e-ISSN 2716-697X

# Total Phenolic, Flavonoid Content, Antioxidant Activity and Phytochemical Screening of Donax grandis (Miq.)Ridl.

Senty Vun-Sang, Caysandra Audrey Michelle Elwon and Mohammad Iqbal\*

Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia \*Corresponding author's email: miqbal@ums.edu.my https://doi.org/10.51200/bijb.v3i.4060

Received: 15 July 2023 | Accepted: 26 September 2023 | Published: 1 December 2023

#### **ABSTRACT**

Numerous studies have provided evidence supporting the significant role of oxidative stress in the development and progression of various diseases. The significance of the capacity of antioxidant status to recuperate from specific diseases has been demonstrated to be crucial for enhancing human health. Donax grandis, a plant species belonging to the Marantaceae family, is used by the indigenous population of Sabah to treat a range of ailments. This study aimed to chemically analyse the aqueous extract of D. grandis for the presence of bioactive compounds. The analysis revealed the detection of tannins, saponins, and flavonoids within the extract. Quantification of phenolics and flavonoids was conducted to determine their overall content. In-vitro antioxidant activity was investigated using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, employing a UV-VIS spectrophotometer. The phenolic compound concentration was  $34.34 \pm 1.37$  mg/g gallic acid equivalent, while the flavonoid content was 3.93  $\pm$  0.12 mg/g catechin equivalent. The IC<sub>50</sub> value of *D. grandis* in the DPPH assay was determined to be  $303.68 \pm 0.4 \,\mu g/ml$ . The findings have revealed significant phytochemical properties found in D. grandis, which are anticipated to have therapeutic potential in managing diseases associated with oxidative stress.

**Keywords:** *Donax grandis*, aqueous extraction, phenolics, flavonoids, radical scavenging activity

#### INTRODUCTION

Malaysia is fortunate to possess a distinctive array of biological diversity and a significant abundance of medicinal plants. However, it should be noted that a relatively small number of approximately 300 species, which are indigenous to this nation, have been utilised for consumption, subjected to clinical investigations, and recorded in documented sources (Abd Aziz et al., 2017). According to the World Health Organisation (WHO), approximately 88% of the population in developing countries relies on traditional medicine as their primary source of healthcare (WHO, 2020). The phenomenon in question has garnered significant interest from numerous researchers who are now directing their attention towards the scientific assessment of indigenous medicinal flora.

Antioxidants are chemical compounds that impede the process of oxidation in other molecules by inhibiting the chain reactions initiated by free radicals, which can lead to cellular damage and death (Chaudhary et al., 2023). In recent times, there has been a significant surge in the examination and application of plant antioxidants for both scientific investigation and various industrial applications, such as in the fields of dietetics, pharmaceuticals, and cosmetics (Vun-Sang et al., 2017; Hoang et al., 2021). The scrutiny of synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), and propyl gallate (PG), has primarily been driven by concerns regarding their potential toxic and carcinogenic effects (Azlim Almey et al., 2010). Hence, assessing the antioxidant activity of plant extracts and their capacity to neutralise free radicals is regarded as a significant undertaking. These natural sources are deemed preferable to synthetic alternatives due to their perceived health benefits and enhanced safety, making them more appealing to contemporary consumers.

Donax grandis, alternatively referred to as Donax canniformis or Clinogyne grandis, is classified within the Marantaceae family (Daud et al., 2011; Silalahi & Wakhidah, 2021). D. grandis is commonly referred to as 'Lias' by the indigenous population in Sabah, and 'Bemban' by the Malay population in Peninsular, Malaysia (Ridley & Curtis, 1902; Silalahi & Wakhidah, 2021). D. grandis is distributed across various countries in the Southeast Asia region, including Thailand, Malaysia, Brunei, Singapore, Papua New Guinea, the Philippines, and Polynesia. D. grandis is commonly observed in moist habitats such as secondary forests and areas densely populated with bamboo (Ibrahim & Mohd Zain, 2017; Silalahi & Wakhidah, 2021).

D. grandis possesses numerous medicinal properties, and the utilisation of D. grandis rhizomes is effective in the treatment of shingles. Furthermore, the infusion of leaves and roots from *D. grandis* has the ability to lower body temperature, thereby providing a potential treatment for fever when administered through bathing. In addition, it has been reported that the juice derived from the stems of D. grandis exhibits efficacy in the treatment of snake bites (Daud et al., 2011; Silalahi & Wakhidah, 2021). According to a study conducted by Hanum and Hamzah (1999), it was found that the poultice derived from the stem and leaves of D. grandis possesses properties that make it suitable for use as eye refreshment. The utilisation of D. grandis by the indigenous

population of Sabah, Malaysia, for the treatment of ocular infections through the application of the aqueous content derived from the youngest shoot of D. grandis, has been a longstanding practice (Wani et al., 2020). Yeoh et al. (1986) conducted a study on the leaves of D. grandis, which demonstrated the presence of elevated concentrations of amino acids. Additionally, Daud et al. (2011) conducted a separate investigation, revealing the presence of various chemical compounds in D. grandis, including phenolic compounds, alkaloids, tannins, phytosterols, cardiac glycosides, terpenoids, steroids, saponins, and flavonoids (Yeoh et al., 1986; Daud et al., 2011).

The purpose of this investigation was to evaluate the existence of bioactive compounds and the antioxidant potential of the aqueous extract derived from *D. grandis*. Many studies have been conducted on the medicinal potential of solvent extraction of D. grandis; however, the study on aqueous extraction was limited. Therefore, in this study, aqueous extracts of leaves of D. grandis from Sabah, Malaysia, were qualitatively screened for phytochemicals, their antioxidant activity, and the determination of their phenolic and flavonoid content using standard assays. The findings of this study have the potential to contribute valuable insights into the medicinal properties of the plant, thereby warranting further investigation in future studies.

# **MATERIALS AND METHODS**

#### Chemicals

Folin-Ciocalteu reagent (FCR), sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), sodium chloride (NaCl), sodium nitrate (NaNO<sub>3</sub>), aluminium chloride (AlCl<sub>3</sub>), sodium hydroxide (NaOH), 2, 2-diphenyl-2-picrylhadrazyl (DPPH), gallic acid, catechol, and ferric chloride (FeCl3) were purchased from Sigma Aldrich (St. Louis, MO, USA). Chemicals of analytical or gas chromatography (GC) quality were purchased from Fisher Scientific (Hampton, New Hampshire, USA) and J.T. Baker® (Phillipsburg, New Jersey, USA), respectively.

# **Preparation of Plant Extract**

The leaves of D. grandis (Figure 1) were collected from Kota Marudu (6°29'23"N 116°44'10"E), Sabah, and identified by a botanist from the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The young leaves of D. grandis were cleaned under running tap water to eliminate dirt and extraneous contaminants. D. grandis leaves were dried in the shade for 3 to 4 weeks. Shade drying is frequently used when the purpose is to preserve the natural components contained in the item being dried. Sun drying and oven drying can expose the material to greater temperatures and more direct sunlight, which can lead to the breakdown of sensitive substances such as phytochemicals, antioxidants, and essential oils (Jayasuriya et al., 2023; Benjamin et al., 2022). After the plant samples had dried thoroughly in the shade, they were crushed into a coarse powder with a heavy-duty blender and

stored in airtight containers. The plant sample was then extracted using the method described by Thanebal et al. (2021) with slight modification. One hundred grams of plant sample were placed in 1,000 mL of boiling distilled water (100°C) using a ratio of 1:10 (weight of plant sample: volume of distilled water). The 100 g plant sample was boiled for 10 minutes in boiling distilled water before being cooled at room temperature for around 1 hour. The cooled extracts were then filtered into a beaker using a funnel to remove the coarse extracts. To get a clear solution, the extracted solution was filtered again using filter paper (Whatman Filter No. 1, England). The filtered extract solution was then put into 50 mL falcon tubes (25 mL in each falcon tube) and stored in a -80°C freezer for 24 hours before lyophilisation. The extract solution was freeze-dried for 3 to 4 days until no moisture remained. Finally, the freeze-dried extracts were put in a  $-20^{\circ}$ C freezer before further analysis.



Figure 1 Donax grandis (Elwon, 2015)

## **Phytochemical Screening (Qualitative Analysis)**

Phytochemical tests are conducted on the aqueous extract of each plant sample and also on the powdered form of the plant samples using standard methods as described by Krishnaiah et al. (2009) with slight modifications. The extracts obtained were submitted to six qualitative phytochemical screenings for tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides, as described by Krishnaiah et al. (2009). The stock solution of the aqueous extract (1 mg/mL) of D. grandis was prepared and used for phytochemicals screening.

#### **Test for Tannins**

Approximately 0.5 g of powdered plant samples were boiled and filtered in 20 mL of distilled water. Then, a few drops of 0.1% iron chloride (FeCl<sub>3</sub>) were added to the extract and observed for brownish green or dark blue colouration, indicating tannins' presence.

#### Test for Phlobatannins

About 10 ml of aqueous extract was boiled with 1% hydrochloric acid (HCl) in a conical flask. A deposition of red precipitates indicates the presence of phlobatannins.

#### **Test for Saponins**

Approximately 2 g of powdered extract was boiled with 20 ml of distilled water and filtered through filter paper (Whatman Filter No. 1, England). After filtration, 10 mL of the solutions were added with 5 mL of distilled water and violently shaken until stable foam was formed. Three drops of olive oil were added to the foam, and emulsion formation indicated the presence of saponins.

#### Test for Flavonoids

In test tubes, 5 mg of plant extracts were mixed with 5 mL of distilled water (1:1) to make aqueous extracts. Then, a few drops of a 1% ammonia (NH<sub>3</sub>) solution were added to the tubes containing the plant extracts, and the emergence of yellow colouration detected the presence of flavonoids.

#### **Test for Terpenoids**

In test tubes, 5 mg of plant extracts were mixed with 5 ml of distilled water to the ratio of 1:1, and then mixed with 2 mL of chloroform (ChCl<sub>3</sub>). Following that, 3 mL of concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added to the mixture to generate a layer in which an interface with a reddish-brown colouration indicates the presence of terpenoids.

## **Test for Cardiac Glycosides**

In test tubes, 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was prepared. Then, 5 mg plant extracts were mixed with 5 mL of distilled water (1:1) and then mixed with 2 mL of glacial acetic acid (CH<sub>3</sub>COOH) containing 1 drop of FeCl<sub>3</sub>. The combination above is carefully added to 1 mL of concentrated H<sub>2</sub>SO<sub>4</sub>, with the concentrated H<sub>2</sub>SO<sub>4</sub> beneath the mixture. A brown ring will develop in the sample if cardiac glycoside is present, indicating the presence of the cardiac glycoside ingredient.

#### **Determination of Total Phenolic Content**

The total phenols in the aqueous extract of *D. grandis* were estimated by Folin-Ciocalteu's phenol reagent according to Shingleton et al. (1999) method with slight modifications. A blank was made by combining 1.5 mL of 10% (v/v) Folin-Ciocalteu's phenol reagent, 1.5 mL of sodium carbonate, and 0.2 mL of distilled water. A stock solution of plant extracts and gallic acid at a concentration of 1 mg/mL was prepared. As standards, 0.01, 0.02, 0.04, 0.08, 0.1, and 0.2 mg/mL gallic acid concentrations were generated. In test tubes, 0.2 mL of plant extracts were mixed with 1.5 mL of 10% (v/v) Folin-Ciocalteu's phenol reagent and left in the dark for 5 minutes at room temperature. The solutions in the test tubes were added with 1.5 mL of 60 g/L sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution. The samples were then left in the dark at room temperature for 90 minutes before the total phenolic content was determined using a spectrophotometer (Genesys 20, Thermo Scientific, model 4001/4) with a 725 nm wavelength and expressed in milligrams of gallic acid equivalents per gram of plant extracts.

#### **Determination of Total Flavonoid Content**

The total flavonoid content in the *D. grandis* extracts was determined using an aluminium chloride (AICI<sub>3</sub>) colourimetric test (Atanassova et al. 2011) with slight modifications. A standard catechin stock solution was prepared at a concentration of 1 mg/mL. From the stock solution, eight different concentrations of catechin (0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, and 0.2 mg/mL) were made in triplicate. The reaction mixtures were made by combining 0.25 mL of the sample solution, 1.25 mL of distilled water, and 0.75 mL of a 5% sodium nitrite (NaNO<sub>2</sub>) solution and incubating them in the dark for 5 minutes. The mixtures were then treated with 0.15 mL of 10% AICl₃ solution and kept in the dark for another 5 minutes at room temperature before being treated with 0.5 ml of 1 molar NaOH solution and vortexed immediately. The volume was then increased to 3.0 mL by adding 0.1 mL of distilled water. The absorbance of the mixes was then measured with a spectrophotometer at 510 nm. The total flavonoid content of the plant extracts was reported in milligrams of catechin equivalents per gram.

## **Determination of Free Radical Scavenging Activity**

The capacity of plant extracts to scavenge DPPH free radicals was assessed using the Hatano et al. (1988) technique. Stock solutions of plant extracts and ascorbic acid were produced to reach 5 mg/ml stock solutions. Then, eight different concentrations of the stock solution were prepared in triplicate: 10, 25, 75, 150, 300, 600, 1,200, and 2,400 µg/mL. Then, the volumes were made up to 0.3 mL by adding distilled water followed by 2.7 mL of DPPH solution and vigorously agitating with a vortex machine. After 60 minutes in the dark, the absorbance was measured with a spectrophotometer at 517 nm. The formula for calculating free radical scavenging activity is presented below.

% Inhibition = 
$$\frac{A \ of \ control - A \ of \ sample}{A \ of \ control} \times 100$$

The IC<sub>50</sub> value of the plant samples was determined by plotting the graph of radical scavenging activity versus sample concentration, and the percentage of inhibition was computed using the equation provided.

# **Statistical Analysis**

All experimental measurements were carried out in triplicate and are expressed as the average of three analyses  $\pm$  standard deviation.

# **RESULTS**

# Qualitative analysis of phytochemical screening

Several phytochemicals in D. grandis aqueous extract were confirmed by phytochemical screening employing qualitative analysis. D. grandis includes bioactive components such as tannins, saponins and flavonoids. Phlobatannins, terpenoids and cardiac glycosides, on the other hand, were not found, suggesting that the phytochemical elements were absent in the plant extract. Table 1 contains information on the phytochemical screening findings.

Phytochemical	Observation	Inferences
Tannins	Brownish green colouration	Present
Phlobatannins	No red precipitates	Absent
Saponins	Formation of stable emulsion foam	Present
Flavonoids	Yellow colouration	Present
Terpenoids	No reddish-brown colouration	Absent
Cardiac glycosides	No brown ring formation	Absent

**Table 1** Phytochemical constituent of *D. arandis* 

#### **Total Phenolic Content**

The total phenols of *D. grandis* were expressed as milligram gallic acid equivalents (GAE) per gram dry weight of the extract. The total phenolic concentration in the aqueous extracts of D. grandis was determined using a series of gallic acid concentrations to which the absorbance values were plotted to obtain a linear calibration curve (y = 0.0106x + 0.0223) with a coefficient ( $R^2$ ) of 0.9988 (Figure 2). The total phenolic content in *D. grandis* is  $34.34 \pm 1.37$  mg/g. The result is summarised in Table 2.

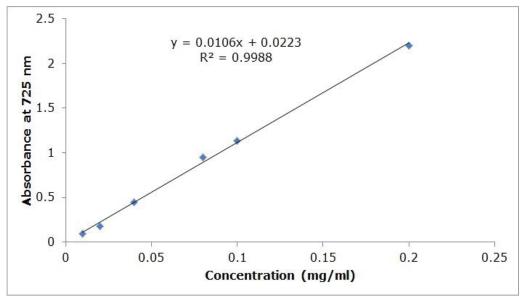


Figure 2 Linear curve of gallic acid concentration vs. absorbance for determination of total phenolic content.

#### **Total Flavonoid Content**

The content of flavonoids was expressed in terms of catechin equivalent, mg/g of extract. The flavonoid content was estimated from a linear calibration curve for a range of catechin concentrations (y = 0.0087x + 0.0685) with a coefficient ( $R^2$ ) of 0.9684 (Figure 3). The concentration of flavonoids in *D. grandis* is  $3.93 \pm 0.12$  mg/g. The results obtained are summarised in Table 2.

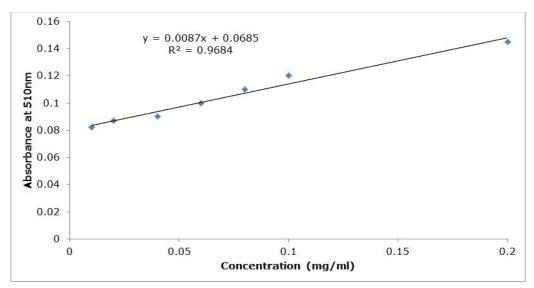


Figure 3 Linear curve of catechin concentration vs. absorbance for determination of total flavonoid content.

Table 2 Total phenolic content and total flavonoid content of aqueous extract of D. grandis

Plant Extract	Total phenolic content (mg GAE/g dried weight)	Total flavonoids content (mg of CE/g of extract)
Donax grandis	34.34 ± 1.37	$3.93 \pm 0.12$

Data are presented as mean  $\pm$  SD of three replicates.

# Radical Scavenging Effect of Donax grandis

Ascorbic acid was used as a reference standard to compare the antioxidant potential of *D. grandis*.  $IC_{50}$  is the sample concentration required to reduce the free radicals by 50%. The lower the IC<sub>50</sub> value, the better the radical scavenging activity of the sample. A lower IC<sub>50</sub> value means the antioxidant is more effective at neutralising the DPPH radicals. It indicates a higher ability to donate electrons or hydrogen atoms to the radicals, thereby reducing their reactivity and potentially damaging cells. A higher IC<sub>50</sub> value suggests that the antioxidant is less effective at neutralising the DPPH radicals. It indicates that more antioxidants are required to achieve the same level of radical scavenging. Based on the results obtained, D. grandis shows weaker radical scavenging activity with an IC<sub>50</sub> value of 303.68  $\pm$  0.4  $\mu$ g/mL. While ascorbic acid, its IC<sub>50</sub> value is 15.52  $\pm$  0.59  $\mu$ g/ mL. The results are summarised in Table 3.

Table 3 IC<sub>50</sub> values of *D. grandis* by free radical scavenging method

Sample	Radical scavenging activity (DPPH) IC₅₀ (μg/mL)
Ascorbic acid	$15.52 \pm 0.59$
Donax grandis	$303.68 \pm 0.4$

Data are presented as mean  $\pm$  SD of three replicates.

#### DISCUSSION

Phytochemicals encompass a diverse array of plant compounds that have the potential to enhance human health and mitigate the occurrence of non-communicable diseases, including diabetes and cancer (Câmara et al., 2021; Rudrapal et al., 2022). According to Pinto et al. (2021), polyphenolic compounds are the predominant bioactive compounds obtained from food and plants.

An initial phytochemical screening of the leaves of *D. grandis* revealed that the aqueous leaf extract contained tannins, flavonoids and saponins but was absent of phlobatannins, terpenoids and cardiac glycosides. These results are in agreement with those of Daud et al., (2011), who also found the presence of tannins and saponins and the absence of terpenoids and cardiac glycosides in both methanol and aqueous extracts of D. grandis leaves. However, the findings of flavonoids present in aqueous extracts in this study do not align with Daud et al., (2011) where flavonoids in D. grandis leaves were absent in hexane, dichloromethane, methanol and aqueous extracts.

Tannins are polyphenols that occur naturally in plants and are divided into two groups, condensed tannins and hydrolysable tannins (esters of phenolic acids) (Liu et al., 2005). Another term for condensed tannins is phlobatannin or catechol tannin. Some of the common tannins are aflavins, daidezein, genistein, and glycitein. A study by Liu et al. (2005) found that tannin-related compounds can stimulate glucose transport by activating the insulin-mediated signalling pathway in adipocytes (Liu et al., 2005). Tannins are also reported to have anti-HIV-1 properties (Lu et al., 2004), antibacterial properties against Staphylococcus aureus (Akiyama et al., 2001), and antiparasitic properties (Kolodziej & Kiderlen, 2005). In addition, Souza et al. (2007) found that tannin-rich fractions from the stem bark of the Myracrodruon urundeuva plant have antioxidant, anti-inflammatory, and anti-ulcer properties in mice.

Saponins are glucosidic plant compounds that possess forming properties. Saponins isolated from numerous plants have shown properties of hypoglycaemia (Lee et al., 2000) and antioxidants (Hu et al., 2002). Saponin may also lower blood cholesterol levels (Egbuna & Ifemeje, 2016) by combining with cholesterol to form insoluble complexes that are passed out of the body through the bile. Cholesterol reabsorption is inhibited by it, which results in lower serum cholesterol (Krishnaiah et al., 2009). In addition, antifungal and antiviral effects are also attributed to the saponins (Mengoni et al., 2002).

Flavonoids are a type of polyphenol that has received the most attention due to their natural occurrence in plants and potential health benefits. Derivatives of flavonoids have been shown to possess various properties, such as anti-allergic, anti-inflammatory, antibacterial, antiviral, antioxidant, and anticarcinogenic activities (Yao et al., 2004; Saeed et al., 2012). Additionally, according to Mishra et al. (2015), flavonoids may have different mechanisms that control their anti-obesity and anti-diabetic effects (Coman et al., 2012). Flavonoids are highly effective in scavenging various oxidants, including singlet oxygen and other free radicals associated with various diseases (Jing et al., 2010; Saeed et al., 2012).

According to Amoussa et al. (2015), there is a direct relationship between antioxidant activity and the reducing power of bioactive chemicals. The presence of phenolic compounds in D. grandis may explain their antioxidant activity. According to the current study, the total phenolic content of *D. grandis* is  $34.34 \pm 1.37$  mg GAE/g dry weight. The current phenolic content of D. grandis plants employed in this study exhibited different values than prior investigations. Daud et al. (2011) discovered a total phenolic content of  $0.53 \pm 0.11$  mg GAE/g plant extract in a study on *D. grandis* utilising methanolic extraction. Another study by Ibrahim and Mohd Zain (2017), reported 7.12 mg/g GAE dry weight of total phenolic content from *D. grandis* leaves from 80% ethanolic extraction. A recent study by Mohd Yunus (2020) on methanolic extract of D. grandis leaves reported a total phenolic content of 1021.8 mg GAE/ dry weight. D. grandis leaves in this study show a high level of total phenolic content which is >20 mg GAE/g according to Bunzel and Schendel (2017). Bunzel and Schendel (2017) classified total phenolic content as low (<10 mg GAE/g), medium (10 - 20 mg GAE/g) and high (>20 mg GAE/g). According to Stankovic (2011), phenolic chemicals directly contribute to plant antioxidant activity. The capacity of phenolic compounds to scavenge free radicals by donating hydrogen atoms underpins their antioxidant action (Nadhiya & Vijayalakshmi, 2014; Stankovic, 2011). Antidiabetic, antibacterial, antioxidative, and anti-inflammatory properties have been identified for phenolic compounds (Nadhiya & Vijayalakshmi, 2014).

The total flavonoid content of D. grandis extracts may contribute to their antioxidant activities. According to Amoussa et al. (2015), phenolic compounds and flavonoids are naturally occurring substances that have demonstrated various biological properties associated with antioxidant mechanisms. The present investigation reveals that the concentration of total flavonoids in D. grandis extract is  $3.93 \pm 0.12$  mg/g. The result obtained from this study shows a similar trend of the total flavonoid content of 3.21 mg/g dry weight from 80% ethanolic extract of D. grandis leaves reported by Ibrahim and Mohd Zain (2017). Stankovic (2011) states that flavonoids possess notable antioxidant and chelating properties. According to Amoussa et al. (2015), flavonoids possess antioxidant properties that enable them to counteract the harmful effects of free radicals through their interference with biochemical pathways. According to a report by Nadhiya and Vijayalakshmi (2014), flavonoids have been found to exhibit antioxidant, anti-inflammatory, and anticarcinogenic properties. The plants' observed free radical scavenging activities can be attributed to the presence of phenolic compounds and flavonoids.

The analysis of DPPH radical scavenging is widely employed in assessing antioxidant capacity owing to its high level of accuracy (Amoussa et al., 2015) and sensitivity (Tupe et al., 2013). DPPH radical scavenging activity is based on the idea that an antioxidant compound can reduce DPPH by giving it an electron or hydrogen radical. This interaction results in a noticeable alteration in colour, shifting from purple to yellow, which can be measured at a wavelength of 517 nm (Tupe et al., 2013; Amoussa et al., 2015). According to Amoussa et al. (2015), the decrease in the absorbance measurement determines the assessment of radical scavenging capacity. The findings of this study indicate that D. grandis exhibited a weaker level of radical scavenging activity, as evidenced by its IC<sub>50</sub> value of 303.68  $\pm$  0.4  $\mu$ g/mL. However, the IC<sub>50</sub> values reported for *D. grandis* in this study do not align with those reported by other researchers. According to the study conducted by Daud et al. (2011) regarding D. grandis, the researchers reported an  $IC_{50}$  value of 16760 µg/mL. In recent study on methanolic extract of D. grandis leaves also reported an IC<sub>50</sub> value of 87550  $\mu$ g/ml (Mohd Yunus, 2020). The observed decrease in the reducing capacity of *D. grandis* in this study suggests a notable manifestation of antioxidant activity. When the  $IC_{50}$  value is lower than 50 µg/mL, the antioxidant activity is categorised as being very strong. The level of antioxidant strength is divided into four levels: very strong (IC<sub>50</sub> <50 μg/mL), strong  $(IC_{50}: 50-100 \,\mu g/mL)$ , moderate  $(IC_{50}: 101-150 \,\mu g/mL)$ , and weak  $(IC_{50}: 250-500 \,\mu g/mL)$ (Setha et al., 2013; Jumina et al., 2019).

There were variations observed in the IC<sub>50</sub> values, total phenolic contents, and phytochemical contents between the present study and a previous study conducted on D. grandis. These differences could be attributed to variations in the origin of the plants, discrepancies in the storage conditions of the samples, and the potential loss of certain antioxidant compounds during the sample preparation process (Rabeta & Nur Faraniza, 2013). Furthermore, the observed discrepancies in  $IC_{50}$  values and total phenolic contents between this study and previous research could be attributed to variations in the choice of solvent used to extract antioxidant compounds (Rabeta & Nur Faraniza, 2013). The impact of various drying methods on overall antioxidant activity has been documented in previous studies (Bernard et al., 2014).

# CONCLUSION

The findings from the phytochemical analysis demonstrate that the leaf extracts of D. grandis possess promising qualities as a viable source of bioactive compounds, which could be utilised in developing pharmaceutical drugs for contemporary medical applications. Additional research is necessary to assess the potential of these substances in terms of their antimicrobial, antihyperglycemic, anti-inflammatory, and antihelminthic properties. Furthermore, isolating, purifying, and characterising the active constituents is imperative to facilitate novel and intriguing investigations on the plant.

#### **ACKNOWLEDGMENTS**

Financial support for this research was provided by UMS Priority Area Scheme SBK0192-SKK-2015. The authors would like to thank Professor Dr Lee Ping Chin, Director of the Biotechnology Research Institute, Universiti Malaysia Sabah, for her support and encouragement.

## **AUTHOR CONTRIBUTIONS**

Senty Vun-Sang prepared the manuscript. Caysandra Audrey Michelle Elwon conducted the experiment. Associate Professor Dr Mohammad Igbal helped in designing the experiment and proofread the manuscript.

#### **DECLARATION OF COMPETING INTEREST**

The authors ensure that the work presented in this article is free of financial interests or personal relationships that may have influenced its outcome.

## REFERENCES

- Abd Aziz, A. F., Bhuiyan, M. S. A., & Iqbal, M. (2017). An evaluation of antioxidant and antidiabetic potential of Cynometra cauliflora (Nam-nam, Fabaceae). Transactions on Science and Technology, 4 (3-3), 372 – 383. https://tost.unise.org/pdfs/vol4/no3-3/4x3-3x372x383.
- Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., & Iwatsuki K. (2001). Antibacterial action of several tannins against Staphylococcus aureus. Journal of Antimicrobial Chemotherapy, 48 (4), 487 – 491. https://doi.org/10.1093/jac/48.4.487
- Amoussa, A. M. O., Sanni, A., & Lagnika, L. (2015). Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of Acacia ataxacantha. Journal of Pharmacognosy and Phytochemistry, 4 (2), 172 – 178. https://www.phytojournal.com/ vol4lssue2/lssue\_Jul\_2015/4-2-35.1.pdf
- Atanassova, M., Georgieva, S., & Ivancheva, K. (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of the *University of Chemical Technology and Metallurgy*, 46 (1), 81 – 88. https://journal.uctm. edu/node/j2011-1/12\_Maria\_Atanasova.pdf
- Azlim Almey, A. A., Ahmed Jalal Khan, C., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M. R., & Kamarul Rahim, K. (2010). Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. International Food Research Journal, 17, 1077 – 1084. http://ifrj.upm.edu.my/17%20(04)%202010/(28)%20 IFRJ-2010-035%20Kamarul%5B1%5D.pdf
- Benjamin, M. A. Z., Ng, S. Y., Saikim, F. H., & Rusdi, N. A. (2022). The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules, 30, 27 (19), 6458. https://doi.org/10.3390/molecules27196458

- Bernard, D., Kwabena, A. I., Osei, O. D., Daniel, G. A., Elom, S. A., & Sandra, A. (2014). The effect of different drying methods on the phytochemicals and activity of Ceylon Cinnamon (Cinnamomum zeylanicum) plant parts radical scavenging. European Journal of Medicinal *Plants*, 4 (11), 1324 – 1335. https://doi.org/10.9734/EJMP/2014/11990
- Bunzel, M., & Schendel, R. R. (2017). Determination of (total) phenolics and antioxidant capacity in food and ingredients. In S. S. Nielsen (Eds.), Food analysis (Food Science Text Series). Springer. https://doi.org/10.1007/978-3-319-45776-5\_25
- Câmara, J. S., Albuquerque, B. R., Aguiar, J., Corrêa, R. C. G., Gonçalves, J. L., Granato, D., Pereira, J. A. M., Barros, L., & Ferreira, I. C. F. R. (2021). Food bioactive compounds and emerging techniques for their extraction: Polyphenols as a case study. Foods, 24, 10(1), 37. https:// doi.org/10.3390/foods10010037
- Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., Calina, D., & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry, 11, 1158198. https:// doi.org/10.3389/fchem.2023.1158198
- Coman, C., Rugina, O. D., & Socaciu, C. (2012). Plants and natural compounds with antidiabetic action. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40 (1), 314 – 325. https://doi. org/10.15835/nbha4017205
- Daud, J. M., Mohd Hassan, H. H., Hashim, R., & Taher, M. (2011). Phytochemicals screening and antioxidant activities of Malaysian Donax grandis extracts. European Journal of Scientific Research, 61 (4), 572 - 577. https://citeseerx.ist.psu.edu/document?repid=rep1&type= pdf&doi=3eb1ed3107e0a44185393f99b4840e5ac6832b8b
- Egbuna, C., & Ifemeje, J.C. (2015) Biological function and anti-nutritional effects of phytochemicals in living system. IOSR Journal of Pharmacy and Biological Science, 10 (2), 10 – 19. https:// www.iosrjournals.org/iosr-jpbs/papers/Vol10-issue2/Version-3/C010231019.pdf
- Elwon, C. A. M. (2015). Hypoglycemic and hypolipidemic effects of herbal plants against alloxan induced diabetes mellitus in rats. (Unpublished Master thesis), Universiti Malaysia Sabah.
- Hanum, I. F., & Hamzah, N. (1999). The use of medicinal plant species by the Temuan Tribe of Ayer Hitam Forest, Selangor, Peninsular Malaysia. Journal of Tropical Agriculture Science, 22 (2), 85 – 94. http://www.pertanika.upm.edu.my/resources/files/Pertanika%20PAPERS/ JTAS%20Vol.%2022%20(2)%20Sep.%201999/05%20PAGE%2085-94.pdf
- Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. Chemical & *Pharmaceutical Bulletin* (Tokyo), *36* (6), 1090 – 2097 https://doi.org/10.1248/cpb.36.2090
- Hoang, H. T., Moon, J. Y., & Lee, Y. C. (2021). Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives. Cosmetics, 8 (4), 106. https://doi.org/10.3390/cosmetics8040106
- Hu, J., Lee, S. O., Hendrich, S., & Murphy, P. A. (2002). Quantification of the group B soya saponins by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry, *50* (9), 2587 – 2594. https://doi.org/10.1021/jf0114740
- Ibrahim, M. H., & Mohd Zain, N. A. (2017). Nutritional composition and antioxidant activity of leaves and rhizomes of Bemban (Donax grandis) grown under glasshouse conditions. Annual Research & Review in Biology, 17 (3), 1 - 11. https://doi.org/10.9734/ARRB/2017/36041
- Jayasuriya, H., Pathare, P. B., Al-Attabi, Z., & Al-Hamdani, A. (2023). Drying kinetics and quality analysis of coriander leaves dried in an indirect, stand-alone solar dryer. Processes, 11, 1596. https://doi.org/10.3390/pr11061596

- Jing, L. J., Mohamed, M., Rahmat, A., & Abu Bakar, M. F. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (Boesenbergia rotunda, Boesenbergia pulchella var attenuata and Boesenbergia armeniaca). Journal of Medicinal Plants Research, 4 (1), 27 – 32. https://academicjournals. org/article/article1380372995\_Jing%2520et%2520al.pdf
- Jumina, J., Siswanta, D., Zulkarnain, A., Triono, S., Priatmoko, P., Yuanita, E., Fatmasari, N., & Nursalim, I. (2019). Development of C-Arylcalix[4] resorcinarenes and C- Arylcalix[4] pyrogallolarenes as antioxidant and UV-B protector. Indonesian Journal of Chemistry, 19 (2), 273 – 284. https://doi.org/10.22146/ijc.26868
- Kolodziej, H., & Kiderlen, A. F. (2005). Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitized RAW 264.7 cells. Phytochemistry, 66 (17), 2056 – 2071. https://doi.org/10.1016/j.phytochem.2005.01.011
- Krishnaiah, D., Devi, T., Bono, A., & Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research, 3 (2), 67 – 72. https://academicjournals.org/article/article1380370874\_Krishnaiah%20et%20al.pdf
- Lee, K. T., Sohn, I. C., Kim, D. H., Choi, J. W., Kwon, S. H., & Park, H. J. (2000). Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their antioxidant activity in vitro. Archives of Pharmacal Research, 23 (5), 461 – 466. https://doi.org/10.1007/BF02976573
- Liu, X., Kim, J.K., Li, J. & Liu, F., & Chen, X. (2005). Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. The Journal of Nutrition, 135 (2), 165-171. https://doi.org/10.1093/jn/135.2.165
- Lü, L., Liu, S. W., Jiang, S. B., & Wu, S. G. (2004). Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacologica Sinica, 25 (2), 213 – 218. http://cdn.amegroups.cn/journals/aps/files/ journals/26/articles/7866/public/7866-PB1-R1.pdf
- Mengoni, F., Lichtner, M., Battinelli, L., Marzi, M., Mastroianni, C. M., Vullo, V., & Mazzanti, G. (2002). In vitro anti-HIV activity of oleanolic acid on infected human mononuclear cells. Planta *Medica*, 68 (2), 111 – 114. https://doi.org/10.1055/s-2002-20256
- Mishra, C., Singh, B., Singh, S., Siddiqui, M. J. A., & Mahdi, A. A. (2015). Role of phytochemicals in diabetes lipotoxicity: an overview. International Journal of Research and Development in Pharmacy and Life Sciences, 4 (4), 1604 – 1610. https://ijrdpl.com/index.php/ijrdpl/ article/view/446
- Mohd Yunus, N. S. N. (2020). Antioxidant and antibacterial activities of *Donax grandis* leaves against food borne bacteria (Staphylococcus aureus and Escherichia coli). (Unpublished bachelor's degree thesis), Universiti Teknologi MARA. https://ir.uitm.edu.my/32872/1/32872.pdf
- Nadhiya, K., & Vijayalakhsmi, K. (2014). Evaluation of total phenol, flavonoid contents and in vitro antioxidant activity of Benincasa hispida fruit extracts. International Journal of Pharmaceutical, Chemical & Biological Sciences, 4 (2), 332 – 338. https://www.ijpcbs. com/articles/evaluation-of-total-phenol-flavonoid-contentsand-invitro-antioxidantactivity-of-benincasahispida-fruit-extracts.pdf
- Pinto, T., Aires, A., Cosme, F., Bacelar, E., Morais, M. C., Oliveira, I., Ferreira-Cardoso, J., Anjos, R., Vilela, A., & Gonçalves, B. (2021). Bioactive (Poly)phenols, volatile compounds from vegetables, medicinal and aromatic plants. Foods, 6, 10 (1), 106. https://doi.org/10.3390/ foods10010106
- Rabeta, M. S., & Nur Faraniza, R. (2013). Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of Garcinia atrovidis and Cynometra cauliflora. International Food Research Journal, 20 (4), 1691 – 1696. http://www.ifrj.upm.edu.my/20%20(04)%20 2013/26%20IFRJ%2020%20(04)%202013%20Rabeta%20(018).pdf

- Ridley, H. N., & Curtis, C. (1902). Malay plant names. Journal of the Straits Branch of the Royal Asiatic Society, 38, 39 – 122. https://journals.scholarsportal.info/details/23047534/ v1902i0038/39\_mpn.xml
- Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stressinduced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. Frontiers in Pharmacology, 13, 806470. https://doi.org/10.3389/ fphar.2022.806470
- Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 12, 221. https://doi.org/10.1186/1472-6882-12-221
- Setha, B., Gaspersz, F., Idris, A. P. S., Rahman, S., & Mailoa, M. N. (2013) Potential of seaweed Padina sp. as a source of antioxidant. International Journal of Scientific & Technology Research, 2 (6), 221 – 224. https://paper.researchbib.com/view/paper/5578
- Silalahi, M., & Wakhidah, A. Z. (2021). Donax canniformis (G. Forst.) K. Schum. Marantaceae. In F. M. Franco (Ed.), Ethnobotany of the mountain regions of Southeast Asia. Springer Nature. https://doi.org/10.1007/978-3-030-38389-3\_116
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 299, 152 – 178. https://doi.org/10.1016/S0076-6879(99)99017-1
- Souza, S. M., Aquino, L. C., Milach, A. C. Jr, Bandeira, M. A., Nobre, M. E., & Viana, G. S. (2007). Antiinflammatory and antiulcer properties of tannins from Myracrodruon urundeuva Allemão (Anacardiaceae) in rodents. Phytotherapy Research, 21 (3), 220 - 225. https:// doi.org/10.1002/ptr.2011
- Stankovic, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. Journal of Science, 33, 63 – 72. https://www.pmf. kg.ac.rs/KJS/en/images/volumes/vol33/kjs33mstankovic63.pdf
- Thanebal, S. P., Vun-Sang, S., & Iqbal, M. (2021). Hepatoprotective effects of Pandanus amaryllifolius against carbon tetrachloride (CCl<sub>4</sub>) induced toxicity: A biochemical and histopathological study. Arabian Journal of Chemistry, 14, 103390. https://doi. org/10.1016/j.arabjc.2021.103390
- Tupe, R. S., Kemse, N. G., & Khaire, A. A. (2013). Evaluation of antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts. International Food Research Journal, 20 (3), 1053 – 1063. http://ifrj.upm.edu.my/20%20(03)%202013/4%20IFRJ%20 20%20(03)%202013%20Tupe%20(364).pdf
- Vun-Sang, S., Teoh, P. L., Rodriques, K. F., & Iqbal, M. (2017). Phytochemical analysis and antioxidant activity of Ficus lepicarpa leaves from Sabah, Malaysia. Transactions on Science and Technology, 4 (3-3), 360 – 365. https://tost.unise.org/pdfs/vol4/no3-3/4x3-3x360x365. html
- Wani, C., Vikhe, S., & Rao, P. (2020). Natural antioxidants: Their existence in medical plants -Review. World Journal of Pharmaceutical Research, 9 (7). https://www.wjpr.net/abstract\_ file/14667
- World Health Organization. Regional Office for South-East Asia. (2020). Regional workshop on clinical research methodologies in traditional medicine for the WHO South-East Asia Region: Report of the regional workshop. World Health Organization. Regional Office for South-East Asia. https://apps.who.int/iris/handle/10665/338750

- Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R., & Chen, S. S. (2004). Flavonoids in food and their health benefits. Plant Foods for Human Nutrition, 59 (3), 113 – 122. https://doi.org/10.1007/s11130-004-0049-7
- Yeoh, H. H., Wee, Y. C., & Watson, L. (1986). Taxonomic variation in total leaf protein amino acid compositions of monocotyledonous plants. Biochemical Systematics and Ecology, 14 (1), 91 – 96. https://doi.org/10.1016/0305-1978(86)90092-X