



Ultrasonic Extraction, Antioxidant and Anti-glycation Activities of Polysaccharides from *Gynura Divaricata* Leaves

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ABSTRACT

To date, there are numerous studies on *Gynura divaricata* mainly focused on isolation and biological activities of small chemical compounds such as polyphenols. Only few attentions have been paid on polysaccharides from *G. divaricata* leaves regarding the extraction, structural characterization and biological functions. Therefore, in this study, ultrasonic extraction, an efficient technique compared to the conventional methods, was applied via response surface methodology to determine the optimum conditions, which maximized the yield of *G. divaricata* polysaccharides (GDP). Moreover, its physico-chemical and biological properties including *in vitro*-antioxidant and anti-glycation activities were investigated. The GDP yield under the optimal condition (148 W ultrasonic power, 15 min extraction time and 18.5 ml/g ratio of water to raw material) was found to be $6.45\% \pm 0.13$, which was very close to the value predicted by the mathematical model. The primary characterization using various physico-chemical approaches revealed that GDP is a polysaccharide mainly composed of uronic acid (57.38%) and neutral sugars (40.94%) with molecular weight approximately of 960 kDa. In addition, GDP exhibited remarkable metal chelating antioxidant up to 30% of activity at 60 mg/100 ml and anti-glycation capacity reached 90% at 100 mg/100 ml. Ultrasound-assisted extraction was found to positively affect the GDP yield. The GDP exhibited highly potent antioxidant and anti-glycation properties which could be explored as a novel natural antioxidant and natural glycation inhibitor for food, pharmaceutical and cosmetics industries.

Keywords: polysaccharides, purple velvet, anti-AGE formation, antioxidant, optimization

1. INTRODUCTION

Gynura divaricata, belonging to Asteraceae family, is a tropical plant and grows well all year around. It widely distributes in various parts of Asian countries including China, Vietnam and Thailand. In Thailand, *G. divaricata*, commonly known as “purple velvet” or “jak-na-rai”, is not only served as a traditional Thai medicine but its leaves are also used as a vegetable by the locals in the west of Thailand [1]. It has been reported that *G. divaricata* has been employed as a folk medicine for treatments of diabetes and hypertension [1]. Several biological activities of this plant are also shown to inhibit *in vitro* α -amylase activity [1], *in vitro* α -glucosidase activity [2], as well as to promote hypoglycemic effect in mice [3]. Many constituents such as flavonols, polyphenols, polysaccharides, alkaloids, terpenoids, and sterols were found in *G. divaricata* leaves [2, 4, 5].

To date, studies on *G. divaricata* mainly focus on isolation and biological activities of small chemical compounds such as polyphenols and alkaloids [2, 4, 5]. Only few attentions have been paid on polysaccharides from *G. divaricata* leaves regarding the extraction, structural characterization and biological functions [6]. Hence, we focused on such concerns because various plant polysaccharides have been demonstrated to possess a wide range of bioactivities. For examples, polysaccharides from mulberry leaves had significant anti-hyperglycemic and anti-hyperlipidemia effects [7]. Polysaccharides extracted from mushroom (*Inonotus obliquus*) have been shown to exhibit anti-cancer and anti-inflammatory properties [8]. Finger citron polysaccharides have been identified as dietary free radical scavengers for oxidative damage prevention [9]. In addition, these polysaccharides are generally non-toxic and do not cause significant side effects [10].

Nowadays, ultrasound-assisted treatment has been applied for extracting the plant polysaccharides; this method is simpler, shorter

extraction time, uses lower temperature and gives higher extraction rate than the traditional one [11, 12]. However, it is well known that extraction efficiency of this technique is influenced by multiple parameters such as ultrasonic power and ratio of solvent to raw material. These effects could be either independent or interactive [13]. When many factors and interactions affect desired response, response surface methodology (RSM) is used as an effective tool for optimising the process. RSM has been applied widely in optimising the extraction process variables such as polysaccharides and phenolic compounds from various materials [12, 13, 14].

In the present study, optimization of ultrasonic-assisted extraction was carried out using RSM for the maximum GDP polysaccharides yield. In addition, characterization, antioxidant and anti-AGEs formation capacities using various *in vitro* assays of GDP were investigated.

2. MATERIALS AND METHODS

2.1 Plant Material

The clean dried purple velvet leaves (*Gynura divaricata*, DC) were kindly provided by Mahidol University, Kanchanaburi Campus, Thailand. They were ground using a blender (Philips, Japan) and then sieved through a 60-meshes sieve. The fine powders were then defatted with petroleum ether and pretreated with 80% (v/v) ethanol for 3 h twice in order to remove any impurities before extractions.

2.2 Ultrasonic Assisted Extraction

Extractions through an ultrasonic homogenizer (Model HD 2200, Bandelin Sonopuls, Germany) were performed according to the method described [15] with some modifications. Briefly, powders of the *G. divaricata* leaves (2 g dry basis) were ultrasonically extracted with deionized water at designed extraction times, ultrasonic powers and ratios of water to raw material. Temperature was kept constantly below 50 °C throughout the

extraction procedure. Then, the supernatant was collected by centrifugation (Sorvall RC-5C PLUS, USA) at 5000 \times g for 20 min and deproteinized using the Sevag method [15]. After that, 95% v/v ethanol (5 fold volumes) was slowly added to the supernatant and left for 1 h. The resulting precipitate was collected by centrifugation at 5000 \times g for 20 min and washed successively twice with absolute ethanol and acetone, respectively. The GDP was then hot air dried at 40 °C and kept in desiccator until used.

2.3 Optimization the Extraction Condition

Ultrasonic-assisted extraction conditions were optimized using response surface methodology (RSM), with the standard of five levels and three factors central composite design (CCD). Three independent variables were ultrasonic power (X_1), extraction time (X_2) and ratio of water to raw material (X_3). In each factor, the experimental range was determined based on the preliminary results of single factor experiment. Independent variables and their experimental ranges are present in Table 1. The completed design consisted of 19 experiments including eight factorial points, six axial points and five replicates of the center points (Table 2). Yield of GDP was selected as the response for the combination of the independent variables. Analysis of the experimental design and calculation of the predicted data were carried out using Design-Expert (version 8.0, State-Ease Inc., Minneapolis, MN, USA). The model was built based on the variables with confidence levels

of 95%. Finally, three additional experiments were performed to verify the validity of the statistical model.

2.4 Characterization of GDP

2.4.1 GDP composition

Total sugar content was determined by phenol-sulphuric acid method using D-glucose as a standard [16], whereas total uronic acid content was determined calorimetrically by hydroxydiphenyl assay with D-galacturonic acid as a standard [17]. Protein content was examined by Coomassie Brilliant Blue with bovine serum albumin as a standard [18]. Total phenolic content was measured by Folin-Ciocalteu assay using gallic acid as a standard [4]. Yield of GDP extracted from leaves of *G. divaricata* (w/w) was calculated based on total sugar content using the following formula: (Total sugar content in dry sample (g)/Dry leaves weight (g)) \times 100.

2.4.2 FT-IR analysis

Functional groups present in GDP were investigated by Fourier transform-infrared spectroscopy (FT-IR). Samples, in triplicate, were incorporated into KBr (spectroscopic grade) and pressed into a 1 mm pellet. FTIR spectra were recorded in the range 4000-400 cm^{-1} through a spectrophotometer (Shimadzu, Japan).

2.4.3 Average molecular weight

The molecular weight and its distribution of GDP were determined by gel permeation

Table 1. Independent variables and their levels used in the central composite design (CCD).

Independent variables	Levels				
	-2	-1	0	1	2
Ultrasonic power X_1 (w)	20	52	100	148	180
Extraction time X_2 (min)	1	5	10	15	18
Ratio of water to raw material X_3 (ml/g)	13	15	17.5	20	22

Table 2. Experimental conditions from CCD and yield of *G. divaricata* polysaccharides (GDP).

Run	Ultrasonic power (W)	Extraction time (min)	Ratio of water to leaf powder (ml/g)	GDP yield ^a (%)
1	1	-1	1	6.21
2	-1	-1	1	5.72
3	0	0	0	5.85
4	-1	1	-1	5.04
5	1	1	-1	6.00
6	0	-2	0	5.81
7	-1	-1	-1	5.08
8	0	0	-2	4.17
9	0	2	0	6.24
10	1	1	1	6.40
11	1	-1	-1	6.12
12	0	0	0	5.60
13	0	0	0	5.52
14	2	0	0	5.40
15	0	0	0	5.72
16	0	0	2	6.11
17	-2	0	0	4.29
18	-1	1	1	5.51
19	0	0	0	5.71

^a Each value represented the mean (n = 3)

chromatography (GPC) with dextran (MW4,400-401,000 Da) as a standard according to the method described [19]

2.5 Antioxidant Properties *in vitro*

2.5.1 ABTS scavenging assay

The ABTS scavenging activity was determined according to the method described [4] with some modifications. The GDP and a reference compound (ascorbic acid, ASC) were dissolved in deionized water yielding series of sample solutions with different concentrations. Two hundred microliters of the prepared ABTS^{•+} solution was transferred into a 96 well microplate (Costar[®], Corning, USA). Aliquot of 50 μ L of the sample solution was added and mixed at 1200 rpm for 30 s using a thermomixer (Eppendorf, Germany). The absorbance was recorded at 734 nm using a microplate reader (Spectramax 190, Molecular devices, USA) at 6 min, 15 min, 30 min, 1 h,

6 h, 12 h and 24 h. The ABTS^{•+} scavenging activity (%) was calculated using the formula: $[(A_{ABTS} - A_{Sample}) / A_{ABTS}] \times 100$, where A_{ABTS} and A_{Sample} are absorbances of the ABTS^{•+} solution without and with samples, respectively.

2.5.2 Conjugated autoxidizable triene (CAT) assay

The GDP was freshly prepared in phosphate buffer solution (PBS, pH 7.2) yielding a series of sample with different concentrations. Stripped tung oil (Sigma-Aldrich, 5 mg) was mixed with 25 ml of the PBS solution containing 34 μ M Brij 35 as an emulsifier by Ultra Turrax homogenizer (Model T25, IKA, USA) at 6,500 rpm for 90 s. The emulsion solution (125 μ L) was pipetted into a UV-grade-96 well microplate followed by adding the sample (50 μ L) then mixed and pre-incubated at 1,200 rpm, 37 $^{\circ}$ C for 5 min by using a thermomixer (Eppendorf, Germany). Twenty-five microliters of a freshly prepared

AAPH (2,2'-Azobis(2-methylpropionamide) dihydrochloride, Wako, Japan) solution was then added into the mixture. The decrease in absorbance at 273 nm was recorded every minute for 3 hour at 37 °C with 5 s agitation before each measurement using a microplate reader. The net protection area (AUC) providing by a sample was calculated as previously described [20].

2.5.3 Ferrous ion chelating (FIC) assay

The GDP and EDTA (as a standard compound) were dissolved in deionized water yielding a series of sample solutions with different concentrations. Aliquot of 100 µl of the sample solution was added into 100 µL of FeSO₄ solution (0.1 mM). Then, 100 µl of ferrozine solution (0.25 mM) was added and allowed to stand at room temperature in the dark for 10 min. The absorbance was measured at 562 nm using a microplate reader. The metal chelating capacity of the samples was calculated as previously described [4].

2.6 Anti-glycation Property *in vitro*

The anti-glycated activity of GDP was carried out according to the method described [4]. Bovine serum albumin (BSA, Fluka, 7 mg/ml) was mixed with glucose (25 mM) and sodium azide (0.02% w/v) in phosphate buffer (100 mM, pH 7.4). The sample was dissolved in deionized water with a series of concentrations. The sample solution (500 µl) was added to the mixture then incubated at 37 °C for 5 days. Aminoguanidine hydrochloride (AG, Sigma-Aldrich, 10 mg/100 ml) was used as a control standard. The content of AGEs was determined at excitation wavelength 330 nm and emission wavelength 410 nm by fluorospectrophotometric method and the percentage of anti-glycated activity was finally calculated [4].

3. RESULTS AND DISCUSSION

3.1 Model Fitting and Statistical Analysis

As shown in Table 2, yield of GDP ranges from 4.17 % to 6.40% depending on extraction parameters. The maximum value was obtained using the ultrasonic power of 148 W for 15 min with the ratio of water to raw material of 20 ml/g. By applying multiple regression analysis on the experimental data, a fitted quartic model demonstrating correlation between the dependent and independent variables was obtained as follows:

$$Y = 7.0144 - 0.2195X_1 - 0.1551X_2 + 0.1901X_3 + 0.0028X_1X_2 + 0.0124X_1X_3 + 0.0013X_2X_3 + 0.0012X_1^2 - 0.0005X_2^2 - 0.0162X_3^2 - 0.0275 \times 10^{-3}X_1^2X_2 - 0.0651 \times 10^{-3}X_1^2X_3 + 0.7406 \times 10^{-6}X_1^2X_2^2$$

Where, Y is yield of GDP polysaccharides and X₁, X₂, X₃ represents ultrasonic power, extraction time and ratio of water to raw material, respectively. Hence, the predicted polysaccharide yield could be obtained using this equation. However, it is worth noting that optimization of a fitted response surface could produce poor or misleading results, unless the model exhibits a good fit. Therefore, it is necessary to check the model validity.

Statistical testing of the obtained model was performed in form of analysis of variance (ANOVA) as shown in Table 3. The goodness of the model can be checked by the determination coefficient (R²) which was found to be 0.982 for our studies. This signified a reasonable fit of the model to the experimental data. The adjusted determination coefficient of the model (R²_{adj} = 0.946) assured a high degree of correlation between the observed and predicted data of the GDP yield. A low value of coefficient of variation (CV of 2.53%) clearly showed a high degree of precision and a good reliability of the experimental performed. From the analysis, the F-value (F = 27.1180) and the P-value (0.0003)

Table 3. Analysis of variance (ANOVA) for the quartic model of GDP extraction.

Source ^a	Degree of freedom	Sums of squares	Mean squares	F-value	P- value ^b
X ₁	1	2.0158	2.0158	100.3278	< 0.0001
X ₂	1	0.0924	0.0924	4.6014	0.0756
X ₃	1	1.8818	1.8818	93.6608	< 0.0001
X ₁ X ₂	1	0.0128	0.0128	0.6371	0.4552
X ₁ X ₃	1	0.0480	0.0480	2.3915	0.1730
X ₂ X ₃	1	0.0024	0.0024	0.1219	0.7389
X ₁ ²	1	0.9960	0.9960	49.5746	0.0004
X ₂ ²	1	0.1700	0.1700	8.4630	0.0270
X ₃ ²	1	0.4166	0.4166	20.7336	0.0039
X ₁ ² 2X ₂	1	0.0749	0.0749	3.7277	0.1017
X ₁ ² 2X ₃	1	0.4704	0.4704	23.4121	0.0029
X ₁ ² X ₂ ²	1	0.6298	0.6298	31.3467	0.0014
Model	12	6.6380	0.5450	27.1180	0.0003
Residual	6	0.1206	0.0201		
Lack of fit	2	0.0572	0.0286	1.8028	0.2766
Pure error	4	0.0634	0.0158		
Cor total	18	6.6587			

R² = 0.982 R²_{adj} = 0.946 C.V.= 2.53%

^a X₁, Ultrasonic power (W); X₂, extraction time (min); X₃, ratio of water to raw material (ml/g).

^b P- value < 0.05 indicates statistical significance.

indicated that the model itself was significant. F-value for the lack-of-fit was insignificant ($p > 0.05$), thereby confirming the validity of the model. All these results indicate that the model was adequate for predicting the yield of GDP polysaccharides under the range of experimental variables.

The significance of each coefficient was checked by P-value. If P-value is less than 0.05, it indicates that the model terms are significant. The smaller the value of P, the more significant it tends to be. It is notable that the terms X₁, X₃, X₁², X₂², X₃², X₁²2X₃, and X₁²X₂² significantly also affected the extraction yield of GDP (Table 3). Overall, it can be concluded that the ultrasonic power (X₁) and ratio of water to raw material (X₃) were the most significant factors affecting the yield of GDP.

3.2 Effect of Extraction Conditions on the Yield of GDP

Three dimension response surface (3D) plots were constructed using the predicted model to visualize the relationship between the independent and dependent variables. Each plot demonstrated the effect of two parameters, while the other one factor was fixed at its zero level (center value of the testing ranges) as shown in Figure 1. Different shapes of 3D signified different interaction between variables. As shown in Figure 1A – 1C, the ultrasonic powers (X₁) and ratios of water to raw material (X₃) had positive effects on the extraction yield of GDP, while the GDP yield changed slightly when the extraction time (X₂) was in the range of 5-15 min. The GDP yield increased progressively with increasing the ultrasonic power (X₁), but beyond 148 W, it obviously decreased as the

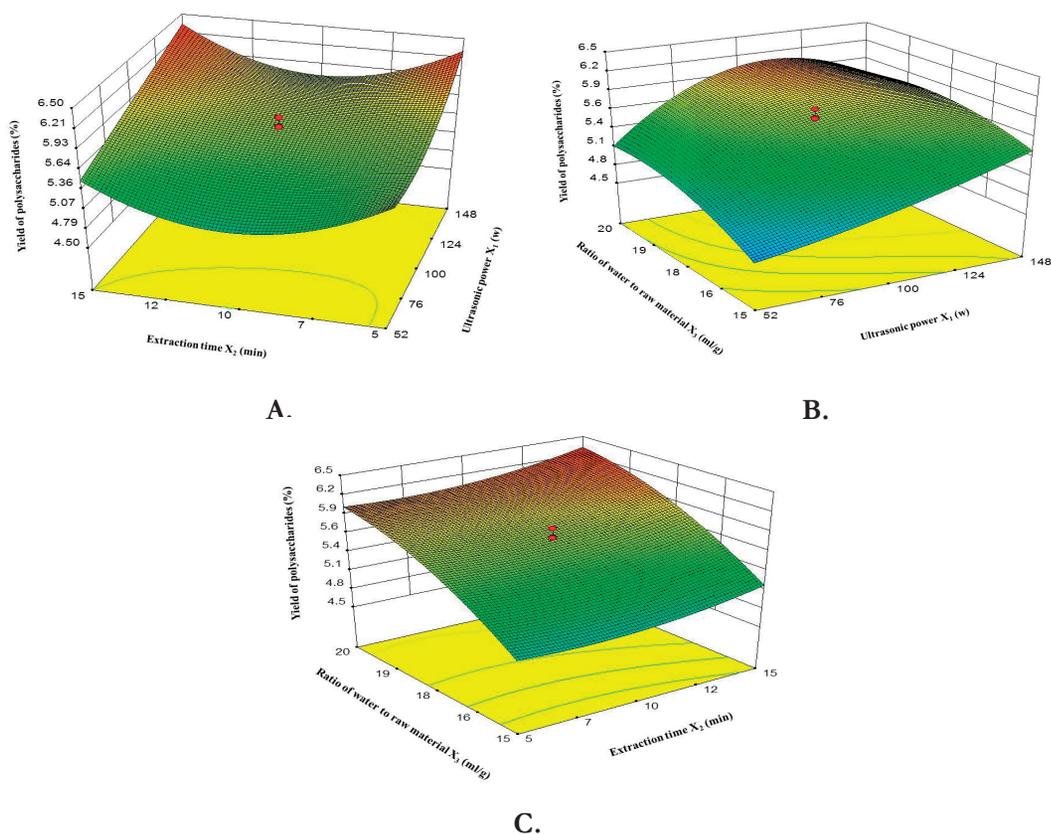


Figure 1. Response surface curves (a, b and c) showing the interactive effects of ultrasonic power (X_1), extraction time (X_2) and ratio of water to raw material (X_3) on the extraction yield of GDP.

ultrasonic power declined. In addition, the GDP yield was enhanced by extending the ratios of water to raw material (X_3) with a critical value at 20 ml/g when the ultrasonic power was set at 100 W. It was found that an excessive ratio did not significantly enhance the GDP yield (data not shown).

It could be said that the enhancement of ultrasonic power and ratios of water to raw material in a certain extent could effectively increase the polysaccharide yield. Since sonication generates the number and collapse microscopic bubbles, which are positively correlated to the amplitude of ultrasonic waves. Higher extraction efficiency of polysaccharides at higher ultrasonic power is due to the increase in the number of cavitation bubbles formed causing the relative high temperature and pressure at

the micro-environment level, which disrupts the cell wall and increase mass transfer rates [13]. In contrast, very high ultrasonic power decreases the extraction efficiency through depolymerisation of a part of polysaccharides into some free sugars [21]. For the ratio of water to raw material, it is consistent with the mass transfer principles, where the driving force during the mass transfer is the concentration gradient between the solid and the bulk of the liquid, which increases when a higher solvent per sample ratio is used. On the other hand, excessive amount of solvent will not further enhance the driving force [22].

3.3 Validation of the Predicted Model

Using computing program, the optimum condition for the highest yield of GDP were

the ultrasonic power, extraction time and ratio of water to raw material of 147.6 W, 14.9 min and 18.5 ml/g, respectively. Such condition would predict the expected maximum GDP yield of 6.40%. However, for practical use, the optimum condition was slightly modified as follows: ultrasonic power of 148 W, extraction time of 15 min and ratio of raw material of 18.5 ml/g. To verify the validity of the model, additional experiments under the modified optimal condition were carried out. It was found that the polysaccharides yield was 6.45% \pm 0.13 (n = 3), which was in good agreement with the predicted value. Subsequently, the GDP extracted under the optimum condition was characterized and analyzed for antioxidant and anti-glycation properties.

3.4 Polysaccharide Characterization

Total sugar, uronic acid, protein, polyphenolic and moisture contents of the GDP from the optimum condition were calculated and normalized as 40.94%, 57.38%, 0.71%, 0.80% and 0.17%, respectively. Its molecular weight determined by high performance gel permeation chromatography was calculated as approximately 960 kDa.

FT-IR was carried out in order to identify functional groups presented in the GDP and

the spectrum was shown in Figure 2. A broad and intense peak at around 3419 cm^{-1} and a weak band at 2930 cm^{-1} were assigned to hydroxyl stretching vibration and C-H stretching vibrations, respectively. In addition, the medium to weak bands ranged from 1250 to 896 cm^{-1} are mainly due to C-C and C-O stretching in pyranose ring and to C-O-C stretching of glycosidic bonds [23]. The absorption peaks in these regions are commonly found in all polysaccharides. Moreover, the GDP shows characteristic of β -anomeric configuration with an absorption band at 896 cm^{-1} . The presence of band at 1730 and 1627 cm^{-1} was assigned to carbonyl ester and anti-symmetric stretching of carboxylate group (COO), indicating the presence of uronic acid and its ester [24]. This result was in good agreement with the uronic content (ca. 62%) previously obtained from composition analysis.

From the chemical composition and the FTIR analysis, the GDP was suggested as polysaccharides predominately consisting of uronic acid and/or its ester.

3.5 Antioxidant Properties of GDP

Free radicals are well-known to strongly associate with lipid oxidation leading to the occurrence of rancidity in many products such

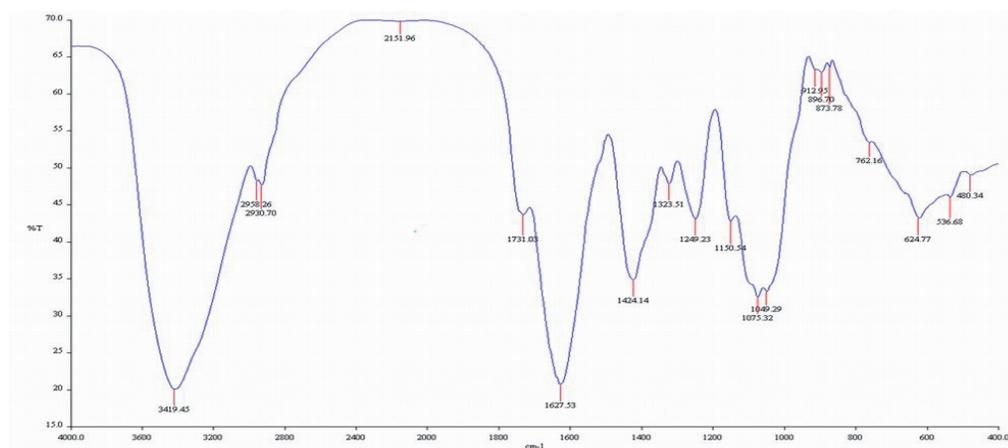


Figure 2: Fourier transforms infrared (FT-IR) spectrum of GDP.

as foods and cosmetics and also a number of human degenerative diseases such as cancers, diabetes and Alzheimer's symptoms [25]. To investigate further potential applications of GDP, the antioxidant properties were analyzed in terms of free radical scavenging and metal chelating activities, which are two major antioxidant mechanisms in inhibition of the free radicals. Metal chelators can inhibit free radicals by suppressing the formation hydroxyl radicals and/or lipid alkoxyl radicals via Fenton reactions [26]. In this study, free radical scavenging activities of GDP were assessed using ABTS and CAT assays. In addition, metal chelating capacity of GDP was carried out using FIC assay.

3.5.1 Free radical scavenging activity

3.5.1.1 ABTS assay

ABTS^{•+} scavenging activity of GDP was evaluated and compared with ascorbic acid (ASC) as a control standard (Figure 3). Kinetic pattern of reaction with ABTS^{•+} of GDP and ASC was also established. It was noticeable that the reaction rate of GDP with ABTS^{•+} increased slowly during the incubation and reached stable at 24 h (data not shown). Unlike ASC, the reaction time was achieved end point at 6 min and its capacity remained stable until 24 h. This signified the stability of ABTS^{•+} and ASC

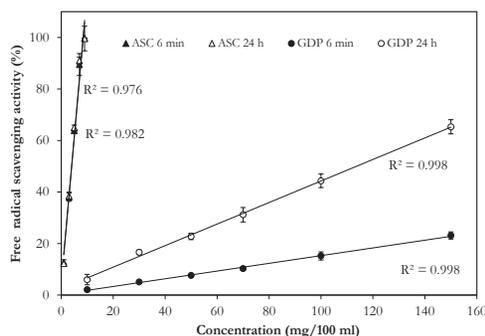


Figure 3: ABTS radical scavenging activity of GDP at 6 min and 24 h in comparison with ascorbic acid.

activities during the incubation time. Difference in reaction time between GDP and ASC could be due to chemical structure, molecular size and conformation of the compounds. It has been reported that the chemical structure of antioxidants such as the presence of quinic acid group may provide the steric hindrance to the access of ABTS^{•+} [27]. This is also the first evidence showing that the reaction rate of plant polysaccharides with ABTS^{•+} was slow and required up to 24 h for incubation to reach the end point. Furthermore, to obtain clearly information concerning on antioxidant capacities of these polysaccharides, kinetic profile should be taken with caution and end point of measurement should be taken at a time. Our results show a linear dose-response relationship of the GDP concentration against ABTS^{•+}. When the concentration of GDP increased from 10 to 150 mg/ 100 ml, the ABTS^{•+} scavenging activity increased from 2.47%±0.21 to 19.29% ±0.45 at 6 min and from 7.40%±0.15 to 62.38% ±1.48 at 24 h of incubation period. In the other words, the activity increased from 11.23 to 34.78 mg (increased 2.86 times) of ASC per one gram of GDP.

3.5.1.2 CAT assay

Free radical scavenging capacity of GDP was also carried out using the CAT assay in order to determine its performance in heterogeneous oil-in-water emulsion system. As illustrated in Figure 4A, kinetics of relative absorbance bleaching suggested that GDP did perform as a free radical scavenger antioxidant in the heterogeneous system and its antioxidant behavior was rather able to stabilize the initiator peroxy radicals derived from AAPH than to scavenge the propagator lipoperoxy radicals from stripped tung oil. This could be due to a high polarity of GDP [20]. Estimated from net protection AUC (Figure 4B), the CAT value of GDP increased in a dose-dependent manner

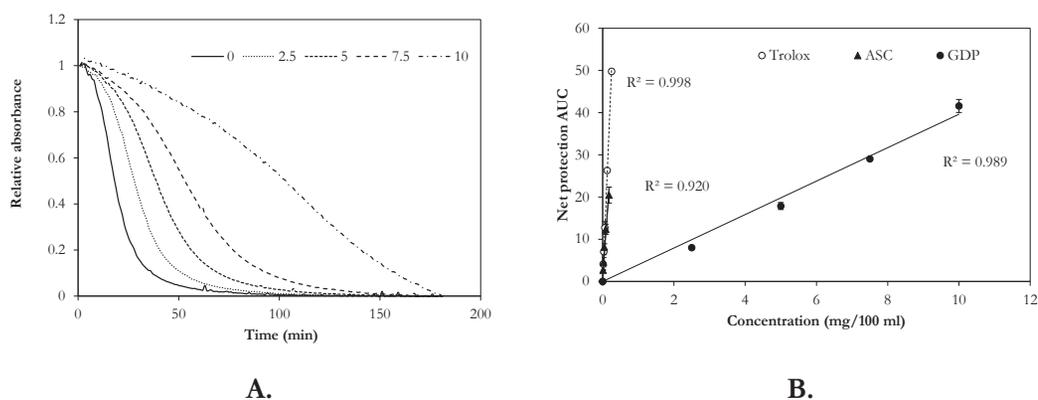


Figure 4. Kinetics of relative absorbance bleaching in the presence of different GDP concentrations (A); Net protection AUC versus concentrations of GDP, compared with those of trolox and ascorbic acid (B). Values are the means of three independent replicates.

and one gram of sample was equivalent to 21.2 mg of trolox or 29.6 mg of ASC.

Therefore, from our results, GDP did perform as the free radical scavenger in both homo and heterogeneous oil-in-water emulsion systems. Free radical scavenging activity of plant polysaccharides have been reported in literatures and their activities are generally lower than that of ascorbic acid and trolox. The scavenging activity was varied depending on polysaccharides concentration used and their origins [9, 15, 28]. For examples, the scavenging activity of *Dendrobium nobile* polysaccharides increased from less than 10 to 20% when the concentration was increased from 50 to 200 mg/100 ml [28]. Polysaccharides extracted from finger citron showed the scavenging activity of less than 10% at a concentration of 10 mg/100 ml and increased to 70% at 80 mg/100 ml concentration [9]. Although, GDP had a good effect on the free radical scavenging activity (ca. 62% at 150 mg/100 ml), its capacity was lower than that of ASC and trolox at all investigated concentrations and all test assays.

3.5.2 Metal chelating activity

When compared with EDTA as a control standard for FIC assay, GDP had a lower metal

chelating ability as shown in Figure 5. The metal chelating activity of GDP increased in a dose-dependent manner up to 30% \pm 0.66 inhibition at a concentration of 60 mg/100 ml. One gram of GDP had metal chelating capacity equivalent to 0.025 g of EDTA. The metal chelating activity of plant polysaccharides has only a few reports. For instances, *Ampelopsis grossedentata* polysaccharides exhibited 20% metal chelating activity at a concentration of 100 mg/100 ml [29], while *Agaricus blazei Murrill* polysaccharides displayed 20% of the activity at a concentration of 120 mg/100 ml [30]. Polysaccharides obtained from *Herba Lophatheri* showed 50.1% metal chelating capacity at 320

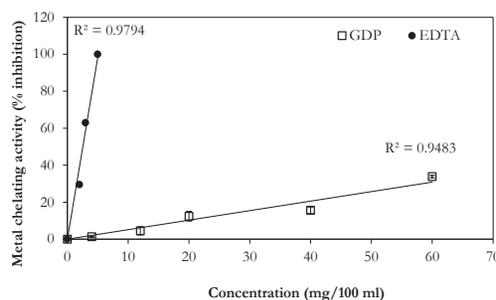


Figure 5. Metal chelating capacities of different concentrations of GDP and EDTA. Values are the means of three independent replicates.

mg/100 ml [31]. The GDP exhibited up to 30% metal chelating activity when used at a relatively low concentration of 60 mg/100 ml, indicating more potent metal chelating activity than most plant polysaccharides demonstrated so far. The metal chelating capacity of polysaccharides may be due to the formation of cross-bridge between carboxyl group in uronic acid and divalent ion [31]. The GDP metal chelating activity probably provides additional benefits, especially in combination with other antioxidants. For examples, in case of pharmaceutical drug, antioxidant with iron chelator property played a major role in the prevention of neurodegeneration in a variety of cellular and animal models of neurodegenerative diseases [32]. In case of food application, the addition of metal chelator with free radical scavenging antioxidants inhibited oxidation in fish oil salad dressing during 6 weeks of storage period [33].

3.5.3 Anti-glycation property of GDP *in vitro*

Advance glycation end products (AGEs) are produced from glycation process. This process begins with chemical reactions between available amino groups of amino acid residues in proteins and reducing sugars. Accumulation of AGEs is toxic to cells and can accelerate diabetic complications including retinopathy, cataract and atherosclerosis [34]. Aminoguanidine (AG) is well-known as a powerful therapeutic agent to relieve diabetic complications by inhibiting the glycation process. However, its practical application is limited because of its potential toxicity and side effects [35]. Hence, there is an increasing interest in searching new effective AGE inhibitors from natural sources due to their low toxicity. In this study, anti-AGEs formation capacity of GDP in the BSA/glucose system was investigated using aminoguanidine (AG) as a control standard. GDP exhibited anti-glycation capacity in a dose-dependent manner as shown in Figure 6. Its activity reached $90.56\% \pm 6.42$ at the concentration

of 100 mg/100 ml. However, the GDP activity was still lower than that of AG ($90.51\% \pm 2.8$ at 10 mg/ml). Nevertheless, the anti-glycation property of plant polysaccharides has rarely been demonstrated. Only *Polygonum multiflorum* Thunb., *Dendrobium huoshanense*, longan (*Dimocarpus longan* Lour.) and pomegranate (*Punica granatum*) polysaccharides have been reported to have this activity [15, 36]. *Polygonum multiflorum* Thunb polysaccharides had 17.4% and 10.5% anti-glycation activity at 150 and 100 mg/100 ml, respectively [36] while longan polysaccharides had 45.3% at a concentration of 50 mg/100 ml [15]. Interestingly, the GDP exhibited that capacity higher than most plant polysaccharides reported.

4. CONCLUSIONS

Ultrasonic extraction exhibited as a very effective method for the GDP extraction. The maximum yield under the optimal extraction condition (148 W ultrasonic power, 15 min extraction time and 18.5 ml/g ratio of water to raw material) was $6.45\% \pm 0.13$. For polysaccharide characterization, the results clearly showed that GDP with molecular weight of 960 kDa mainly consisted of uronic acid and its ester. In addition, GDP showed excellent antioxidant and anti-glycation capacities, especially the

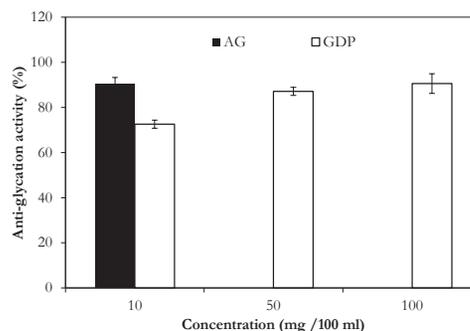


Figure 6: Anti-glycation activities of different GDP concentrations (10-100 mg/100 ml) and aminoguanidine (AG, 10 mg/100 ml). Values are the means of three independent replicates.

metal chelating antioxidative and anti-AGEs formation abilities. Thus, GDP could be a new choice of natural antioxidant and natural AGE inhibitor, effectively preventing lipid oxidation and diabetic complication illness. These studies provide information for further researches, development and utilization of the *G. divaricata* polysaccharides. Works on purification, structural elucidation and structure-function relationships of the GDP are being carried out.

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