MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY OF *Ganoderma boninense*

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History
Received: 2nd August 2020
Accepted: 13th October 2020

Keywords:

Ganoderma boninense; phytochemical; phenolic; flavonoid; antioxidant.

Abstract

Ganoderma lucidum is known as a functional mushroom and is traditionally being used as medicine but *G. boninense* is well known as the causal pathogen of basal stem rot disease of oil palm. Numerous secondary metabolites of mushrooms from this genus have been reported with various biological activities. However, there are not many reports report on the medicinal benefits of *G. boninense.* Hence, this study was designed to investigate the phytochemical constituents and antioxidant activities of the fruiting body *G. boninense* extracts. Various groups of phytochemicals were extracted using solvents with different polarities such as chloroform, ethyl acetate, acetone, methanol, ethanol and distilled water. Results confirmed the presence of numerous phytochemicals in *G. boninense* fruiting body. Ethanolic extract of *G. boninense* has the highest phenolic (33.05 ± 1.374 mg GAE/g DW) and total flavonoid (8.20 ± 0.059 mg QE/g DW) content. Furthermore, ethanolic extract of *G. boninense* also exhibited the greatest potency in antiradical activity with IC_{50} of 113.9 ± 2.78 µg/ml. Correlation coefficient, $R^2 = 0.97$ suggested phenolic acids of ethanol extract was contributed to its radical scavenging activity.

INTRODUCTION

Phytochemical is a group of naturally occurring organic chemicals that can be found in living organisms, especially plants but not limited to others such as fungi. These natural products also known as bioactive compounds, are non-essential nutrient elements for plants but possess biological activities [1]. Enormous varieties of bioactive compounds such as alkaloids, flavonoids, phenolics, tannins, steroids, terpenes are synthesized and accumulated by plants to accommodate changes such as biotic stresses, defensive response, molecules signaling interactions and others [2]. Importantly, the abundance of these compounds is playing a vital role owing to their versatile applications in the pharmacological or toxicological industry [3-4]. Interestingly, large amounts of phytochemical constituents are reported as good antioxidant agents [5-6].

Medicinal herbs contain appreciable amounts of natural antioxidants in the form of secondary metabolites [2]. Derivatives of phenolic acid and flavonoid are recognized as the most abundant groups which are characterized by positive correlation with antioxidant activity [7-11]. However, not all phenolic and flavonoid content is exclusively associated with antioxidants [12-16]. Antioxidant capacity contributed by other groups of secondary metabolite is noteworthy [17-19]. Consumption of natural antioxidants help scavenge free radicals because the endogenous antioxidants in our immune system may not be sufficient [20]. Free radicals are atoms or molecules of reactive oxygen species (ROS) with an unpaired electron. They are unstable, highly reactive and toxic with the ability to oxidize and change molecules nearby to gain an electron. This will lead to the damage, destruction and death of cells [21]. The accumulation of a high level of ROS leads to

oxidative stress conditions that can affect numerous physiological processes and cause pathological diseases [22].

Ganoderma boninense, a species under the basidiomycetes genus of *Ganoderma* and belongs to the family of *Polyporaceae*, is well-recognized as basal stem rot (BSR) causal pathogen that has caused severe losses in oil palm plantation [23-24]. However, *Ganoderma lucidum* has been widely used as traditional medicine and is known to possess various biological activities such as anti-tumor, anti-cancer, anti-viral, anti-microbial, anti-tubercular and many more [25- 26]. Reports revealed that all these activities correlated to the presence of bioactive compounds isolated from *Ganoderma* spp [27-28]. There are some reports on the biological properties of *G. boninense* [29] and its antibacterial activity [30]. Nonetheless, more efforts are needed to further explore the potential of these fungi. Hence, this study aims to discover the effect of various solvents in extracting phytochemical constituents and antioxidant activity of *G. boninense* extracts.

MATERIALS AND METHODS

Materials

Solvents such as chloroform, ethyl acetate, acetone, methanol, and ethanol were purchased from Sigma-Aldrich., USA. Chemical reagents such as sodium hydroxide, ferric chloride, acetic anhydride, sodium carbonate, aluminium chloride, sodium acetate, gallic acid, quercetin, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), Mayer's reagent, Dragendroff's reagent and Benedict's reagent were purchased from Merck Milipore., USA

Methods

Collection of *Ganoderma spp* Fruiting Bodies

Unknown *Ganoderma* spp were collected from infected oil palm trees in Sawit Kinabalu Oil Palm Plantation at Langkon, Sabah Malaysia. The fresh fruiting bodies were cleaned under running tap water to remove surface dirt before freeze-dried. The fruiting bodies, also known as basidiocarp were first identified as *G. boninense* using molecular identification technique before extraction [31].

Preparation of *G. boninense* Fruiting Bodies Extracts

Fruiting bodies identified as *G. boninense* were pooled together and grounded with liquid nitrogen using mortar and pestle. For solvent extraction, approximately 10 g of grounded fine powder was macerated with 400 ml of absolute chloroform, ethyl acetate, acetone, methanol, and ethanol, respectively for five days at room temperature (26±2 °C) and shaken at 150 rpm [32-33]. For water extraction, the solvent was replaced with sterilized distilled water and extraction was conducted in a water bath at 90 °C and shaken continuously [34-35]. All supernatant was filtered through Whatman No. 1 filter paper and concentrated using a vacuum rotary evaporator. Crude extracts were kept in -20 °C until further use. The extraction yield for different solvents was calculated as followed:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract after evaporating solvent and freeze drying}}{\text{Dry weight of the sample}} \times 100\%$$

Phytochemical Analysis

Quantification of phytochemical constituents in the extract of *G. boninense* from various solvents extraction was conducted using the following protocols but with slight modifications. Detailed methods are as follows: [36-38]:

Test for Flavonoids

Shinoda's test: Extracts were dissolved in 5 ml of ethanol. A few pieces of magnesium were added followed by a few drops of concentrated hydrochloric acid. The formation of pink, reddish, brown or occasionally green to blue colour indicates the presence of flavonoids.

Alkaline reagent test: A few drops of sodium hydroxide were added to the extracts. The formation of intense yellow colour which turned into colourless after the addition of a few drops of diluted acetic acid indicates the presence of flavonoids.

Ammonia test: Five ml of dilute ammonia solution were added to the extracts followed by an addition of concentrated H_2SO_4. The formation of a yellow colour and disappears after some time indicates the presence of flavonoids.

Test for Phenolic Compounds

Ferric chloride test: A few drops of neutral 5% ferric chloride were added to the extract. The formation of blue, green colour indicates the presence of phenolic compounds.

Gelatin test: A few drops of 10% gelation solution were added to the extract. The formation of white precipitate indicates the presence of phenolic compounds.

Test for Terpenoids

Salkowski's test: Extracts were added with 3 ml of chloroform. The mixture was allowed to stand for some time followed by a few drops addition of concentrated sulphuric acid. The mixture was shaken well and kept aside for a particular time. The formation of the reddish-brown colour of the interface indicates the presence of terpenoids.

Libermann-Burchard's test: A few drops of acetic anhydride were added to the extract followed by a few drops of concentrated sulphuric acid which were slowly added along the sides of the test tube. The formation of pink or red colour indicates the presence of terpenoids.

Test for Alkaloids

Mayer's test: A few drops of Mayer's reagent were added to the extract. The formation of white or pale yellow precipitate indicates the presence of alkaloids.

Dragendroff's test: A few drops of Dragendroff's reagent were added to the extract. The formation of orange precipitate indicates the presence of alkaloids.

Test for Steroids

Liberman-Burchard's test: Two ml of acetic anhydride was added to the extract followed by a few drops of concentrated sulphuric acid which were slowly added along the sides of the test tube. The formation of green colour indicates the presence of steroids.

Test for Glycosides

Borntrager's test (Anthraquinone glycosides): Extracts were heated in concentrated hydrochloric acid and filtered. One ml of the filtrate was added with two ml of chloroform and shaken. The chloroform layer was separated and a few drops of 10% ammonia were added. The formation of pink to red colour indicates the presence of anthraquinone glycoside.

Keller-Killiani's test (Cardiac glycosides): Two ml of glacial acetic acid was added to the extract containing a few drops of ferric chloride. One ml of concentrated sulphuric acid was slowly added along the sides of the test tube. The formation of a brown ring at the interface or the formation of greenish colour throughout the solution indicates the presence of cardiac glycosides.

Test for Saponins

Froth test: Three ml of distilled water was added to the extract. The mixture was shaken vigorously. The formation of foam indicates the presence of saponins.

Test for Carbohydrates

Benedict's test: Three ml of distilled water was added to the extract. The mixture was shaken vigorously and filtered. The filtrates were added with 5 ml of Benedict's reagent and boiled for 5 minutes. The formation of orange-red precipitate indicates the presence of reducing sugars.

Determination of Total Phenolic Content (TPC)

Total phenolic content was conducted using the Folin-Ciocalteu method according to Barku and colleagues [39] with slight modifications. Briefly, the 10% (w/v) of Folin-Ciocalteu reagent was prepared by diluting with distilled water. Then, each crude extract was dissolved in methanol (5 mg/ml). A total of 5 ml of 10% Folin-Ciocalteu was mixed with 1 ml of *G. boninense* extract. The mixture was shaken for 1 min and incubated for 5 min. A total of 4 ml of 10% (w/v) sodium carbonate solution was added followed by gentle shaking for 1 min and incubated in dark for 2 h at room temperature (26±2 °C). After that, absorbance was measured at 765 nm using methanol as blank. Standard curve of gallic acid with serial dilution (10 µg/ml – 100 µg/ml) were generated to calculate the total phenolic content using the following formula where the result was expressed as mg gallic acid equivalents (GAE) per gram of extract (dry weight):

$$TPC = \frac{c \times V}{m}$$

c is sample concentration from the calibration curve (mg/ml), V is the volume (ml) of the solvent used in extraction and m is the weight (g) of the dried sample used.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was conducted using aluminium chloride colorimetric method as described by Ahmed and others [40] with slight modifications. Firstly, the 10% (w/v) of aluminium chloride solution was prepared and each crude extract was dissolved in methanol (5 mg/ml). A total of 1 ml of 10% aluminium chloride was added with 1 ml of *G. boninense* and 1.5 ml of 1 M sodium acetate. The mixture was mixed and incubated in the dark at room temperature (26±2 °C) for 2.5 hours. After that, the absorbance was measured at 415 nm using methanol as blank. Standard curves of quercetin with serial dilution (10 µg/ml – 100 µg/ml) were generated to calculate the total flavonoid content by using a formula as described by Alara and co-workers [41]. The output was expressed as mg quercetin equivalents (QE) per gram of extract (dry weight).

$$TFC = \frac{c \times V}{m}$$

Where c is the sample concentration from quercetin calibration curve (mg/ml), V is the volume (ml) of solvent used in extraction and m is the weight (g) of the dried sample used.

Determination of Antioxidant Activity against 1,1-diphenyl-2-picryl hydrazyl radical (DPPH)

The radical scavenging activity of the *G. boninense* extracts was determined using DPPH assay conducted in 96-well plate according to Chandra and colleagues [42] with some modifications. Different concentrations of extracts (20 µg/ml – 200 µg/ml) were prepared. One hundred µl of extract solution was mixed with 100 µl of 0.004% (w/v) of DPPH solution. The mixture was incubated in the dark for 30 min at room temperature (26±2 °C). The absorbance was measured at 517 nm. The 0.004% (w/v) of DPPH solution was used as a negative control. The percentage of DPPH scavenging ability was calculated as followed:

$$\% \text{ Inhibition} = \frac{A_0 - A}{A_0} \times 100$$

where A is the absorbance containing extract and A_0 is the absorbance of negative control. Ascorbic acid (20 µg/ml – 200 µg/ml) was used as positive control. The concentration of sample required for resulting 50% inhibition of DPPH (IC_{50}) was calculated by plotting the percentage inhibition against the sample concentration.

Experimental Design and Statistical Analysis

All the experiments were performed in triplicate. The obtained data were shown in mean values with standard deviation (± SD) and statistical multiple comparisons were conducted by using the analysis of variance (AVOVA) Tukey's test with $p < 0.05$ as significant different using Statistical Package for Social Science (SPSS) program version 26. IC_{50} values of the antioxidant inhibition were calculated by non-linear regression using GraphPad Prism 8.

RESULTS AND DISCUSSION

Extraction Yield of *G. boninense* using Different Solvents

Bioactive compounds in natural resources are usually present in low concentrations. Hence, the extraction technique is notably crucial for the separation of the biologically active metabolites to obtain high recovery and a variety of extracts with minimal alteration of the metabolite's properties. To achieve this, several parameters have to be taken into consideration before separating the phytochemical constituents. This includes matrix properties, nature of constituents, method and technology, cost, efficiency and others [43]. In this study, the maceration technique was adopted by immersing the powders of *G. boninense* fruiting body in different absolute solvents for a period of time with agitation. The process is designed to soften and break the sample's cellulose-based cell wall and consequently releasing soluble metabolites based on the law of similarity and inter-miscibility [44]. Therefore, diverse compounds could be extracted by using solvents with different polarities.

Different solvents in varying polarity were selected in this study. Water is the most polar followed by ethanol, methanol, acetone, ethyl acetate and chloroform being the least polar. Results showed that different solvent polarity affected the extraction yield as presented in Figure 1. Water extraction resulted the highest yield (7.76% ± 0.27 w/w) followed by ethanol (5.08% ± 0.23 w/w), methanol (4.75% ± 0.23 w/w), ethyl acetate (3.63% ± 0.10 w/w), chloroform (2.99% ± 0.16 w/w) and acetone (2.16% ± 0.11^e w/w). The higher extraction yield was observed in polar solvents (water, ethanol, methanol) compared to lower polarity solvents such as acetone, ethyl acetate and chloroform indicated that the solubility of compounds is in favour of highly polar solvents. The current finding is similar to the work of Ismail and colleagues [29], in which the authors have also reported polar solvent such as methanol is having a better extraction yield in comparison to non-polar solvents. Moreover, water has the highest extraction yield probably due to elevated temperature in the extraction process. Theoretically, high temperature will destroy the integrity of cell membrane structure by softening or weakening the interaction between cell membranes [45]. As a result, it increases the solubility and diffusion of solutes from the sample matrix.

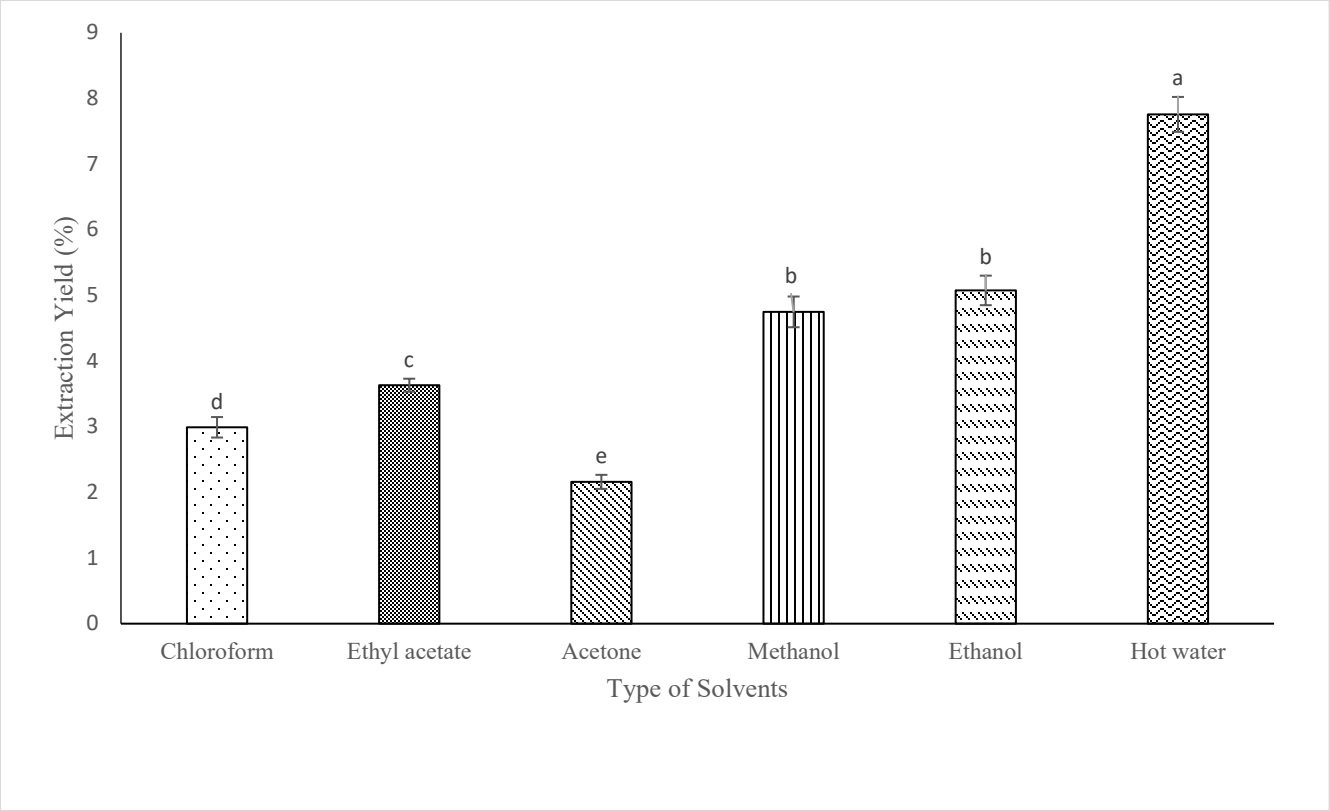

Figure 1. Extraction yield of crude extracts from fruiting body of *G. boninense* in different solvents. Results expressed as mean ± SD, $n = 3$ and different lowercase alphabets represent statistically significant difference at $p < 0.05$ using One Way ANOVA Tukey's test.

Qualitative Phytochemical Analysis of *G. boninense* Crude Extracts Using Different Solvents

The result of the qualitative analysis of phytochemical constituents that present in the fruiting body of *G. boninense* is shown in Table 1. The phytochemical screening tests revealed that the palm tree decaying *Ganoderma* contains numerous groups of phytochemicals based on the extraction solvents used in separating the compounds. These constituents included flavonoids, phenolics, alkaloids, glycosides, terpenoids, sterols, carbohydrates and saponin. The extractability of different categories of compounds can be observed based on the polarity of the solvent used. Less polar solvents (chloroform, ethyl acetate) tends to extract compounds like terpenoids, steroids while higher polar solvents (acetone, methanol, ethanol) tends to extract compounds like flavonoids, phenolics, and carbohydrates. This is simply based on the "like dissolve like" principle. However, alkaloids were found in all types of solvents. There are two types of alkaloids: freebase alkaloid which is soluble in nonpolar solvents and a water-soluble salt of organic acid which is soluble in polar solvents [46]. Moreover, cardiac glycosides are sparingly soluble in water and freely soluble in organic solvent due to their structure comprising steroids on aglycone [47].

The finding was similar to studies conducted on other *Ganoderma* species such as *G. lucidum* [48]; *G. philippii* [49]; and wild *Ganoderma* spp [50]. *Ganoderma* mushrooms were well-recognized as a traditional medicinal herb since ancient times for body resistance enhancement and promotion of longevity. Besides, modern studies have proven their biological activities in anti-cancer, anti-tumor, anti-inflammatory, anti-viral, antioxidant, immunomodulating and others due to the presence of a high variety of bioactive compounds like carbohydrates, triterpenes, proteins, sterols and many more [27,51].

Table 1. Qualitative analysis of different solvent extracts from fruiting body of *G. boninense*

Phytochemical Constituents and Chemical Tests	Solvent Extracts					
	Chloroform	Ethyl acetate	Acetone	Methanol	Ethanol	Hot water
Flavonoids						
Shinoda's test	+	+++	++	++	++	-
Alkaline reagent test	-	++	+	+	++	++
Ammonia test	-	+++	++	+	+	+
Phenolic Compounds						
Ferric chloride test	-	+	+	++	+++	+
Gelatin test	-	-	+	+	+	-
Terpenoids						
Salkowski's test	++	+++	++	-	-	-
Liberman-Burchard's test	+++	++	-	-	-	-
Alkaloids						
Mayer's test	++	++	+	+	-	-
Dragendroff's test	+	+	+	+	++	++
Steroids						
Liberman-Burchard's test	++	+++	+++	-	-	-
Glycosides						
Borntrager's tes	-	-	-	-	-	-
Keller-Killiani's test	+	+++	+++	++	++	+
Saponins						
Froth test	+++	-	-	++	-	+
Carbohydrates						
Benedict's test	-	+	+	++	++	+++

(+++ indicates strongly present; ++ indicates moderate present; + indicates slightly present; - indicates absent)

Total Phenolic Content of *G. boninense* Crude Extracts

Phenolic compounds are secondary metabolites with one aromatic ring being structurally attached with monosaccharide to form a simple phenolic acid molecule or more aromatic rings polymerized with hydroxy groups to form polyphenol [52]. Hence, they have been classified into several groups including flavonoids, tannins, coumarins, phenolic acids and play numerous roles in plant growth [53]. In this study, the Folin-Ciocalteu assay was adopted because of the ability of phosphomolybdic acid and phosphotungstic acid to react with phenol group forming molybdenum blue and tungsten blue by reduction process under alkaline condition [54].

Figure 2 showed the means total phenolic content (TPC) crude extracts of *G. boninense* using different solvents. Ethanol extract obtained the highest TPC value (33.05 ± 1.37 mg GAE/g DW) followed by methanol (24.21 ± 0.44 mg GAE/g DW), ethyl acetate (21.23 ± 2.18 mg GAE/g DW), hot water (18.93 ± 0.71 mg GAE/g DW), acetone (16.80 ± 0.98 mg GAE/g DW) and the least was chloroform extract (12.39 ± 1.11 GAE/g DW). Among all the extracts, ethanol and chloroform extracts have shown statistical significance compared to other extracts ($p < 0.05$). On the other hand, ANOVA analysis showed no significant difference between methanol and ethyl acetate extract; ethyl acetate and hot water extract; hot water and acetone extract ($p > 0.05$). All the TPC values were obtained from the linear gallic acid calibration curve $y = 0.01x + 0.0134$, $R^2 = 0.9975$ and expressed as mg gallic acid equivalents (GAE)/g DW.

Results suggest that extraction of phenolic compounds favour in high polarity solvents as most of the phenolic compounds are polar soluble substances due to the presence of hydroxyl group. However, hot water extraction tends to extract more non-phenol compounds such as saccharide polymers or depleted by heat. Hence, the value of TPC is lower in water extract compared to other polar solvents like ethanol and methanol. According to Do and co-workers [55], ethanol is known to perform good extractability of polyphenols while methanol is more suitable for extraction of lower molecular weight polyphenols. Abundant of phenolic compounds such as p-hydroxybenzoic acid, protocatechuic, syringic acid, p-coumaric and cinnamic acids were identified in ethanol extract of *G. lucidum* [56]. Likewise, phenolic compounds such as apigenin, benzoic acid, catechin, epicatechin, chlorogenic, coffeic acid and rosmarinic acids also reported in other methanol and water extract of *Ganoderma* spp [57].

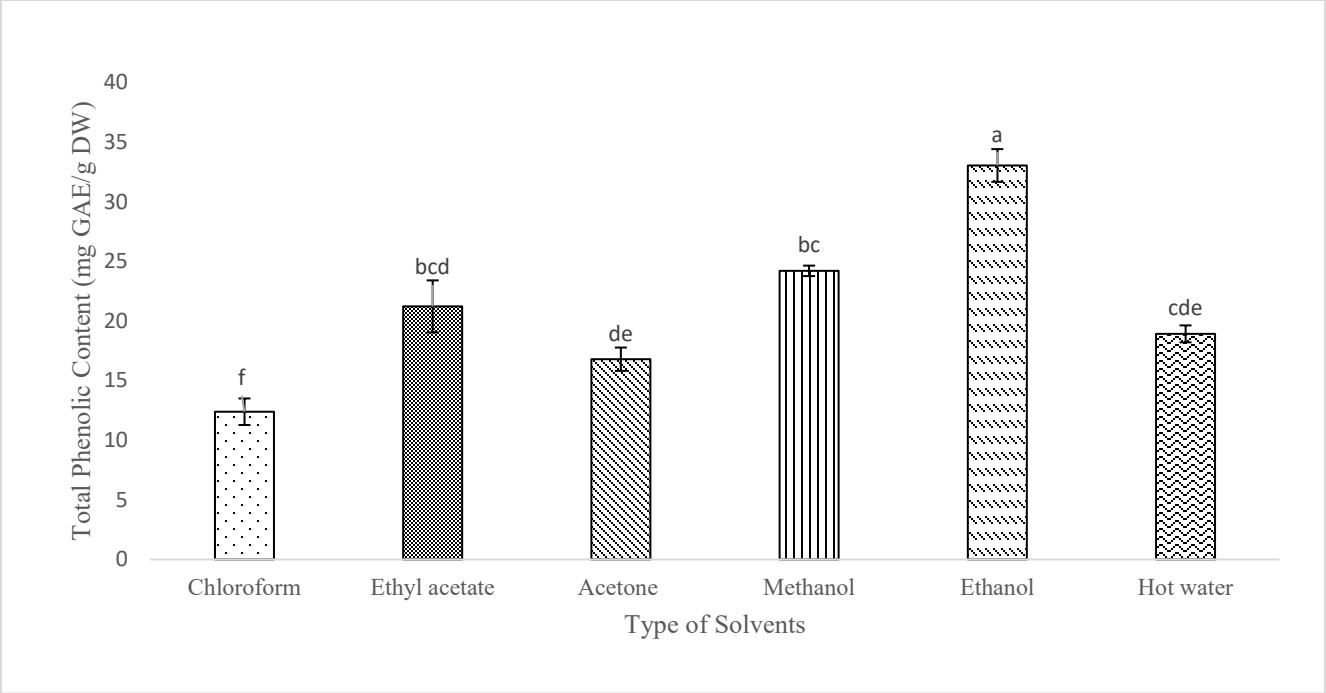

Figure 2. Total phenolic content of crude extracts from fruiting body of *G. boninense* in different solvents. Results expressed as mean ± SD, $n = 3$ and different lowercase alphabets represent statistically significant difference at $p < 0.05$ using One Way ANOVA Tukey's test.

Total Flavonoid Content of *G. boninense* Crude Extracts

Flavonoid is the subclass of phenolic compounds and this class of secondary metabolites presents abundantly in nature. Flavonoids have a C_6-C_3-C_6 skeleton where the structure is composed of two benzene rings bonded with a heterocyclic pyrin ring [58]. The variation and pattern of substitution on the heterocyclic further classified flavonoids into various groups such as flavonols, flavanones, flavanols, isoflavones, anthocyanidines, and others [59]. In this study, the total flavonoid content of *G. boninense* extracted by various solvents was determined using aluminium chloride colorimetric method because of its capability to react with C_4-keto group and C_3 or C_5 hydroxyl group of flavones and flavonols to form acid-stable complexes. Furthermore, aluminium chloride also can react with orth-dihydroxyl groups in both C_6 rings of flavonoids to form acid-labile complexes [60]. The formation of the red coloured acidic complexes was read at 415 nm using a spectrophotometer.

Figure 3 showed the means total flavonoid content (TFC) of crude extracts of *G. boninense* using different solvents. Ethyl acetate extract obtained the highest TFC value (8.20 ± 0.06 mg QE/g DW) followed by ethanol (7.86 ± 0.20 mg QE/g DW), acetone (7.06 ± 0.24 mg DE/g DW), hot water (6.61 ± 0.20 mg QE/g DW), methanol (6.24 ± 0.22 QE/g mg DW), and the least was chloroform (5.66 ± 0.06 mg QE/g DW). ANOVA analysis showed that ethyl acetate and ethanol statistically have no significant difference ($p > 0.05$) although ethyl acetate extract revealed the highest mean of TFC value. Meanwhile, the TFC value of hot water extract also shown no significant difference with acetone and methanol extracts. Lastly, chloroform extract showed a significant difference ($p < 0.05$) compared to other solvent extracts. All the TFC values were obtained from the linear gallic acid calibration curve $y = 0.0206x - 0.2749$, $R^2 = 0.9977$ and expressed as mg quercetin equivalents (QE)/g DW.

Based on the result, the finding implied that the occurrence of the flavonoids in *G. boninense* are mostly less polar or semi-polar. According to Awouafack and colleagues [61], flavonoid glycosides are soluble in more polar solvents whereas semi-polar solvents such as ethyl acetate, dichloromethane and diethyl ether are suitable to extract flavonoid groups which include isoflavones, flavanones, methylated flavones and flavonols. Non-polar solvents like hexane and chloroform extracted mostly aglycone group. A previous study reported polar extract of *G. lucidum* and other medicinal mushrooms contain flavonoids such as catechin, naringin, myricetin, quercetin, biochanin A, formononetin, hesperetin, kaempferol and others [62]. Nonetheless, the presence of flavonoids in mushrooms was questioned by Gil-Ramírez and co-workers [63] due to the absence of enzymes for flavonoid biosynthesis but the authors suggested that mushrooms obtain flavonoids from their host, soils or neighbouring plants. This result is substantiated by the study of TFC *Dacryodes rostrata* pulp in which ethyl acetate extract had the highest TPC value and has shown no significant difference with ethanol extract [64].

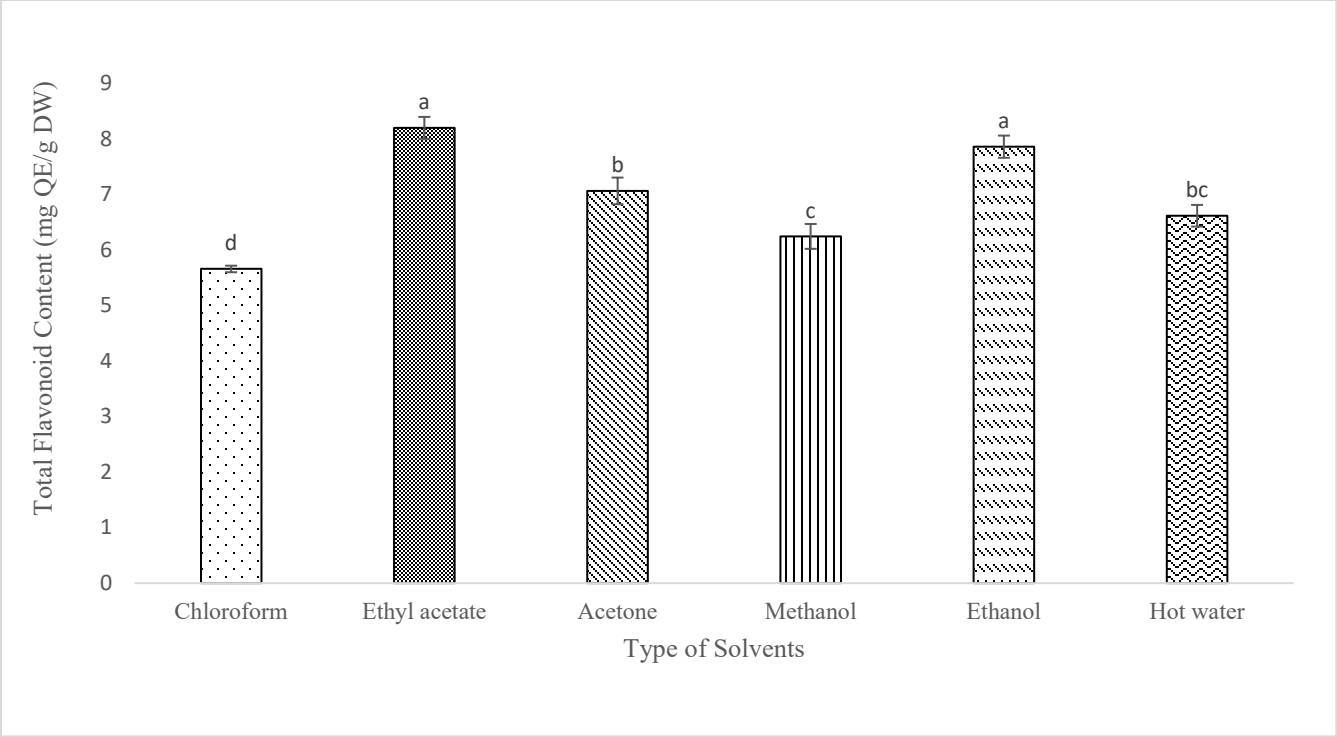

Figure 3. Total flavonoid content of crude extract from the fruiting body of *G. boninense* in different solvents. Results expressed as mean ± SD, $n = 3$ and different lowercase alphabets represent statistically significant difference at $p < 0.05$ using One Way ANOVA Tukey test.

DPPH Radical Scavenging Activity

DPPH (2,2'-diphenyl-1-picryhydrazyl) is a stable free radical that can be reduced by donating a hydrogen atom from other compounds [65]. Hence, by donating more hydrogen atoms, the intensity of the violet colour is reduced. The degree of discoloration captures by absorbance indicates the scavenging potential of compounds. Meanwhile, the half-maximal inhibitory concentration (IC_{50}) is the concentration of a particular drug to achieve inhibition of a biological process in half.

The antioxidant activity of different *G. boninense* extracts was indexed by the potency of scavenging DPPH radicals. As illustrated in Figure 4, all the extracts showed concentration-dependent increases in anti-radical potency. However, different extracts possessed varying degrees of scavenging activity. Among all the extracts, ethanol extract exhibited the greatest potency in scavenging DPPH radical with the statistically significant ($p < 0.05$) and lowest IC_{50} value of 113.9 ± 2.78 µg/ml, followed by methanol extract 241.0 ± 10.8 µg/ml, ethyl acetate extract (289.6 ± 20.37 µg/ml), acetone extract (480.8 ± 38.43 µg/ml). The non-linear regression analysis of hot water and chloroform extract was interrupted due to the lack of data information for the calculation of the best-fit curve. Hence, the values of IC_{50} for these solvent extracts were unable to be obtained. The obtained IC_{50} values were tabulated in Table 2.

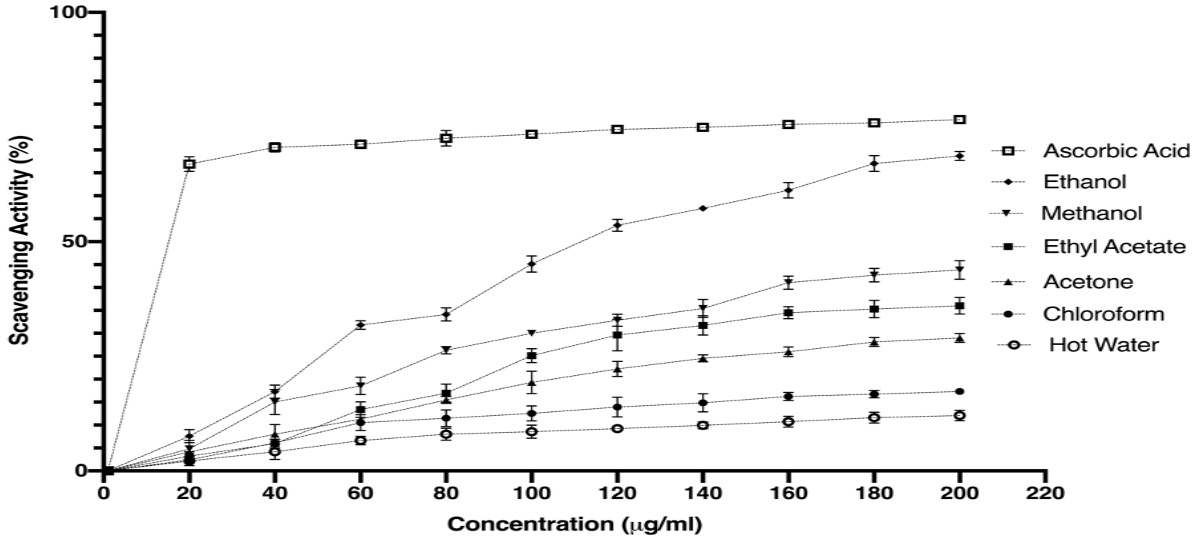

Figure 4. DPPH scavenging activity of different *G. boninense* extracts at different concentrations showed a concentration-dependent relationship. Results expressed as mean ± SD, $n = 3$.

Table 2. Non-linear regression analysis was performed to obtain the half-maximal inhibitory concentration (IC_{50}) values of DPPH scavenging activity of different *G. boninense* extracts.

Extracts	IC_{50} value (μg/ml)
Chloroform	NA
Ethyl acetate	289.6 ± 20.37^{b}
Acetone	480.8 ± 38.43^{a}
Methanol	241.0 ± 10.8^{b}
Ethanol	113.9 ± 2.78^{c}
Hot water	NA
Ascorbic acid (control)	16.48 ± 0.91^{d}

Values are expressed as mean ± SD, $n = 3$ and different lowercase alphabets represent statistically significant differences at $p < 0.05$ using One Way ANOVA Tukey's test.
NA = Not able to calculate due to the best-fit curve was not obtained.

Correlation relationship between Antioxidant Activity, and Total Phenolic, and Total Flavonoid Content

Bivariate Pearson Correlation analysis was performed to investigate the relationship between antioxidant with DPPH activity (expressed as reciprocal of the IC_{50} values) and total phenolic and flavonoid content. As illustrated in Figure 5, the phenolic content exhibited a high positive association with scavenging activity ($R^2 = 0.97$) at a 95% confidence level. However, a weak correlation was observed between flavonoid content and DPPH scavenging activity ($R^2 = 0.11$). It is possible to suggest that phenolic compounds of *G. boninense* are highly responsible for radical scavenging activity. This can be explained by the reduction capability of phenolic compounds as a hydrogen donor to form stable intermediate with free radicals [66]. No significant correlation of flavonoid with DPPH activity was also reported by Olajire and Azeez [67] in their study of Nigerian vegetables and Nickavar and colleagues [68] in their study of *Salvia* species. The antioxidant activity of a polyphenol is greatly influenced by the chemical structure. This was reported by Rice-Evans and co-workers [69] where the 3,4,5-trihydroxybenzoic acid which has three available hydroxyl groups exhibited higher antioxidant activity than 3,4-dihydroxycinnamic acid which has only two available hydroxyl groups. Moreover, the capacity of radical scavenging of flavonoids was reported due to their configuration of di-hydroxyl groups in ring B and C, carbonyl groups in ring C, and the presence of C2-C3 double bond with 4-keto configuration [70].

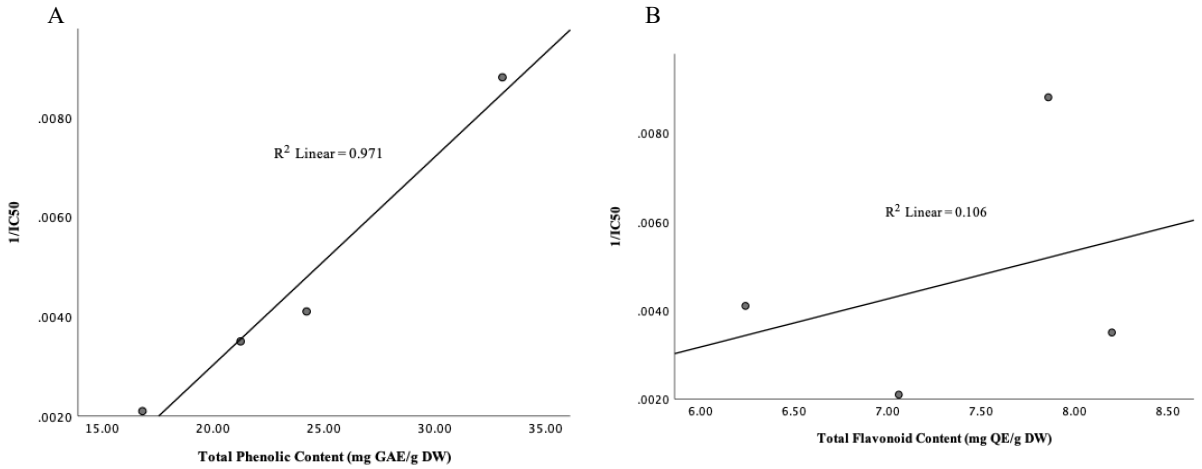

Figure 5. Correlation between the DPPH antioxidant activity of *G. boninense* extracts versus total phenolic content with equation $y = -5.31E^{-3} + 4.17E^{-4} * x$ (A.) and flavonoid content with equation $y = 3.32E^{-3} + 1.08E^{-3} * x$ (B.). The correlation coefficient value for total phenolic ($R^2 = 0.97$) and total flavonoid content ($R^2 = 0.11$) was observed at 95% confidence level.

CONCLUSION

The effect of several common solvents on extraction efficiency, phytochemical content and antioxidant activity of *G. boninense* fruiting body from Sabah, Malaysia is reported where this study suggests ethanol as the solvent of choice for extracting the highest phenolic and flavonoid content with the greatest potency in DPPH radical scavenging activity although hot water yielding the highest extractable solids. Correlation relationship revealed that antioxidant activity is contributed by phenolic acid compounds but not flavonoid. Therefore, future studies on the antioxidant potency of *G. boninense* shall focus on phenolic acids. The results of the current work indicate that, *G. boninense* a pathogen of oil palm, could be a useful resource in the future. However, more investigation is needed to further explore this potential.

ACKNOWLEDGMENT

This research was financially supported by Universiti Malaysia Sabah. The authors would like to thank Sawit Kinabalu Sdn Bhd for the assistance during permitted field sampling.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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