Phytochemicals Screening and Antioxidant Activities of Malaysian Donax Grandis Extracts

Jamaludin Mohd Daud  
*Kulliyyah of Science, International Islamic University Malaysia  
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota  
25200 Kuantan, Pahang, Malaysia*

Husna Hawa Mohd Hassan  
*Kulliyyah of Science, International Islamic University Malaysia  
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota  
25200 Kuantan, Pahang, Malaysia  
Email: husnatm@yahoo.com*

Ridzwan Hashim  
*Kulliyyah of Allied Health, International Islamic University Malaysia  
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota  
25200 Kuantan, Pahang, Malaysia*

Muhammad Taher  
*Kulliyyah of Pharmacy, International Islamic University Malaysia  
Jalan Sultan Ahmad Shah, Bandar Indera Mahkota  
25200 Kuantan, Pahang, Malaysia*

Abstract

Common Donax (*Donax grandis*) is in a family of *marantaceae* and widely distributed in South East Asia region. Phytochemical constituents in different parts of the plant (leaves, fruits, stems and roots) were extracted by soxhlet extractions using hexane, dichloromethane, methanol and water. Methanolic extract showed the highest yield of crude extracts from all parts of the plant as compared to the other solvents, including water. Screening tests of the crude extracts revealed the presence of phenolic compounds, alkaloids, tannins, phytosterols, cardiac glycosides, terpenoids, steroids, saponins and flavonoids. Proximate analysis of saponins have also been done for all plant parts of *D. grandis* using the methanolic extract. The results showed that fruits contained the highest amount of saponins (2.39 wt.%). All parts of the plant samples showed highest total phenolic content (0.18 to 0.65 mg GAE/g). These three solvent fractions possessed strong radical scavenging activity from DPPH, in the ranged from 14.86 to 21.85 mg/mL. The results indicate that *D. grandis* is a potential candidate to be used as an antioxidant and antiproliferative agents.

**Keyword:** *Donax grandis*, soxhlet extraction, total phenolic content, antioxidant, phytochemicals, saponins
1. Introduction
Many herbal plants products have attracted the attention of chemist for exploring of new phytochemical compounds and its antioxidant properties. Common Donax (Donax grandis) is from a family of Marantaceae. Synonym as D. canniformis or Clinogyne grandis. It is widely distributed in Malaysia, Thailand, Singapore, Brunei, Philippine, Papua New Guinea and Polynesia in the South East Asia region. D. grandis has a hollow stem like bamboos with branching at each segment. The leaves are wide and large, varies between ovate to oval shape with a white flower. The fruits are rounded, smooth and green in colour when unripe, then turns to yellow when they are ripe. D. grandis is distributed in jungle along the river and usually found in the wet places in the secondary forest and bamboo thicket. It can be propagated by seed and easily by rhizome (Ong, 2008).

Fatan, (1990) reported that D. grandis rhizomes are used traditionally in Malay culture to cure shingles. Medicinally the leaves and roots decoction is taken in bath to cool body during fever and the juice from stems effectively used against snake bite. Furthermore, the poultice of leaves and stem also can be used as an eye refreshment (Faridah & Nurulhuda, 1999). Study shown that D. grandis leaves contain high level of amino acids compared to others monocotyledonous plants in the taxonomy variation studies (Yeoh et al., 1986). Although the active compound properties of this plant have not been fully documented, nevertheless from the previous screening test has shown that saponins are the main active compounds in the plant (Rahmani et al., 1985).

The medicinal values of plants have been claimed to lie in their phytochemical components including alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Phan et al., 2001). Thus, the study on phytochemical constituents and of antioxidant properties of Malaysian D. grandis was carried out in order to discover the medicinal potentials of the plant.

2. Method
2.1. Chemicals and Reagent
All the chemicals and reagents used in this experiment were of analytical grade.

2.2. Sample Collection and Preparation
Donax grandis samples were collected along the Pahang River at Temerloh, Pahang, Malaysia in 2010. Their identities were checked by Faculty of Forestry UPM. Their voucher specimens have been deposited at the Kulliyah of Pharmacy, IIUM Kuantan. Each part of the plants (leaves, fruits, rhizomes and stems) was cleaned and dried in a drying cabinet (Protech) at 40ºC for 7 days. The dried samples were ground to powder form using a Disk mill machine (Qingdao Dahua Double Circle). The powder materials were then stored separately in airtight bottles before use in the study.

2.3. Extraction of Plant Materials
Powdered materials were extracted in soxhlet extractors using solvents of increasing polarity (hexane, dicholoromethane (DCM), and methanol (MeOH) separately for 72 hours, following the method by Sengul (2009). The extracts were concentrated under vacuum at 60 ºC using a rotary evaporator (Bucii). The solid residues obtained after rotary evaporation were dried, kept in glass vials and stored in a refrigerator. Portions were taken from the refrigerator to be used for each of the experiments below.

Powdered materials were also soaked in distilled water and incubated at 60ºC for 6 hours. Then, the decoction was filtered. The filtrates were concentrated using a rotary evaporator to a thick paste and dried in a freeze dryer (Zulkhairi, 2009). The solid residues were also kept in glass vials and stored in a refrigerator before use.
2.4. Evaluation of Phytochemicals Screening Test

The extracts were analysed for the presence of phenolic compounds (ferric chloride test and gelatin test), flavonoids, tannins, phytosterols (Libermann-Burchard’s test), terpenoids (Salkowski test), steroids, alkaloids, cardiac glycosides (Keller-Killiani test) and saponins (Adetuyi & Popool, 2001; Egwaikhidi, 2007; Sofowara, 1982; Trease, 1989; Siddiqui & Ali, 1997; and Finar, 1983).

2.5. Saponin Determination

The method used was based on Obdoni & Ochuko (2001). Weight of each 20 g dried ground sample of the plant parts was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath at about 55°C for 4 hours with continuous stirring. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% ethanol. Volume of the combined extracts was reduced to 40 ml by evaporation under reduced pressure using a rotary evaporator (Buccii). The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded. The purification process was repeated.

Then 60 ml of n-butanol was added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated as weight percentage of dried plant sample.

2.6. Determination of Total Phenolic Content

Total phenolic compounds were measured using the modified Folin-Ciocalteu method (Amin et al., 2004). Aliquots 1 ml (1mg/ml) of each of the extracts was mixed with 5 ml Folin-Ciocalteu reagent. The mixture was vortexed and 4 ml sodium carbonate solution (20% m/v anhydrous sodium carbonate in distilled water), was added, vortexed again and left to stand for 60 minutes to obtain maximum colour development. The absorbance was measured at 760 nm using UV-Vis spectrophotometer (Perkin Elmer). Distilled water was used as a blank. The total phenolic contents were expressed as gallic acid equivalents (GAE) in milligrams per gram of extract, using a standard curve generated with 0.2 – 1.0 µg/ml) of gallic acid. All determinations were performed in triplicate.

2.7. DPPH Scavenging Assay

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. Radical scavenging activity was measured by a slightly modified method described by Ayoola et al. (2008). The extracts were prepared at concentrations of 10, 20, 30 and 40 mg/ml in methanol (Analar grade). BHT was used as the antioxidant standard at concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4 mg/ml. One mL of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 ml DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{[\text{Ab} - \text{Aa}]}{\text{Ab}} \times 100$$

where Ab is the absorption of the blank sample and Aa is the absorption of the extract. Antioxidant capacity was expressed as IC50; extract concentration (mg/ml) that required scavenging 50% of DPPH. All measurements were carried out in triplicate.

3. Result and Discussion

Drying and extraction results for different part (leaves, fruit, stem and root) of D. grandis are shown in Table 1. Percentage yield of drying (42.78%) and extraction (13.68%) for fruit were the highest compared to the other parts of the plant.
Table 1: The percentage yield of drying, extraction and saponin content in Malaysian *D. grandis*

<table>
<thead>
<tr>
<th>Plant</th>
<th>% Yield of drying*</th>
<th>% Yield of extraction**</th>
<th>% Of saponin content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>36.90</td>
<td>6.67</td>
<td>1.89</td>
</tr>
<tr>
<td>Fruits</td>
<td>42.78</td>
<td>13.68</td>
<td>2.39</td>
</tr>
<tr>
<td>Rhizomes</td>
<td>27.34</td>
<td>9.30</td>
<td>1.36</td>
</tr>
<tr>
<td>Stems</td>
<td>25.81</td>
<td>13.20</td>
<td>1.26</td>
</tr>
</tbody>
</table>

*%Yield = (weight of dried sample / weight of wet sample) *100
**%Yield = (weight of dried extract / weight of dried sample) *100*

Table 2: Phytochemical Screening of Malaysian *D. grandis* using different solvent for extraction.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Phenolic Compound</th>
<th>Flavanoids</th>
<th>Tannins</th>
<th>Phytosterols</th>
<th>Steroids</th>
<th>Terpenoids</th>
<th>Alkaloids</th>
<th>Saponin</th>
<th>Cardiac Glycoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fruit</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stem</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Root</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fruit</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stem</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Root</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fruit</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stem</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Root</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fruit</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stem</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Root</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

‘+’ sign indicates the present compound
‘–’ sign indicates the absent compound

Result from the phytochemical screening test in Table 2 indicated the presence of phenolic compounds, flavanoid, tannins, phytosterol, terpenoid, steroid, alkaloid, cardiac glycoside and saponin in the different parts of the plant with different polarity of solvents. Phytosterols were abundantly present in all the extracted samples. Furthermore, phenolic compounds were present in the extracts of high polarity solvents (MeOH and water) for each part the plant whereas flavanoids and tannins which is commonly distributed groups of plant phenolic were absent in the medium polar solvent accept in leaves as compare to tannin, that only present in leaves for all solvent.

The presence of phenolic compounds in the plant indicates that these plant may have the ability as an anti-microbial agent (Harisharanraj et al., 2009). These can support the use of *D. grandis* traditionally in treating boils and shingles (Fatan, 1990). Tannins have stringent properties that hasten the healing of wound and inflamed mucous membranes. These must be the reason it has been used by Temuan tribe for snake bite healing (Faridah & Nurulhuda, 1999). The extracts in non-polarity and low polarity solvent showed a positive test of steroids in 3 parts of the plant (except root) whereas terpenoids and alkaloids were positively found in hexane in 3 parts of the plant (accept in leaves) and in DCM extracts for terpenoids only. Cardiac glycosides only present in stems at a low polarity solvent whereas saponins were present on extracts of high polar solvents. Furthermore, the presence of saponins was confirmed quantitatively (in Table 1 and Table 2) and the highest percentage of saponins can be seen in fruits (2.39%) as compare to the other parts of the plant (Table 1). Saponin is a glycoside of triterpenes in which most of anticancer agents come from this group of compound (Bryan, 2005).
Table 3: Total Phenolic Content and antioxidant activity of methanolic extract from different parts of Malaysian D. grandis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolic content (mg GAE/g plant extract)</th>
<th>Antioxidant activity (IC50 value) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits</td>
<td>0.65±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.52±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leaves</td>
<td>0.53±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.76±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roots</td>
<td>0.29±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.86±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stems</td>
<td>0.18±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.85±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>N.T</td>
<td>0.18±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data represent means ± SD of three independent experiments, performed in triplicate

Note: All the data is three replicates of each samples
N.T : Not Tested

The phenolic compounds are considered as being a major group to the number of the secondary metabolites that contributes to the antioxidant activity of the plant. The fruits showed higher activity TPC followed by leaves, roots and stems (Table 3). However, all parts of the plant showed low TPC level which is <1000 mg GAE/100 g, according to Chew (2009).

The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract (Ayoola et al.,2009).

Findings of this study revealed that D.grandis contained low level IC<sub>50</sub>. Furthermore as we can see in Table 3, there are no significant difference in IC<sub>50</sub> between fruits and leaves. According to Kuete and Efferth (2010) this plant can be ranked as low radical scavenging activities (<1000 mg/mL).

There were no correlation found between total phenols and free radical scavenging activities in tested extracts (R<sup>2</sup> = 0.095). There are no significant contribution of TPC in antioxidant properties of D.grandis which is not affected the radical scavenging activities of the plants. It may be due to the presence of other compound such as phytosterol and saponin that may contribute to the scavenging activities of the sample. However the scavenging power of all the extracts were significantly low compared to the standard (BHT).

4. Conclusion
From the results fruits showed higher percentage for the yield of drying, extractions and saponin content. All parts of the plants contained essential phytochemicals. Extracts from D.grandis showed varying in TPC content in following order: fruits, leaves, roots and stem. However all parts of the plants showed low level of antioxidant (radical scavenging). These results will also be used for the next ongoing studies in order to isolate, characterized and elucidate the structure of the active compound from these plant for its anticancer properties which has been claimed by a traditional practitioner.

5. Acknowledgement
Authors are thankful to Br. Muzammil (Biotech Dept, IIUM) and Mr. Azhari Yahya (POLISAS, Kuantan) for their technical support.
References


