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THE TOTAL PHENOLIC CONTENT OF *PANDANUS* EXTRACTS

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ABSTRACT

The Pandanaceae consist of four plant genera. The *Pandanus* is the largest genus, having *P. odorus* as one species, which is synonymous with *P. amaryllifolius*, or *pandan wangi*. The leave extract has medicinal purposes, such as antioxidant activity, due to the polyphenolic content. From the literature search, the powdered forms were found to have the highest total antioxidant activity, total phenolic content (TPC) and scavenging activity, compared to its fresh forms. A polyphenolic compound, such as quercetin, is detected in the methanol (MeOH) extract. The chloroform leaves fraction showed the highest radical scavenging activity while the ether root fraction showed the highest radical scavenging activity. Higher antioxidant activity potential for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was recorded in MeOH root extract, compared to the aqueous extract. Other *Pandanus*, e.g. *P. fascicularis* and *P. odoratissimus* also exhibited their antioxidant activity in MeOH extract. Therefore, a similar approach is taken to evaluate the potential of MeOH extract of *P. pygmaeus*. From the test, the TPC of this extract was found to be (202.62 ± 6.60) mg Gallic Acid Equivalence (GAE) per gram extract. However, this reading is lower than the analysis obtained from dichloromethane extract (243.83 ± 1.65 mg GAE / gram extract), and approximately three folds more than the result from *P. conoideus* MeOH fruit extract. Yet, the powdered *P. odorus* leaves hold the record of the highest TPC, when the data from the literatures were compared. It is expected that lignan could also be one of the chemical compositions of this newly investigated *Pandanus*.

Keywords: *Phenolic, antioxidant, Pandanus*

INTRODUCTION

The Pandanaceae is one of the plant families, which consist of four genera. The *Pandanus* is the largest genus, followed by *Freycinetia, Sararanga* and *Martellidendron* [1]. One of the *Pandanus* species is botanically known as *P. odorus* Ridl., which is also reported as *P. amaryllifolius* Roxb. or *pandan wangi* [2]. From the literature review, the leave extract has medicinal purposes, such as antioxidant activity, due to the polyphenolic content [3]. The powdered forms were reported to have the highest total antioxidant activity, total phenolic content (TPC) and scavenging activity, compared to its fresh forms [4].

A polyphenolic compound, such as quercetin, is detected in the methanol (MeOH) extract. The chloroform leaves fraction showed the highest radical scavenging activity while the ether root fraction showed the highest radical scavenging activity. Higher antioxidant activity potential for 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay was recorded in MeOH root extract, compared to the aqueous extract. Other *Pandanus*, e.g. *P. fascicularis* and *P. odoratissimus* also exhibited their antioxidant activity in MeOH extract.

Therefore, a similar approach is taken to evaluate the potential of MeOH and other non polar extracts of the leaves of *P. pygmaeus* Thouars (*Fig. 1*). This plant is also called as the dwarf *Pandanus*. It is an endemic species in Madagascar and grows to about 50 centimetres tall. The edges of the leaves have sharp prickles. Meanwhile, their stems are simple and usually branched. This plant has adventitious prop roots. It is mostly cultivated as an ornamental plant. *P. pygmaeus* is also placed at the university campus to enhance the landscape at the main circular intersection. The advantage is, it requires little maintenance.

In this experiment, the main objective was to determine the TPC by using Folin-Ciocalteau (F-C) assay, whereby, gallic acid was utilised as the standard phenolic compound (Rohman *et al*. 2010). Finally, the results

were analyzed and compared with the literatures. This work is essentially, one part of a study of bioactive constituents from Pandanaceae [5].

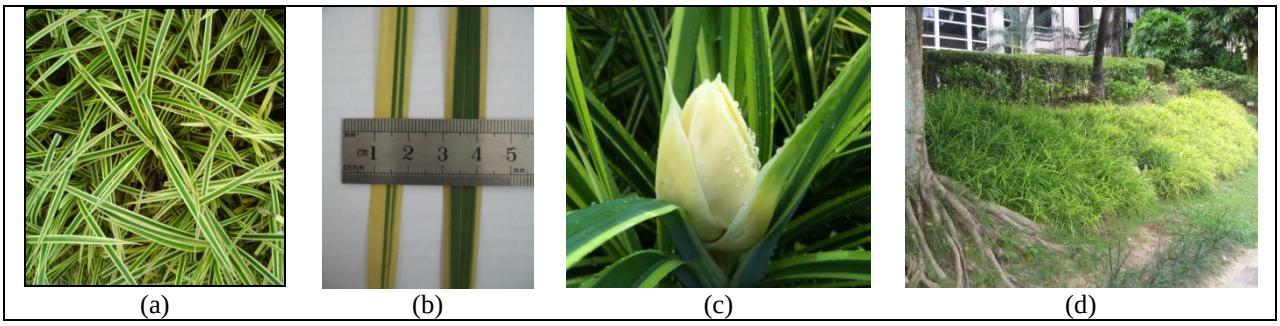

Fig. 1: (a) *Pandanus pygmaeus*, as observed at UiTM Puncak Alam campus, on 15th March 2012. (b) Their green and yellow variegated leaves are about 40 to 70 centimetres in length and one centimetre width. (c) In another specimen, the plant gives a light to bright yellow bud. (d) The difference in the foliage colouration could be noticed. At a shady spot, the leaves appear fully green, as compared to the yellow samples when exposed to the sunlight.

MATERIALS AND METHODS

Chemicals

DPPH, ferrozine, gallic acid, trichloracetic acid (TCA), were obtained from Sigma Chemical Co. (USA). Silica gel, methanol, dichloromethane, hexane, aluminum hydroxide, Folin-Ciocalteu reagent, sodium nitrite, sodium carbonate, potassium ferricyanide, ferric chloride, fero chloride, phosphate buffer, ethylene diamine tetraacetate (EDTA) were purchased from Merck.

Plant material

The fresh leaves of *P. pygmaeus* were collected in the morning of November 2011 from Putrajaya, Malaysia. The leaves were authenticated by one of the authors (IAW) with voucher specimen number PP112011. The samples of this plant were deposited in the Faculty of Pharmacy, UiTM Puncak Alam, Selangor Darul Ehsan, Malaysia. The plant materials were dried under shed at room temperature (28 ± 2°C).

Plant extracts

The dried leaves of *P. pygmaeus* were firstly cut into smaller pieces. After that, the leaves were milled into uniform dry powder by using a grinder. The sample (3.5 kg) was macerated consecutively, with hexane, dichloromethane and MeOH. The extractives were concentrated in *vacuo* (below 40 °C) to afford the three organic crude extracts. They were weighed to obtain the percentage yield and stored in a refrigerator for further experimental procedures.

Thin layer chromatography (TLC)

The chemical compositions of the extracts were detected using TLC (hexane:acetone). A Dragendorff's solution was used as the spray reagent. Yellow spots on the silica plates indicated the presence of alkaloids.

The fractionation of dichloromethane extract

The dichloromethane extract (30 g) was later fractionated using a flash column chromatography, with the stationary phase of silica gel (Merck). The mobile phase was delivered in a gradient manner with the aid or an electrical pump, in an order of dichloromethane : ethyl acetate (v/v) = 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. A total of 2 liters of the mentioned mixtures were introduced, while the eluents were collected by volume in a 1 L conical flask. They were later combined to fractions, based on the similarity of their TLC profiles. Each fraction was evaluated for its total phenolic and total flavonoid contents, its DPPH radical scavenging activity, its reducing power and its metal chelating ability [5].

The determination of total phenolic content (TPC)

Folin-Ciocalteu (F-C) reagent was used for analysis of total phenolics content (TPC) according to [6, 7]. In a 10 ml volumetric flask, a 0.2 ml aliquot of the extracts in methanol (1.0 mg/ml) was mixed with 0.4 ml of Folin-Ciocalteu reagent. The solution was allowed to stand at 25^{o}C for 5 - 8 min before adding 0.2 ml of 4.0 ml of sodium carbonate solution 7.0 % and made to 10.0 ml with distilled water. The mixture was allowed to stand for 2 hr before its absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. TPC was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g).

RESULTS AND DISCUSSIONS

Plant extraction

The extract gave a green to yellowish solution, as shown in *Fig. 2*. Among the three extracts, the MeOH extract showed the highest yield (6 %); followed by dichloromethane (3 %) and hexanoic (1 %) extracts. The TLC plate displayed yellow spots, after spraying with the Dragendorff's reagent (see *Fig. 2*).

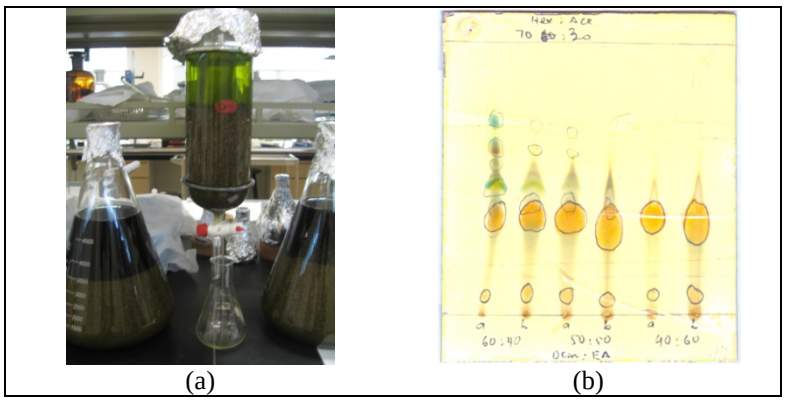

Fig. 2: (a) The sample was subjected to the organic solvents extraction, for example, in a glass extractor (10 cm x 25 cm, width x length). (b)An example of a TLC plate showing yellow spots, from the fractionation of the dichloromethane extract.

The determination of total phenolic content (TPC)

Folin-Ciocalteu (F-C) method is based on oxidation of phenolics by a molybdotungstate in F-C reagent to yield a colored product with λ_{max} 745 – 750 nm [7,8]. A linear calibration curve of gallic acid, in the range of 0.025 – 0.500 mg/mL, with a coefficient of determination (r^2) value of 0.9999, was obtained (*Fig. 3*).

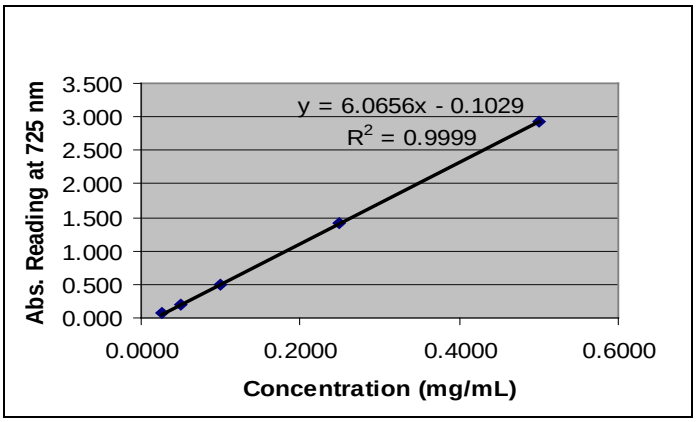

Fig. 3: A calibration curve of the standard gallic acid for the determination of total phenolics content.

From the triplicate tests, the TPC of the MeOH extract was found to be (202.62 ± 6.60) mg Gallic Acid Equivalence (GAE) per gram extract. However, this reading is lower than the analysis obtained from dichloromethane extract (243.83 ± 1.65 mg GAE / gram extract), and approximately three folds more than the

result from *P. conoideus* MeOH fruit extract [7]. Yet, the powdered *P. odorus* leaves hold the record of the highest TPC, when the data from the literatures were compared [4,9].

CONCLUSIONS

It is concluded that the dichloromethane extract of *P. pygmaeus* leaves showed higher TPC than the MeOH extracts. Due to the successive extraction procedure i.e. from nonpolar to polar organic solvents, most of the compounds might be extracted in the dichloromethane. This observation might be different if chloroform was utilised. The impact on the extractive yields was significant, once the maceration of *P. pygmaeus* was changed from dichloromethane to chloroform. On the other note, lignan is expected as one of the chemical compositions of this newly investigated *Pandanus.*

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REFERENCES

[1] Tan, M. A., Nonato, M. G., Kogure, N., Kitajima, M. and Takayama, H. (2011) Secondary metabolites from *Pandanus simplex*. *Biochemical Systematics and Ecology,* 40, 4-5.
[2] Salim, A. A., Garson, M. J. and Craik, D. J. (2004) New Alkaloids from *Pandanus amaryllifolius*. *Journal of Natural Product,* 67, 54-57.
[3] Miean, K. H. and Mohamed, S. (2001) Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. *J. Agric. Food Chem.,* 49, 3106-3112.
[4] Yan, S.W. and Asmah, R. (2010) Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *International Food Research Journal,* 17, 417-423.
[5] Md Yusof, N. (2012) A Study of Bioactive Constituents from *Pandanus Pygmaeus* as Antioxidants. Master Thesis, Universiti Teknologi MARA, Malaysia (in print).
[6] Chun, O. K., Kim, D. O. and Lee, C.Y. (2003) Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal Agriculture and Food Chemistry*, 51, 8067-8072.
[7] Rohman, A. Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R. and Mulatsih, W. (2010) Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). *International Food Research Journal,* 17, 97-106.
[8] Prior, R. L., Wu, X. and Schaih, K. (2005) Standardised methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem., 55*, 2698A – J.
[9] Azlim Almey, A. A., Ahmed Jalal Khan, C., Syed Zahir, I., Mustapha Suleiman, K., 'Aisyah, M. R. and 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.