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Effect of Extraction Solvent Systems on Extract Yield, Phytoconsituent Content and Free Radical Scavenging Activity from *Bougainvillea glabra* (Bunga Kertas) Bract

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In Malaysia, *Bougainvillea glabra* (bunga kertas) is cultivated as flowering ornamental plant for its colourful flower-like bract. The purpose of this study is to determine the solvents extraction effect of polarities on extract yield, phytoconsituent and antioxidant activity in *B. glabra* bract. The *B. glabra* bract was extracted with several solvents (acetone, ethanol, and methanol) at different concentrations (50%, 70% and 100%) and 100% distilled water. The yield percentage of *B. glabra* bract ranged from 38% to 2% with methanol scored the highest yield (38%). *B. glabra* bracts were further tested for phytoconsituent analysis. The 100% methanol and 100% ethanol extracts exhibited high total phenolic and flavonoid content. The total betalain content (betacyanins and betaxanthins) in 100% water extract of *B. glabra* was found to be highest among tested extracts. The 100% methanol and 100% ethanol of *B. glabra* bract extracts showed high antioxidant activities above 70% in three antioxidant pathways of different free radical species and mechanism of actions. Significant positive correlations ($p < 0.01$) of antioxidant activity with TFC and TPC were observed. These findings indicate that chemical properties of solvent such as polarity can influence antioxidant efficiency in the presence of different bioactive compounds. Thus, natural antioxidant substances in *B. glabra* bract scavenge excess free radicals and prevent oxidative damage by keeping the oxidative stress state in balance. In conclusion, the local *B. glabra* bracts can serve as a new source of antioxidants in food and nutraceutical product development.

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Introduction

Epidemiological studies have shown that consumption of plant foods containing phytoconstituent with antioxidant properties is beneficial to human health because it slows down degenerative processes and reduce risk of cancer and heart related diseases (Arabshahi-Delouee and Urooj, 2007). Antioxidants are micro-molecules that involved in structural maintenance of DNA and cell repair (Vimala et al., 2003). They protect DNA and cell membranes against oxidative damage, including induced by carcinogenic agents. Plant food reported to be rich in natural antioxidants that protect the human body against oxidative damage.

Solvent extraction is a common technique used for isolation of plant antioxidant compounds. However, the extract yields and antioxidant activities of the plant materials are strongly dependent on extracting solvent. These are due to the presence of antioxidant compounds of various chemical characteristics and polarities that influence its solubility in a particular solvent. Polar solvents are frequently used for the recovery of polyphenols from a plant matrix (Sultana et al., 2009). The most suitable solvents are (hot or cold) aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Peschel et al., 2006).

The bract of *B. glabra* was selected for the present investigation. The plant of *B. glabra* have long been used in the folk medicine due to their potential health benefits which are mainly due to the presence of antioxidant phytoconstituents such as phenolic and flavonoids (Gupta et al., 2011; Mishra et al., 2009; Schlein et al., 2001; Saikia and Lama, 2011). It is important to establish suitable extraction solvent system to evaluate and quantify effective antioxidant principles of viable plant materials (Sultana et al., 2009). The solvents of methanol, ethanol and acetone have been extensively used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables etc.) such as plum, strawberry, pomegranate, broccoli, rosemary, sage, sumac, rice bran, wheat grain and bran, mango seed kernel, citrus peel, and many other fruit peels (Sultana et al., 2009).

The present study focused in investigation of the most effective solvent system for extracting potent phytoconstituents especially phenolics, flavonoids and betalains as potent antioxidant from *B. glabra* bract growing locally in Malaysia. In this study, the antioxidant

activities were determined for *B. glabra* bract using ten different solvents; 100% methanol and aqueous methanol (50% and 70%), 100% ethanol and aqueous ethanol (50% and 70%) and 100% acetone and aqueous acetone (50% and 70%). All the *B. glabra* bract extract were evaluated for antioxidant activity via three bioassay systems namely DPPH, ABTS and XOD. Each antioxidant bioassay tested relates to the generation of different radicals which acting through a variety of mechanisms and defense lines.

Materials and Methods

Sample collection

400 g fresh flowers of *B. glabra* were collected from a home garden at Taman Bersatu, Rawang. Mature flowers were pluck to ensure the uniformity in the colour of collected *B. glabra* flower. The sample was transported immediately to Biology Laboratory, FRIM. The plant was identified by a taxonomist at FRIM.

Sample processing

The collected flowers were processed to separate the bracts from flower. Infected and contrast colour of bracts were discarded. 303 g of *B. glabra* bracts obtained upon separation. The processed bracts were cleaned in running tap water to remove impurities. All bracts were oven dried at 30 ^{0}C ± 1^{0}C for 72 hours. 134 g of dried bract then pulverized and sieved. The fine powdered bract was stored in an air tight container until further use.

Preparation of test sample

5 mg of *B. glabra* bract extracts were dissolved in 5 mL of pure methanol to obtain a final concentration of 1.0 mg/mL samples stock solution for the testing. All the sample solutions were sonicated for 10 minutes at room temperature to facilitate the dissolubility. Then, sample solution (extract with solvents) was filtered through a 0.22 µm regenerated cellulose (RC) membrane filter (Sortorius).

Polyphenols extraction using different solvent systems

The extraction method was performed as described by (Addai et al., 2013) with some modification. The fine powder of *B. glabra* bract (10 g) was weighed in a conical flask. Then, soaked in 1 L solvent of different types and polarities using methanol, ethanol, and acetone as well as their respective aqueous solution at 50% and 70% concentrations. The conical flask then sealed with aluminium foil to avoid evaporation of the solvent. The fine powder of *B. glabra* successively extracted with its respective solvent for 72 hours with shaking at 200 rpm at room temperature to achieve maximum yield. The extracts were filtered using filter paper (Whatman No.4). The same solvent filtrate pooled and were concentrated using rotary evaporator under reduced pressure. The dried extracts were weighed and yield percentage of the extract was determined according to method of Zhang et al., (2007).

Extraction yield (%) = (A x 100) / (B)

A: weight of the *B .glabra* extract
B: weight of the *B. glabra* bracts raw material

The crude extracts placed in an air tight container and stored at 4^{o}C until further experimental investigation.

Determination of total phenol content (TPC)

TPC analysis was carried out based on the method of Musa et al., (2011). Approximately, 0.4 mL distilled water and 0.5 mL diluted Folin–Ciocalteu reagents were added to 100 µL *B. glabra* extracts. The samples (*B. glabra* extracts with Folin– Ciocalteu reagent) were set aside for five minutes before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength using a spectrophotometer after two hours. The calibration curve of gallic acid was used for the estimation of sample activity capacity. The result was recorded in terms of mg of gallic acid equivalents (GAE) per 100 g of extract (mg GA/100 g of extract).

Determination of total flavonoid content (TFC)

The TFC was determined by the colorimetric method as described by Bakar et al., (2009). A total of 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube, followed by the addition of 0.15 mL of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3{\cdot}6H_2O$ solution was added, and the reaction was allowed to stand for another 5 min before 1.0 mL of 1 M NaOH was added. The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm using a spectrophotometer (Epoch, Biotek, USA). The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of extract (mg QE/100 g of extract).

Determination of betalain content

The content of betaxanthins and betacyanins in the *B. glabra* bract was determined according to the methods of (Ravichandran et al., 2013) The *B. glabra* bract extract (100 µL) was dissolved in 3.9 mL distilled water and measured at 538 nm and 480 nm respectively using UV–Vis spectrometer. The betalain content was calculated from the equation (mg/L) = [(A×DF×MW×1000) / (e)], where A is the absorption, DF the dilution factor and l the pathlength (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (e) respectively are (MW=550g/mol; e= 60,000 L/mol cm in H_2O) and (MW=308 g/mol; e=48,000 L/mol cm in H_2O) were applied.

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The anti-free radical activity was carried out based on the method of Musa et al., (2011), the antioxidant activity was assessed using a DPPH scavenging system. The test was carried out using Oxi Count kit from UKM Technology which stored at -20°C. The kit was ready filled with DPPH dry reagent array, 0.2 ml methanol was added to dissolve the DPPH. Approximately 0.3 ml of methanol was added to the reference well to obtained 1.8±0.01 unit at 517 nm wavelength by using a spectrophotometer (POLARstar Omega, BMG). In the dark, approximately 100 µl *B. glabra* bract extracts with methanolic DPPH solution was incubated at room temperature for 30 minutes for scavenging reaction. The sample activity for DPPH free radical system is determined based on negative and positive control. A standard curve of Trolox was generated.

Determination of 2,2'-azino-bis(3-ethylbenzothiazoline-sulphonic acid (ABTS) activity

The ABTS radical quenching assay was carried out using the method of Ozgen et al., (2006) with slight modification. The assay was modified into 96 well microtiter plate with adjustment in experimental volumes. The ABTS radical cation was generated by the interaction of ABTS (250 µM) and $K_2S_2O_8$ (40 µM). After the addition of 900 µl of ABTS solution to 100 µL of *B. glabra* extract, the absorbance at 734 nm was monitored using the FLUOstar Omega spectrophotometer.

Determination of xanthine/xanthine oxidase superoxide scavenging (XOD) activity

The assay system evaluates the scavenging activity of the sample against superoxide free radical anions. The method of Chang et al., (1996) is slightly modified. 4-Nitro-blue tetrazolium chloride (NBT) solution (100 mL of 4.1 mM/L) is prepared by adding 3.15 g TrisHCL, 0.1 g $MgCl_2$, 15.0 mg 5-bromo-4-chloro-3-indolyl phosphate and 34.0 mg NBT to 100 ml of distilled water. The reaction mixture (100 mL) is prepared by dissolving 0.53g Na_2CO_3 (pH 10.2), 4.0 mg EDTA and 50.0 mg xanthine in 0.025 mM NBT solution. The mixture is kept refrigerated at 4 ^{o}C. The reaction mixture (999 µL) is transferred into a microcuvette and placed in a 25 ^{o}C cell holder of a spectrophotometer. Generation of superoxide is initiated by adding 1.0 ml of XOD (20 U/mL). The optical density (OD) measurements are taken at 560 nm for 120 seconds using a spectrophotometer (Perkin Elmer, Lambda 2S). The 200 µL *B. glabra* crude extract stock solutions (1 mg/mL) is added to 799 mL of the reaction mixture to achieved final concentration at 0.2 mg/mL and placed in a cell holder to autozero. 1.0 mL of XOD (20 U/mL) is then added and after thoroughly mixing, similarly measured for the XOD and SOD curves.

Superoxide scavenging activity (%) = [(A control – A sample) / A control] × 100

A control: slope of negative control
A sample: slope of the sample

The sample activity for superoxide scavenging system is determined based on negative control. A standard curve of SOD was generated.

Statistical analysis

All the assays were done in duplicate wells of triplicate individual independent experiments. The results were expressed as the means values ± standard deviation (SD). The MINITAB (14.20) software was used for statistical analysis. Mean of triplicate measurements were compared by analysis of variance (ANOVA). Significant differences between means were determined by Fisher's least significant difference (LSD) test ($P<0.05$). Correlation analysis was performed using Pearson's correlation coefficient (r).

Results and Discussion

Influence of extraction solvents on the extract yield

Previous research reported that chemical structure and its polarity of extraction solvent influenced the yield of plant extracts (Goli et al., 2005). The yield obtained from various plant sources is attributed by different soluble polyphenol compounds. Polar solvents are frequently used for recovering polyphenol from plant matrix. The most suitable solvents reported are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Do et al., 2014). Literature reported that aqueous methanol was found to be more effective in recovering highest amount of phenolic compounds from rice bran (Chatha et al., 2006) and *Moringa oleifera* leaves (Siddhuraju and Becker 2003).

In current study, the bract of *B. glabra* were collected and extracted in different types solvent and polarities using methanol, ethanol, and acetone as well as their respective aqueous solution at 50% and 70% concentration. Solvents with different polarities were used with the aim to investigate the recovery of polyphenolic compounds from B. *glabra* bract. Yields (%) of 10 crude extracts are shown in Table 1.

The yield percentage of *B. glabra* bract ranged from 38% to 2% in descending order of methanol (100%) > methanol (70%) > ethanol > (100%) > acetone (100%) > methanol (50%) > ethanol (70%) > ethanol > (50%) > acetone (70%) > water (100%) > acetone (50%). The extraction yield of 100% methanol (38.00 %) is higher than that of 100% ethanol (26.00 %) and 100% acetone (22.00 %). Data obtained also showed extraction yield increases with decreasing polarity of the solvent used. Current findings are in agreement with studies of Anokwuru et al., (2011) which methanol has been

reported to be more efficient in extraction of plant polyphenols than other solvents such as acetone and ethyl acetate. In addition, according to Anokwuru et al., 2011, methanol extract of *Hibiscus sabdariffa* calyx demonstrated highest yield among other solvents such as acetone and ethanol.

A study conducted by Hassim et al., (2015) also reported that highest yield (31.17%) was obtained in *Polygonum minus* via methanol extraction. Furthermore, methanol solvent polarity plays an important role in increasing phenolic solubility (Naczk and Shahidi, 2006; Abaza et al., 2011) and efficient in extraction of lower molecular weight polyphenols (Dai and Mumper 2010). Results of current study were found to be vise versa from previous reports which indicate strong positive correlation between extract yield and polarity of the solvent used (López et al., 2011). In this investigation, the yield of aqueous solvent showed decreased yield as the concentration of aqueous solvent increased. It is also found that the yield of the water extract (9.00 %) is the lowest among all the obtained extracts. Thus, extraction of polyphenol compounds in *B. glabra* bract was found to be more efficient in 100% methanol medium compared to aqueous methanolic medium. Usually, the least polar solvents are considered to be suitable for the extraction of lipophilic phenols. Therefore, chemical components present in *B. glabra* bract are lipophilic compounds.

Table 1. Solvent polarity index and yield of *B. glabra* bract

Solvent system	Polarity Index (P*i*) [a]	Yield (% g/g sample)
Acetone		
50%	7.05	2.00
70%	6.27	16.00
100%	5.1	22.00
Ethanol		
50%	7.1	17.50
70%	6.34	19.00
100%	5.2	26.00
Methanol		
50%	7.05	20.00
70%	6.27	37.00
100%	5.1	38.00
Water		
100%	10	9.00

[a] Solvent polarity index cited from Musa et al (2011). P*i*= Ø*a* P*a* + Ø*b* P*b*. Ø*a* and Ø*b* are the volume fractions of solvents 1 and solvent 2 respectively, and P*a* and P*b* are polarity indices of solvent 1 and solvent 2, respectively.

Kaisoon et al., (2011) in a study reported that lyophilised hydrophilic extract of *B. glabra* flower obtained yield of 36.47 %. The differences in findings could be attributed to the different extraction methods (Uma and Parvathavarthini 2010). Extract yields from the *B. glabra* bracts in the present analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants (Hsu et al., 2006). Among other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds is important (Siddhuraju and Becker, 2003; Sultana et al., 2007). Endogenous compounds (protein, polysaccharides, terpenes, cholorophyll, lipids and inorganic compounds) together with phenols contributed to highest percentage of yield in methanol extraction (Anokwuru et al., 2011). As reported in literature, results of this study are in agreement with the extraction yields of rice bran (Chatha et al., 2006) and some medicinal plants (Sultana et al., 2009).

The efficiency of polyphenols recovery from plant materials is influenced by the solubility of the phenolic compounds in the solvent used in the extraction process. Individual plant is unique with complex chemical component profile, thus development of a standard extraction solvent system is important for maximum polyphenols recovery for measurement of potential antioxidant activity in plants.

Influence of solvent extraction on TPC and TFC

Polyphenols were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity (Liu et al., 2009). TPC was determined by Folin Ciocalteu method and the result was expressed in terms of mg GAE/100 g extract. TFC was investigated using the aluminum chloride colorimetry and the result was expresses as mg QE/100 g extracts. In literature, *B. glabra* flower extracts was reported previously for the presence of phenolics and flavonoids (Saxena and Sahu, 2012).

The TPC and TFC of 10 extracts of *B. glabra* bract were evaluated in several solvent systems. The extracts were analysed at final concentration of 0.33 mg/mL and the amount of TPC and TFC extracted for each solvent at different polarities in *B. glabra* bract as shown in Table 2. All the tested extract showed presence of TPC and TFC with significant different values ($p<0.05$). The TPC in the extracts ranging from 18.94±4.83 mg GAE/100 g to 76.74±2.38 mg GAE/100 g and decrease in the following order: 100% ethanol > 100% methanol > 70% methanol > 70% ethanol > 50% acetone > 50% methanol > 70% acetone > 50% ethanol > 100% acetone > 100% water. The TFC values range in the extract obtained were from 9.33±0.58 to 250.10± 2.86 mg QE/100 g and decrease in the following order: 100% ethanol > 100% methanol > 50% ethanol > 50% methanol > 70% ethanol >70% methanol > 50% acetone > 70% acetone > 100% water > 100% acetone.

Current result demonstrated that 100% ethanol extract of *B. glabra* bract has the highest TPC (76.74±2.38 mg GAE/100g) and TFC (249.53±26.20 mg QE/100g) values among the tested extracts. 100% ethanol and methanol (lower polarity solvent) exhibited higher ability in extracting phenolic compounds compared to the polar solvents as reported in literature (Gironi and Piemonte 2011; Schäfer 1998). In contrast, Quezada and Cherian (2012) reported that low polarity is less efficient than polar

solvents in extraction of TPC from flaxseeds. Ethanol was found to be efficient in extraction of phenolic and flavonoid compound from *B. glabra* bracts due to its low polarity in this study.

Table 2. Total phenolic content and total flavonoid content of *B. glabra* bract

Solvent and polarity	TPC (mg GA/100 g)	TFC (mg QE/100 g)	Total Betalain Content	
			Betacyanins (mg/L)	Betaxanthins (mg/L)
Acetone				
50%	42.45±0.51^{h}	42.32±213^{h}	19.20±0.02^{g}	16.21±0.44^{gh}
70%	27.67±0.46^{i}	23.29±4.11^{j}	7.55±0.49^{j}	3.94±0.07^{klm}
100%	17.36±0.74^{k}	9.33±0.58^{l}	1.75±0.21^{lm}	2.55±0.04^{m}
Ethanol				
50%	23.85±1.03^{ij}	192.47±3.25^{b}	41.23±1.31^{c}	33.50±2.13^{d}
70%	48.31±1.13^{g}	93.63±1.52^{d}	17.41±1.54^{gh}	7.55±0.16^{j}
100%	76.74±2.38^{e}	250.10±22.59^{a}	12.87±0.15^{i}	5.98±0.74^{jk}
Methanol				
50%	28.19±2.77^{f}	137.9±14.63^{c}	25.91±0.67^{f}	29.93±0.035^{lm}
70%	49.54±6.30^{g}	93.15±6.85^{d}	16.44±1.50^{h}	12.78±0.90^{i}
100%	63.92±0.90^{e}	249.5±26.20^{a}	3.91±0.90^{lm}	5.31±0.50^{kl}
Water				
100%	18.94±4.83^{k}	18.18±0.07^{k}	115.87±2.10^{a}	102.48±1.93^{b}

Values presented are averages ± SD of three replicates. Mean that do not share a letter are significantly different (P< 0.05) by Fisher's least significant difference (LSD) test

B. glabra bract in 100% ethanol may possess more phenol groups or have higher molecular weights than the phenolics in the aqueous solvent systems. Research findings reported high solubility of phenolics are found in polar a solvent which is in contrast with the current findings (Mohsen and Ammar 2009; Zhou and Yu 2006). Many researchers have stated clearly that phenolic has strong antioxidant activity (Fu et al., 2014; Demiray et al., 2009). Acetone (100%, 70% and 50%) and water extracts showed low TPC and TFC below 50 mg GAE/100 g. Research conducted by Musa et al., (2011) has confirmed the ineffectiveness of acetone and water for the extraction of total phenols in grapes seeds (*Vitis vinifera*). Based on the current TPC and TFC findings, the best extracting solvent was 100% ethanol for *B. glabra* bract. The result of this study is in agreement with Do et al., (2014) which ethanol was the best solvent for TPC and TFC extraction from *L. Aromatica*. TFC was observed to be high, thus indicating that *B. glabra* bracts consist of hydrophobic and water-insoluble flavanoids. The least polar quercetin and aglycones of flavanoid group (mythoxylated isoflavones, flavanones and flavonols) may be present in *B. glabra* bracts contributing to its antioxidant activity. Quercetin may be a potential active compound in flowers that scavenge harmful free radical and exhibits anti-oxidative properties (Vanisree et al., 2004). Plant phenolics are known as powerful antioxidants that scavenge free radicals. Phenolics have the ability to neutralize free radicals due their hydroxyl group (Hatano et al., 1989). Current research finding concluded that the bract of *B. glabra* rich in phenolics and flavonoids which provide a good source of natural antioxidants that may be of interest for nutraceutical product development.

Betalains are known as pigments in petals of many flowering plants, but differs from typical anthocyanins that contain nitrogen. Previous study reported that, the principle pigments present in *B. glabra* bract are form of components of betalains (betanidins) with high hydrophobicity (Wybraniec et al., 2010). Betalains and anthocyanins are two different families of pigments that are never found together in the same plant (Gandía-Herrero and García-Carmona, 2013). Betalains consist of two components: betacyanins which are red-violet pigments and betaxanthins are yellow-orange pigments (Delgado-Vargas et al., 2000; Stintzing and Carle 2004; Ravichandran et al., 2013; Azeredo et al., 2007). The *B. glabra* bract extracts were tested for two betalain components as shown in Table 3.11, 100% water extracts showed significantly (p< 0.05) the highest contents of total betacyanins and betaxanthins 115.87±2.10 and 102.48±1.93 mg/L, respectively.This finding may be due to the fact that betalains are water-soluble nitrogen-containing pigments as described by Moreno et al., (2008). Abou-Elella and Ali (2014) has reported that important physicochemical properties of betalain plant pigments are their significant polarity and ionization (dissociation, zwitter-ionic behavior) in aqueous solutions. Previous finding also reported that an increased in polar character of betacyanins and betaxanthins results in insolubilities in any of the organic polar or semi-polar solvents except of water (Stintzing and Carle 2004; Wybraniec and Mizrahi 2002). The presence of betalains was shown for all aqueous solvents. However, the lowest amounts of betalains were measured in 100% ethanol, methanol and acetone extracts extracts of *B. glabra* bract. Research evidence reported that besides betacyanins, *B. glabra* bracts accumulated large amounts of flavonols (kaempferol and quercetin conjugates) (Heuer et al., 1994).

Influence of extraction solvents on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The extracts of *B. glabra* bract were tested for DPPH free radical scavenging activity using Oxi Count Kit form UKM. The extracts were analysed at concentration of 0.33 mg/ml and results obtained as shown in Table 3. The ten extracts of *B. glabra* bract possessed DPPH free radical scavenging activity ranging from 27.27 % to 88.21 %. The highest scavenging activity (%) was observed in pure methanol extract of *B. glabra* bract (88.21), followed pure ethanol extract (84.81), methanol 50 % (80.89), methanol 70 % (75.99), ethanol 70 % (67.89), acetone 50 %

(46.25), acetone 70 % (42.60), pure water (30.98) and acetone 100 % (37.27) in comparison with Trolox standard.

Based on antioxidant activity range, the DPPH scavenging activity seen in the present study was found that methanol (50 %, 70 % and 100 %) and ethanol at 100 % showed high DPPH free radical scavenging activity above 70 % in different solvent polarities. Although DPPH scavenging activities of methanol 100% extract from *B. glabra* bract were found to be higher than those corresponding methanol (50% and 70%) and ethanol 100% extracts, the difference were not statistically significant (P>0.05). The ethanol 70 %, acetone 50 %, acetone 70 % showed moderate activity, while ethanol 50 %, pure acetone and water showed low activity. Solvent used for polyphenolic extraction had significant effect on antioxidant activity (Tatiya et al., 2011). As from the Table 1, the increased in the ratio of the water increases the polarity index of the mixture. Each solvent seemed to have distinct specificities in the extraction of the antioxidants (Musa et al., 2011).

Table 3. DPPH radical scavenging activity of *B. glabra* bract

Solvent system	DPPH free radical inhibition (%)	ABTS Radical Scavenging Activity (%)	Superoxide radical inhibition (%)
Acetone			
50%	46.25±1.25^{bc}	84.38±3.77^{b}	49.12±1.85d
70%	42.60±4.26^{d}	74.19±2.74^{c}	45.00±2.81d
100%	37.27±1.98^{d}	71.81±2.45^{c}	47.35±2.99d
Ethanol			
50%	36.09±3.49^{d}	87.24±7.17^{b}	57.29±1.88c
70%	67.89±13.83^{c}	74.49±2.74^{c}	60.17±5.57a
100%	84.81±4.16^{a}	95.57±0.19^{a}	71.35±1.76b
Methanol			
50%	80.89±5.08^{ab}	57.39±1.81^{d}	61.18±1.31c
70%	75.99±3.78^{abc}	57.98±2.53^{d}	60.49±4.27c
100%	88.21±2.34^{a}	82.13±0.68^{b}	74.36±4.01c
Water			
100%	30.98±5.24^{d}	50.37 ±4.47^{d}	46.05±3.64e

Values presented are averages ± SD of three replicate experiments. Mean that do not share a letter are significantly different (P< 0.05) by Fisher's least significant difference (LSD) test

In the present study, 100% methanol was the best solvent for obtaining extract with high DPPH free radical scavenging activity in *B. glabra* bract. Based on previous research, methanol extract had superior comparatively amount of antioxidant compositions because of the possibility of more polar phenolic compounds and lipids contains in the extract (Iqbal et al., 2008). All extract obtained by using 100% and aqueous organic solvent showed radical scavenging capacity than that of the water extract. A similar trend was observed in the study of DPPH radical scavenging activity of pineapple crude extract (Alothman et al., 2009) and defatted wheat germ (Wijekoon et al., 2011).

DPPH radical is a stable free radical with an absorption band at 517 nm. It decreases in absorption when accepting an electron or free radicals, which results in a rapid discoloration from purple to yellow. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Hseu et al., 2008). The different of free radical scavenging activities showed the ionic transferring within extraction process in different solvents (Dauda et al., 2016). Thus, this indicate that *B. glabra* bract possess potent proton donating ability and could serve as free radical inhibitor. On the basis of our results, *B. glabra* bract appears to have potential for treatment of oxidative stress related diseases. The result of DPPH scavenging activity implies that the plant extract may be useful for treating radical related pathological damages (Wang et al., 1998).

Influence of extraction solvents on the 2,2'-azino-bis(3-ethylbenzothiazoline-sulphonic acid (ABTS) activity

The ABTS radical known as nitrogen-centered synthetic radical is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidant (Chooi et al., 2007). An earlier report by Floegel et al., (2011) suggested that high-pigmented and hydrophilic antioxidants were better reflected by ABTS assay than DPPH assay. Pigmented *B. glabra* bract extracts of different solvent polarities were tested against ABTS radical scavenging activity.

This test indicated that all extracts exhibited ABTS radical scavenging activity. The results presented in Table 3.14 exhibited ABTS radical-scavenging activity at concentration 90.91 µg/mL and ranging from 50.37±4.47 to 95.57±0.19%. Among the tested extracts, seven extracts showed ABTS scavenging activity above 70%. The highest ABTS radical scavenging activity was found to be significant (P<0.05) in ethanol 100% extract with inhibition (%) of 95.57±0.19, followed by ethanol 50%, acetone 50%, methanol 100% , ethanol 70%, acetone 70% and acetone 100%, while the moderate scavenging activity (>49%) was found in methanol 70%, methanol 50% and water 100% extracts. The lowest ABTS radical scavenging activity was found in water extract of *B. glabra* bract. This finding was in agreement with the report that water extract from barley had lowest ABTS radical scavenging activity among all barley extract (Zhao et al., 2006). The ethanol 100% was the effective solvent for extracting antioxidant compounds from *B. glabra* bract. Antioxidants in the *B. glabra* bract have an ability to suppress the reaction by electron donation radical scavenging and inhibit the formation of the colored ABTS radical. In the present study, 100% ethanol was the best solvent for obtaining extract with high ABTS radical scavenging activity in *B. glabra* bracts.

Influence of extraction solvents on the xanthine/xanthine oxidase superoxide scavenging (XOD) activity

Superoxide anion radical is one of the strongest free radicals *in vivo* and is generated in a variety of biological systems, either by oxidation processes or by enzymes. The concentration of superoxide anions increases under conditions of oxidative stress (Lee and Jeong 2002). An *in-vitro* modified NBT assay was employed where SOD activity was measured indirectly by producing O_2^- (Fisher et al., 2004). The extracts were analysed at concentration of 0.20 mg/mL. The Table 3.16 showed the potent superoxide scavenging activities in *B. glabra* bract extracts. The values of O_2^- radical scavenging activity ranged from 46.05±3.64% to 74.36±4.01% indicating that extraction solvent had a significant (P< 0.05) influence on DPPH radical scavenging activity. With exceptional of 100% methanol (74.36%) and ethanol (71.35±1.76%) with high inhibition activity above 70%, the rest of the tested extracts showed moderate superoxide scavenging activities greater than 39% inhibition. 100% acetone and aqueous acetone (70% and 50%) as well as water extracts showed low superoxide radical scavenging activities below 39%.

The superoxide anion scavenging property of *B. glabra* bract extracts at methanol 100% possessed highest activity among the tested extracts may be attributed to both neutralization of superoxide anion radicals via hydrogen donation and inhibition of xanthine oxidase by various phenolic present in the extract. SOD is a naturally occurring enzyme which protects the cell from the reactive and damaging O_2^- by dismuting it into O_2 and H_2O_2. Natural products which can inhibit these enzymes *in vitro* have the potential to prevent radical related diseases and also skin ageing. Thus, the current findings suggest the methanol 100% extract is acting in a similar manner to SOD by inhibiting formazan production directly. High superoxide inhibition activity show the potential of the *B. glabra* bract extract at methanol 100% to act as a second line of antioxidant defense (Noguchi and Niki 1999). A study reported by Afanas'ev (1991) mentioned that second line antioxidant defense system scavenge biological free radicals that are produced as products of normal physiological functions such as metabolism and biochemical reactions. Highly toxic ROS such as superoxides are known to initiate chain reactions that lead to oxidative damage and cell death (Yoshida et al., 1998). Thus, *B. glabra* bract can be potential preventing agent of age-dependent chronic conditions such as inflammatory diseases, aging, cardiovascular disease, diabetes and cancer.

The superoxide anion generated by xanthine/xanthine oxidase reduces NBT. *B. glabra* bract extracts scavenged superoxide anion by inhibiting NBT in a dose-response manner. These experiments show, the activity range between (36.62– 126.2 µg/mL) with significant dose response correlation at p<0.05. Thus, *B. glabra* bract of ethanol 100%, methanol 70% and methanol 100% may be of use in preventing superoxide anion-induced damages. Many studies, reported that superoxide is an important mediator of tissue injury in stroke (Murakami et al., 1998; Kawase et al., 1999). Superoxide generation has been linked to the progression of neurodegenerative disorder (Beal 2003; Brown et al., 2000; Cassarino and Bennett 1999). The a ability of *B. glabra* bract extracts to scavenge superoxide anion, demonstrated in this study may promise its further usage in slowing or preventing diseased conditions related to oxidative damage. *B. glabra* bracts may enhance endogenous defences, or by the use of SOD like therapeutic agents.

Correlation of antioxidant activities between TPC and TFC

Correlation analysis was used to determine the relationship among antioxidant variables and polyphenols (TPC and TFC). Goristein et al., (2010) has recently reported a high correlation between polyphenolic content in three exotic fruits and antioxidant capacities measured using ABTS and DPPH assay. There were analyses reported on high correlation of antioxidant activities between TPC and TPC (Mahattanatawee et al., 2006). In this study, extracts of *B. glabra* bract at various polarities were analysis for its correlation of antioxidant activities (DPPH, ABTS and XOD) between TPC and TFC. The correlation coefficients (r) calculated from linear regression analysis which p<0.01.

Table 4. Pearson's correlation coefficient of antioxidant activities of *B. glabra* bract at different solvent polarities

Pearson correlation coefficient (r^2)	DPPH	ABTS	XOD
TPC	0.18	0.28	0.67
TFC	0.77	0.04	0.80

Table 4 shows DPPH, ABTS and XOD were positively correlated with TPC and TFC. In this study, an outstanding positive correlation was observed in *B. glabra* bracts between FRAP assay and TFC in this study with r^2= 0.85. XOD assay and TFC also showed excellent correlation with r2= 0.80. TPC with XOD exhibited good positive correlation relationships with r^2= 0.67. TPC were shown to be weakly correlated to DPPH and ABTS with r^2= 0.18 and r^2= 0.28 respectively. TFC has good correlation in DPPH assay (r^2=0.77) and poor correlation in ABTS assay (r^2=0.04). A high correlation between the content of TPC and antioxidant activity has been previously demonstrated by Zhou et al (2009). In this study, an antioxidant activity in B. glabra bract is very much associated with their TFC than TPC. This is in an agreement with findings of Addai et al., (2013), antioxidant activities in the extracts of papaya contributed by an active component. Thus, this result indicates that TFC are the dominating phenolic group in *B. glabra* bract with potential to scavenge free radicals and reduce

oxidative stress. This finding is similar to the extraction of phenolics from guava and pisang mas reported by Alothman et al., (2009)

Conclusions

The solvent extraction polarity influences the extract yield, content of phytoconsituents (TPC, TFC and betalain) and antioxidant activities in *B. glabra* bract. Highest extraction yield were obtained in methanol % (38.00 %) followed by ethanol 100% (26.00 %). TPC of methanol 100% and ethanol 100% were 63.92±0.90 and 76.74±2.38 respectively whereas TFC were 249.5±26.20 and 250.10± 22.59 respectively. Methanol 100% and ethanol 100% has suitable polarity to extract TPC and TFC which are believed to be effective antioxidants. Among the tested extracts again methanol 100% and ethanol 100% exhibited high radical scavenging activities above 70% in DPPH, ABTS and XOD. Significant positive correlations ($P< 0.01$) of antioxidant activity with TFC and TPC were observed. These findings indicate that chemical properties of solvent such as polarity can influence antioxidant efficiency in the presence of different bioactive compounds. Ethanol 100% being organic and non toxic is recommended for further analysis as this solvent is efficient in polyphenol extraction and safe for human consumption (Do et al., 2014). Thus, natural antioxidant substances in *B. glabra* bract scavenge excess free radicals and prevent oxidative damage by keeping the oxidative stress state in balance. In conclusion, the local *B. glabr*a bracts can serve as a new source of antioxidants in food and nutraceutical product development.

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Conflict of Interest

All the authors declare that they have no conflict of interest.

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