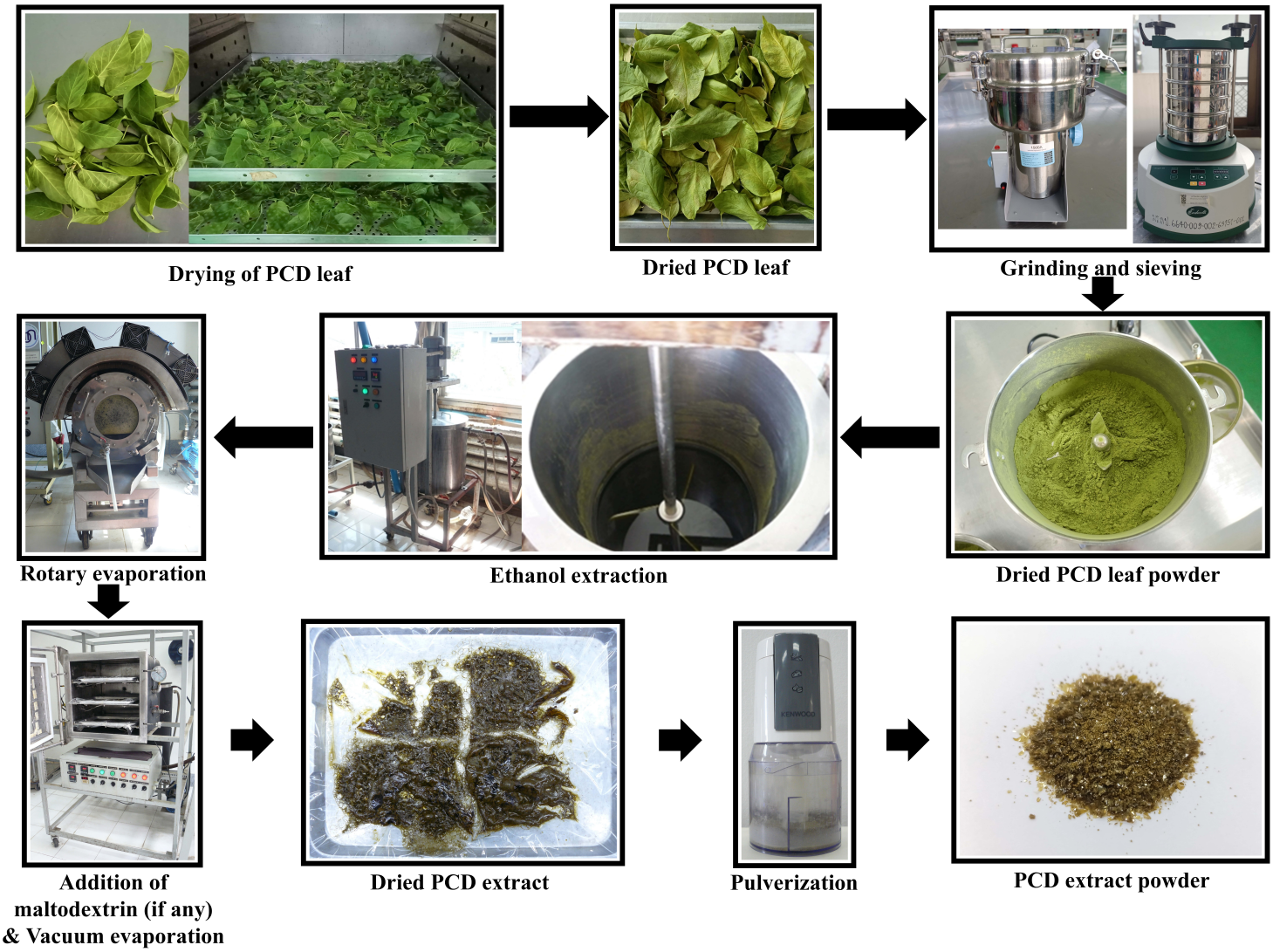


Figure 1

The production process of PCD extract powder from PCD leaves.

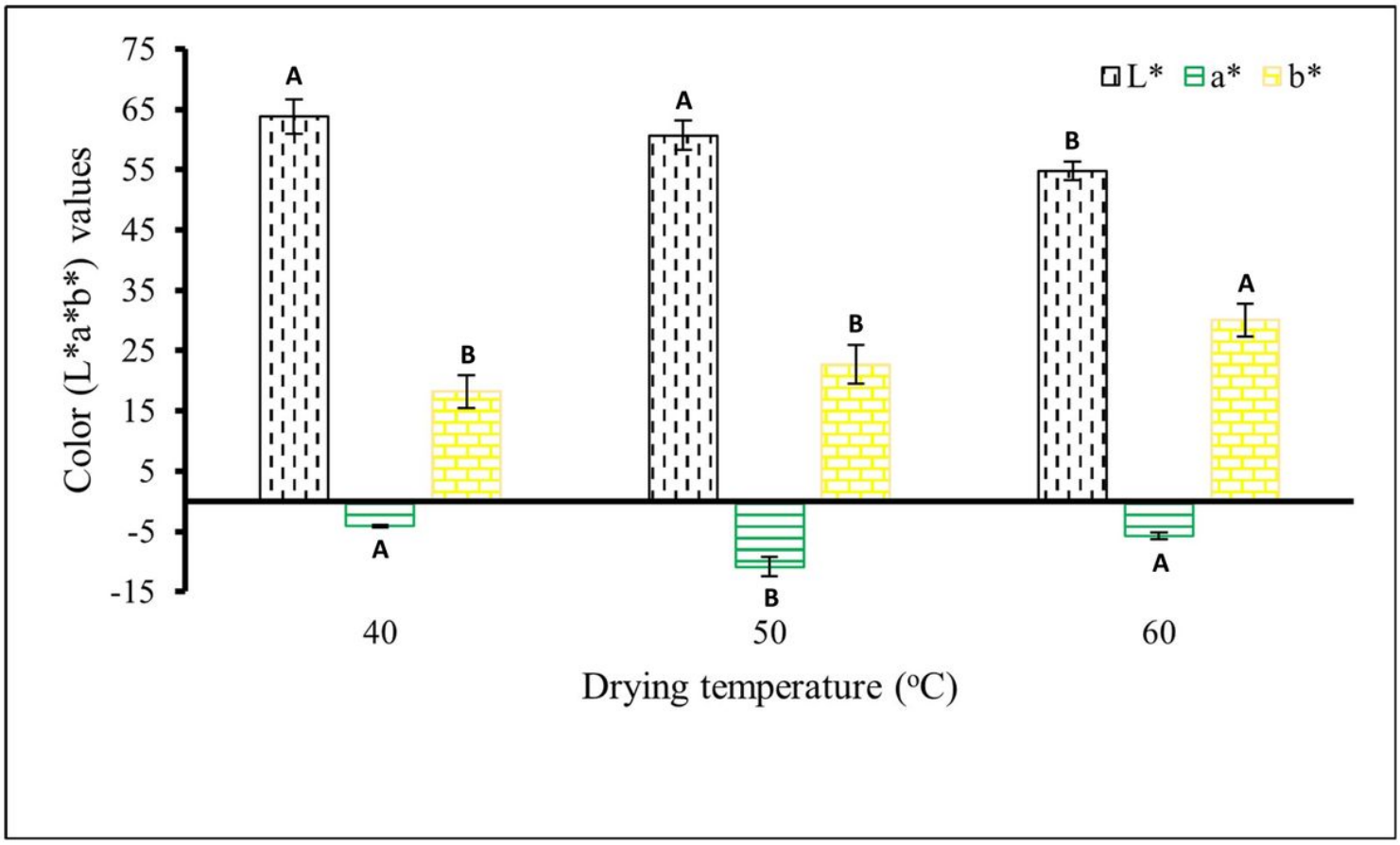


Figure 2

Effect of drying temperature on color parameters of PCD leaves. Error bars indicate the standard errors which deviated from the mean (n = 3). The average values with different alphabets indicated a significant difference ($p \leq 0.05$) among the same type of color value between drying temperatures.

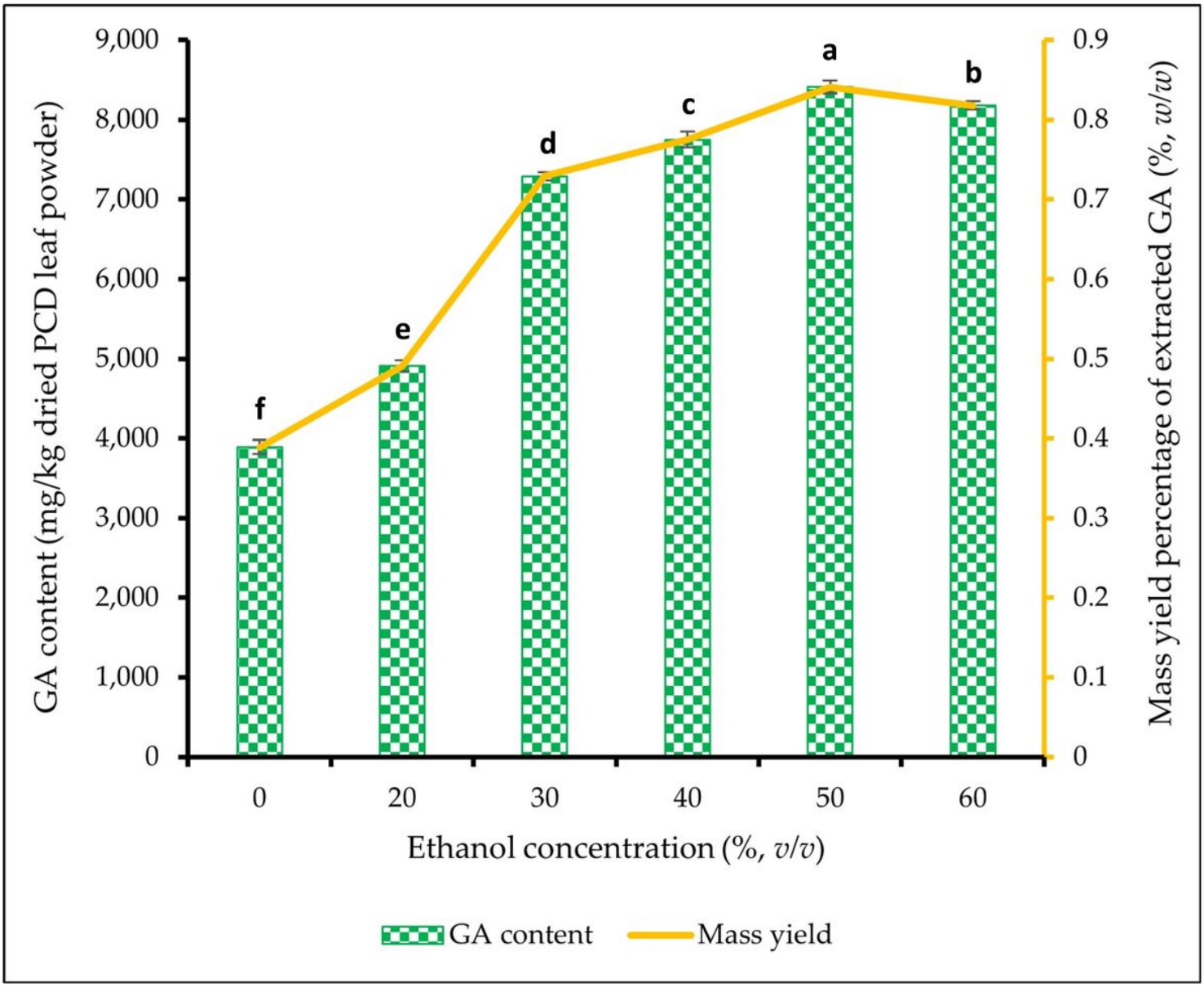


Figure 3

Effect of ethanol concentration on GA content and mass yield percentage of extracted GA of dried PCD leaves powder. A constant extraction temperature of 70 °C for 2 h was maintained while varying different concentrations of ethanol. Error bars indicate the standard error from the mean (n = 3). Means with different lowercase alphabets indicated a significant difference ($p \leq 0.05$) between ethanol concentrations. PCD, Phak Chiang Da; GA, gymnemic acid.

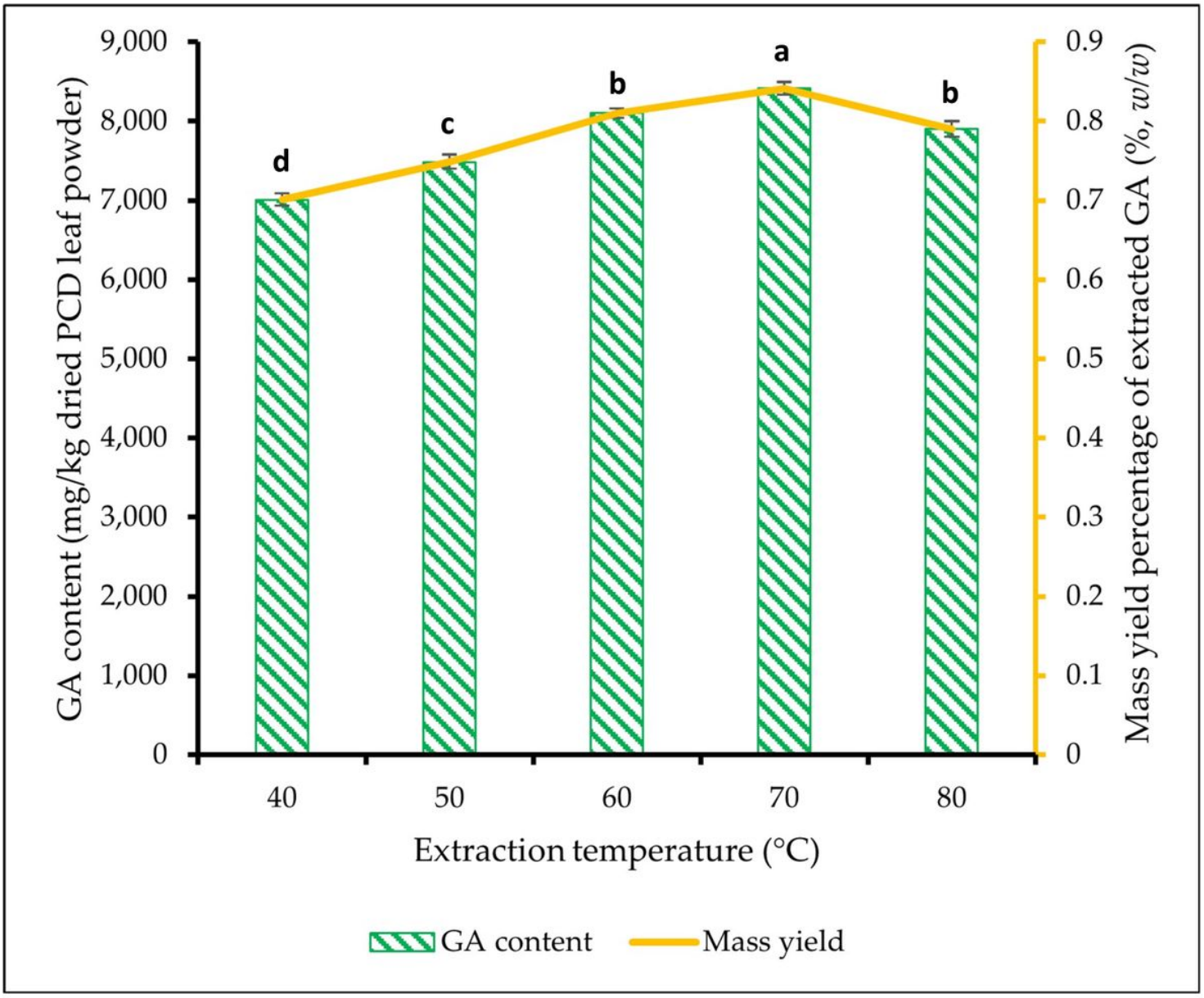


Figure 4

Effect of extraction temperature on GA content and mass yield percentage of extracted GA of dried PCD leaves powder. A constant ethanol concentration of 50% (*v/v*) was maintained while varying different extraction temperatures. Error bars indicate the standard error from the mean (n = 3). Means with different lowercase alphabets indicate a significant difference ($p \leq 0.05$) between extraction temperatures. PCD, Phak Chiang Da; GA, gymnemic acid.

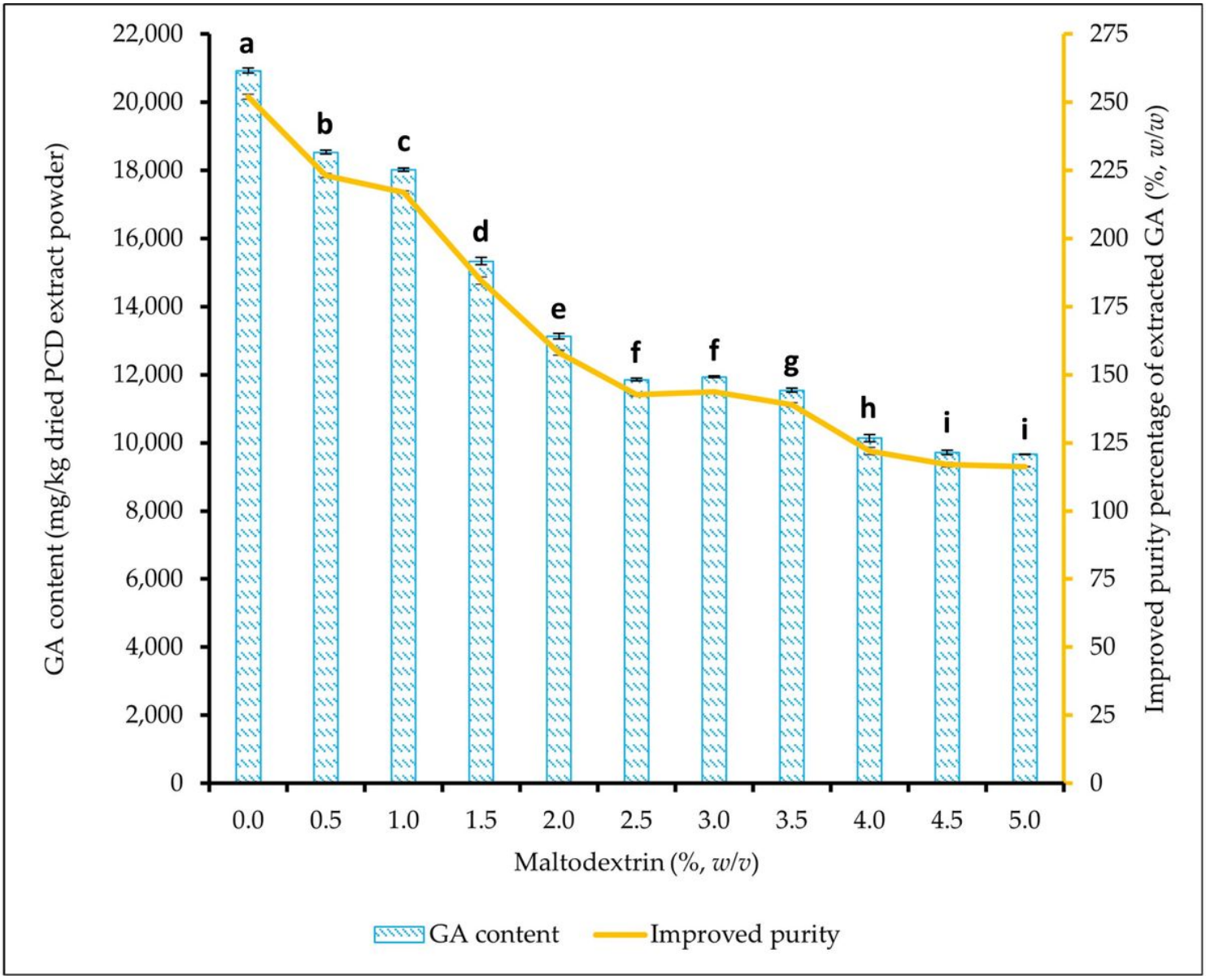


Figure 5

The actual content and improved purity percentage of GA in PCD extract powder prepared with different maltodextrin concentrations relative to dried PCD leaves powder. Error bars indicate the standard errors from the means (n = 3). The averages with different lowercase alphabets indicate a significant difference ($p \leq 0.05$) between maltodextrin concentrations. PCD, Phak Chiang Da; GA, gymnemic acid.