Original article

In vitro antioxidant and in vivo xanthine oxidase inhibitory activities of Pandanus amaryllifolius in potassium oxonate-induced hyperuricemic rats

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Summary Xanthine oxidase (XO) plays an important role in the regulation of uric acid and prevents it from being overproduced as in hyperuricemia disease. The combined effects of antioxidant and xanthine oxidase inhibitor would become a promising approach for hyperuricemia treatment. In this research, the antioxidant and xanthine oxidase inhibitory activities of Pandanus amaryllifolius leaf were evaluated. The leaf water extract (PA-W) showed highest total phenols, and petroleum ether extract (PA-PE) showed highest total flavonoids contents. The antioxidant activity of DPPH, metal chelating and hydrogen peroxide was highest in PA-W extract. The treatment of PA-W extract at 1000 mg kg\(^{-1}\) body weight in potassium oxonate-induced hyperuricemic rats showed significant \((P < 0.001)\) decrease in serum uric acid level by 85% and XO activity by 64%, respectively, as compared to the hyperuricemic rats. In conclusion, the P. amaryllifolius possess the dual effect of antioxidant and XO inhibition as potential therapeutic agents in the hyperuricemia treatment.

Keywords Flavonoids, hyperuricemia, Pandanus amaryllifolius, phenols, xanthine oxidase.

Introduction Xanthine oxidase (XO) plays a crucial role in the degradation of purine in humans (Hille, 2005) with the formation of uric acid (Lima et al., 2015). The hypoxanthine is converted to xanthine and then to uric acid via enzymatic reaction of xanthine oxidase (Mamat et al., 2014), and finally, the uric acid is eliminated in the urine. The excessive formation of the uric acid could consequently result in hyperuricemia and gout (Richette & Bardin, 2010). According to Boffetta et al. (2009), hyperuricemia is a prominent threat factor for gout, hypertension and diabetes. The basis of the disease is uric acid crystallisation and deposition in joints and neighbouring tissues. As uric acid overproduction is the key causation of hyperuricemia, the ultimate assuring target for therapeutic remedy of this disorder is xanthine oxidase (Richette & Bardin, 2010).

The treatment of illness related to hyperuricemia could overcome by keeping the precipitation of plasma urate concentrations at low level and getting rid of the present urate crystals (Kumar & Azmi, 2014). According to Richette & Bardin (2010), the allopurinol and febuxostat are the available drugs used to reduce xanthine oxidase activity and serum uric acid levels. However, the allopurinol has undesirable effects such as fever, skin rash, eosinophilia, hepatitis and renal toxicity. Therefore, the findings of new xanthine oxidase inhibitors with more precise effects and fewer side effects as compared to allopurinol and febuxostat need to be developed in order to inhibit and fight gout and cardiovascular diseases associated with hyperuricemia.

The antioxidant plays an important role in scavenging oxygen free radical that was released during the metabolism of hypoxanthine to xanthine and uric acid (Matata & Elahi, 2007). The superoxide anion and hydrogen peroxide were also generated during the breakdown of hypoxanthine to uric acid, which will lead to the development of hyperuricemia. It is therefore the search of plant-derived antioxidant with anti-hyperuricemic potential provides a fundamental role in the new drug research. The in vitro studies have shown that flavonoids, alkaloids, essential oils, phenolic compounds, tannins, iridoid glycosides and coumarins possessed a dual mechanism as antioxidant and antigout effects via XO inhibitory action. Thus, the research on the dual effect of plant natural phytochemicals would become a promising approach for hyperuricemia treatment.
compounds, antioxidant free radical scavengers and XO inhibition provides a new therapeutic drug for the treatment of hyperuricemia.

The *Pandanus amaryllifolius* from the family of Pandanaceae is commonly known as ‘Pandan’ in Malaysia and has been used traditionally to treat fever, relieving indigestion and flatulence (Cheeptham & Towers, 2002). Ali & Hawa, (2014) and Wang et al. (2012) reported that it contained phenols and flavonoids with antioxidant properties. These suggested that *P. amaryllifolius* has significant dual effects of antioxidants in scavenging free radical activities and highly potential to act as an XO inhibitor to reduce the hyperuricemia effect of uric acid. Therefore, the objectives of this study were to evaluate the dual effect of *P. amaryllifolius in vitro* antioxidant and water as a blank. The results were expressed as mg gallic acid equivalent (mg GAE) g⁻¹ of dry extract.

**Method**

**Collection of plant sample**

The *Pandanus amaryllifolius* leaves were collected from Institute of Biological Sciences garden, University of Malaya, Kuala Lumpur. It authentic was confirmed by plant taxonomist Professor Dr. Ong Hean Chooi from Institute of Biological Sciences, University of Malaya. A voucher specimen no: KLU 49087 was deposited in the Herbarium of University of Malaya, Kuala Lumpur.

**Preparation of plant extracts**

The dried powder of *P. amaryllifolius* was extracted with hexane, petroleum ether, chloroform, methanol and water. The mixture was incubated in a water bath at 40 °C for 8 h. It was then filtered with filtered and concentrated using a vacuum rotary evaporator at 40 °C. The water extract was dried in the freeze dryer machine. All the dried extracts were kept in airtight bottle until further used.

**Total phenolic contents**

The total phenol content and total flavonoid content of *P. amaryllifolius* were determined as described by Ablat et al. (2014). In a 96-well microplate, 20 μL leaf extract was mixed with 100 μL of Folin–Ciocalteu reagent and incubated for five minutes. Then, 75 μL of sodium carbonate solution was added and incubated in darkness for two hours at room temperature. After that, the absorbance was measured at 740 nm using a microplate reader (Tecan Sunrise, Austria). Gallic acid (concentration range: 0.0625–1.0 mM) was used as a standard to construct a linear regression line, and water as a blank. The results were expressed as mg gallic acid equivalent (mg GAE) g⁻¹ of dry extract.

**Total Flavonoid contents**

50 μL of leaf extracts was added into a 96-well microplate along with 15 μL of 5% sodium nitrite solution and 70 μL of distilled water. The mixture was incubated for five minutes at room temperature. Then, 15 μL of 10% aluminium chloride solution was added and incubated for six minutes. Finally, 100 μL of 1 M sodium hydroxide solution was added and the absorbance was measured at 510 nm with a microplate reader (Tecan Sunrise, Austria). Quercetin (concentration range: 0.2–1.0 mM) was used for standard calibration curve. The total flavonoid contents were evaluated according to the linear regression between standard solution and absorbance at 510 nm. The results were estimated as mg quercetin equivalent (mg QE) g⁻¹ of dry extract.

**DPPH radical scavenging activity**

The DPPH radical scavenging activity of *P. amaryllifolius* extracts was determined according to the method as described by Ablat et al. (2014).

**Ferric reducing antioxidant power (FRAP) assay**

The FRAP assay was performed based on the method as described by Ablat et al. (2014).

**Metal chelating activity**

The metal chelating activity was determined according to the method as described by Ablat et al. (2014).

**Hydrogen peroxide scavenging activity**

The *P. amaryllifolius* extract scavenging ability of H₂O₂ molecules was determined based on the method as described by Khan et al. (2012).

**In vitro xanthine oxidase inhibitory activity**

*In vitro* xanthine oxidase inhibitory activity was determined according to the method as described by Azmi et al. (2012). Briefly, 100 μL of extracts (concentration range: 6.25–100 μg mL⁻¹), 300 μL of 50 mM phosphate buffer (pH 7.5) and 100 μL of xanthine oxidase solution (0.1 units mL⁻¹ in 50 mM phosphate buffer, pH 7.5) were incubated for 15 min at 37 °C. The reaction was terminated by adding 200 μL of 0.5 M HCl. The absorbance at 295 nm was measured with UV–vis spectrophotometer-1700 (Shimadzu,
Japan. The inhibitory activity was calculated based on equation (1), and the results were expressed in μg mL⁻¹.

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\text{Xanthine oxidase inhibition (\%) } = \frac{A_{\text{control}} - A_{\text{sample or standard}}}{A_{\text{control}}} \times 100
\] (1)

**Acute toxicity test**

The Sprague Dawley rats of 6–7 weeks old were kept in their cages in the animal house for 2 weeks for adaptation to the standard laboratory conditions. A total of six rats (three male and three female) were used to receive a single oral dose (2000 mg kg⁻¹) of PA-W prepared using distilled water as the vehicle. The rats were kept overnight fasting prior to extract administration. The next day, the weights of the rats were recorded and the extract was administered via oral gavage. After extract administration, food but not water was withheld for further 3–4 h. Observation was performed to individual rat during the first 24 h with extra attention to the first 4 h. For the next 14 days, they were observed daily.

**Experimental animals**

The Sprague Dawley male rats of 6–7 weeks old and 150–130 g weight were used in the hypouricemic activity experiment. The rats were purchased from University of Malaya animal house. The male rats were housed in their cages for 2 weeks prior to dosing to allow for acclimatisation to the laboratory condition according to OECD 423 guidelines. They were maintained under standard laboratory conditions throughout the experiment with the sequence of lighting being 12 h light and 12 h dark, under room temperature (37 °C). Conventional rat pellet diet and unlimited supply of tap water were used for feeding. The protocol used in this experiment fulfilled the ethical code sanctioned by the Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC), University of Malaya (Ethic no: 2015-180908/IBS/R/NAAS).

**Animal experimental design**

Hyperuricemic rat model was assigned to this experiment by intraperitoneal (i.p.) injection of the uricase inhibitor, oxonic acid potassium salt (280 mg kg⁻¹ single dose) according to the method described by Yonetani et al. (1980). The oxonic acid potassium salt solution was prepared by suspending it in 0.9% sodium chloride (NaCl) solution.

**Hypouricemic activity study**

Hypouricemic efficacy of PA-W was investigated using the method described by Umamaheswari et al. (2007) with a few modifications. The high and low doses were selected based on the range of maximum acute toxic dose of 2000 mg kg⁻¹ body weight. The high dose of 1000 mg kg⁻¹ and lower dose of 500 mg kg⁻¹ were the safe dose for hypouricemic animal study. In this experiment, 36 male rats were divided into six groups (n = 6). Group I was normal control group that received 0.5% CMC solution orally. Group II only received high-dose PA-W (1000 mg kg⁻¹) via oral gavage. Group III was the hyperuricemic control that intraperitoneally administered with oxonic acid potassium salt (280 mg kg⁻¹, i.p.). Rats of groups IV and V received high dose (1000 mg kg⁻¹) and low dose (500 mg kg⁻¹) of PA-W by oral gavage, respectively. Group VI was given oral treatment of allopurinol (10 mg kg⁻¹ body weight). PA-W was dissolved in vehicle distilled water while allopurinol was dissolved in 0.5% CMC solution. One hour prior to extracts and allopurinol administration, hyperuricemic rats in groups III, IV, V and VI were injected with oxonic acid potassium salt (280 mg kg⁻¹, i.p.) to elevate the serum uric acid level. One hour after extract and allopurinol administration, small blood samples were collected by tail prick to measure serum urate level using MultiSure Uric Acid Meter and the results were expressed as mg dL⁻¹. The rats were executed, and whole blood samples were collected through intracardiac puncture where the blood was allowed to clot and serum separated. The xanthine oxidase activity of the collected serum was determined using Xanthine Oxidase Activity Kit purchased from Sigma-Aldrich (St. Louis, MO, USA), and the results were expressed as milliunit mL⁻¹.

**Statistical analysis**

The data values were represented as mean ± SEM (standard error of mean) for three replicates. Data were analysed using one-way ANOVA followed by Dunnett’s test for multiple group comparison. The statistical significance was at P < 0.001. The correlation between TPC, TFC and antioxidant activities was assessed using Pearson’s correlation test (P < 0.01 and P < 0.05).

**Results**

The total phenol content of P. amaryllifolius extracts of PA-W at 12.88 ± 0.43 mg GAE g⁻¹ dry extract showed higher phenolic content as compared to PA-M at 11.98 ± 0.40 mg GAE g⁻¹ dry extract, followed by PA-C at 6.42 ± 0.49 mg GAE g⁻¹ dry extract, PA-PE...
at 3.65 ± 0.26 mg GAE g⁻¹ dry extract and PA-H at 2.32 ± 0.27 mg GAE g⁻¹ dry extract. The results of a TPC showed that with the increasing polarity of the solvent, the amount of phenolic compounds increased.

The total flavonoid contents of *P. amaryllifolius* extracts of PA-PE at 15.02 ± 0.58 mg QE g⁻¹ dry extract possessed the highest flavonoid content followed by PA-H at 11.66 ± 1.01 mg QE g⁻¹ dry extract, PA-C at 9.19 ± 1.53 mg QE g⁻¹ dry extract, PA-M at 9.07 ± 0.36 mg QE g⁻¹ dry extract and PA-W at 7.66 ± 1.10 mg QE g⁻¹ dry extract. TFC was found higher in solvent with low polarity of PA-PE and PA-H (Table 1).

The results in Fig. 1 indicate that PA-W showed the highest DPPH inhibition followed by PA-M, PA-PE and PA-H.

The FRAP value of PA-H, PA-PE, PA-C, PA-M and PA-W is 21.21 ± 2.17, 28.64 ± 1.86, 35.91 ± 2.23, 64.39 ± 2.79 and 54.55 ± 1.48 mmol Fe²⁺/g of dry extract, respectively. The reaction involves the formation of blue colour Fe²⁺-TPTZ form in the extracts after the antioxidants react with Fe³⁺-TPTZ complex. The highest FRAP value was detected in PA-M with 64.39 ± 2.79 mmol Fe²⁺/g of dry extract. The reducing ability of PA-H was the lowest as it only showed 21.21 ± 2.17 mmol Fe²⁺/g of dry extract. The reducing power was detected higher in polar solvent as compared to nonpolar solvent.

In metal chelating assay, PA-W portrayed the strongest effectiveness in inhibiting the formation of Fe³⁺-ferrozine complex followed by PA-H, PA-M, PA-PE and PA-C (Fig. 2). The extracts showed metal chelating capability in a dose-dependent manner.

Hydrogen peroxide assay revealed that only PA-W and PA-M possess H₂O₂ scavenging capability among four *P. amaryllifolius* extracts tested (Fig. 3).

The ability of *P. amaryllifolius* extracts in inhibiting xanthine oxidase activity showed that only PA-W, PA-M and PA-H possessed xanthine oxidase inhibition ability with PA-W having the highest activity (>100 μg mL⁻¹) (Fig. 4). As PA-W exhibited the most expressive outcome compared to other extracts, it was chosen to be administered on rats for *in vivo* xanthine oxidase activity.

From the Pearson’s correlation tests summarised in Table 2, TPC of *P. amaryllifolius* extracts showed positive significant correlation with DPPH radical scavenging activity (r = 0.972) and FRAP assay (r = 0.964) at 0.01 level. There was also positive significant correlation between TPC and hydrogen peroxide scavenging activity (r = 0.898) at 0.05 level. TPC demonstrated no correlation with metal chelating activity (r = 0.382) and *in vitro* xanthine oxidase inhibitory activity (r = 0.809). On the other hand, TFC showed negative correlation with all assays.

Sprague Dawley rats of both sexes were fasted overnight before the administration of PA-W by oral gavage. After the study period of 14 days, it was found that the rats were safe at the dose of 2000 mg kg⁻¹. The rats showed normal behaviour pattern throughout the test. There were no signs of toxicity detected and zero occurrence of mortality in all rats. Based on this result, 1000 mg kg⁻¹ (high dose) and 500 mg kg⁻¹ (low dose) were selected for subsequent hypouricemic study.

Administration of oxonic acid potassium salt to rats (280 mg kg⁻¹ body weight, i.p.) caused a significant (P < 0.001) increase in serum uric acid level of Group 3 rats (16.57 ± 2.98 mg dL⁻¹) when compared to normal control rats (Group 1) after two hours (Table 3). Rats in Group 4 and Group 5 that received 1000 mg kg⁻¹ and 500 mg kg⁻¹ dose of PA-W portrayed significant (P < 0.001) decrease in the serum urate level up to 2.55 ± 2.23 mg dL⁻¹ and 6.08 ± 1.00 mg dL⁻¹, when compared to hyperuricemic control, respectively. This showed that PA-W possesses a dose-dependent hypouricemic action in hyperuricemic rats. Group 6 rats treated with allopurinol also significantly reduced the serum uric acid level in hyperuricemic rats and showed practically similar serum urate level as normal control group (Fig. 5).

The results in Fig. 6 showed that Group 3 possessed the highest XO activity followed by Group 5 and Group 4. Group 1, Group 2 and Group 6 exhibited low XO activity (Fig. 6). When compared to a normal control group (1.77 ± 0.43 μmL⁻¹), serum of hyperuricemic rats in Group 3 displayed significant (P < 0.001) increase in XO activity with 10.72 ± 1.17 μmL⁻¹ (Table 4) because of the oxonic acid potassium salt. XO activity in the serum of hyperuricemic rats treated with PA-W (high and low dose) and allopurinol was significantly reduced when compared to hyperuricemic control (Group 3) with 3.84 ± 0.68 μmL⁻¹, 6.35 ± 0.87 μmL⁻¹ and 1.06 ± 0.21 μmL⁻¹, respectively.

**Discussion**

Total phenolic content (TPC) and total flavonoid content (TFC) of *P. amaryllifolius* Roxb. extracts were

| Table 1 | TPC and TFC of *Pandanus amaryllifolius* extracts |
|---------|-----------------------------------------------|
|         | TPC (mg GAE g⁻¹ dry extract) | TFC (mg QE g⁻¹ dry extract) |
| PA-H    | 2.32 ± 0.27                     | 11.66 ± 1.01                 |
| PA-PE   | 3.65 ± 0.26                     | 15.02 ± 0.58                 |
| PA-C    | 6.42 ± 0.49                     | 9.19 ± 1.53                  |
| PA-M    | 11.98 ± 0.40                    | 9.07 ± 0.36                  |
| PA-W    | 12.88 ± 0.43                    | 7.66 ± 1.10                  |
determined to quantify total phenolics and flavonoids available in each extract. According to Karimi et al. (2011), phenolics are prominent secondary metabolites that exhibit extensive pharmacological actions while flavonoids are crucial secondary metabolites that possess a number of bioactive compounds in plants. The
PA-W possessed the highest TPC while the highest TFC is represented by PA-PE. Based on Pearson’s correlation test carried out, TPC and TFC demonstrated negative correlation. Both of these experiments did not reflect on one another. This is because the amount of flavonoids was not in agreement with the amount of phenols represented by each extract. TFC was found higher in extracts of nonpolar solvents. On the other hand, TPC was found higher in extracts of polar solvents. This supports the study by Ablat et al. (2014) as they found that the amount of TPC increased as the polarity of the solvent. According to Kim et al. (2012), the extractants used in the extraction process had an impact on the TPC of extracts. Based on the detection of phytochemical compounds using GC-MS, the highest phenolic content in PA-W may be contributed by the presence of benzoic acid and cinnamic acid. Meanwhile, the present of benzoic acid may lead to the second highest phenolic content rank represented by PA-M. According to Sáyago-Ayerdí et al. (2007), more than one method is required to assess antioxidant potential. Determination of antioxidant activity was carried out using DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) assay, metal chelating activity and hydrogen peroxide scavenging activity. According to Oskoueian et al. (2011), antioxidants aid in obstructing oxidative damage and phenolics can exhibit higher activity than vitamins, but its activity relies on their chemical structure and number of hydroxyl groups. Malta et al. (2011) stated that high amount of phytochemicals such as phenolics, flavonoids, terpenoids and other result in high antioxidant activity. In this study, the antioxidant activities of *P. amaryllifolius* Roxb. extracts were affected by the nature of solvent used.

Bajpai et al. (2017) claimed that DPPH scavenging activity is performed extensively by researchers to estimate antioxidant capabilities within short period of time than other assays. In this study, DPPH assay revealed PA-W as the most potent candidate in scavenging DPPH free radicals. From the result obtained, *P. amaryllifolius* Roxb. extracts showed increasing activity with increasing polarity of the solvent. This could be linked with the TPC results because based on Pearson’s correlation test conducted, there is a significant correlation between TPC and DPPH assay. Therefore, this suggests that phenolic compounds may contribute to DPPH radical scavenging effects in *P. amaryllifolius* Roxb. extracts. Thus, this showed that extraction using different solvents leads to different capability of antioxidant scavenging activity due to varying phytochemical composition present in each extract. Few other studies also proved a significant correlation between TPC and DPPH assay (Ng et al., 2012; Tiveron et al., 2012; Ablat et al., 2014; Saha & Verma, 2016).

Ferric reducing antioxidant power (FRAP) assay is measured via the principle of reducing ferric (III) to ferrous (II) ion (Sahgal et al., 2009). FRAP assay of *P. amaryllifolius* Roxb. extracts also exhibited increasing activity as the solvent polarity increased. Just like

![Figure 4](https://wileyonlinelibrary.com/)

**Figure 4** Effect of *Pandanus amaryllifolius* extracts on *in vitro* xanthine oxidase inhibitory activity. [Colour figure can be viewed at wileyonlinelibrary.com]

| Assays                             | Correlation coefficients (r) |
|------------------------------------|-----------------------------|
|                                    | TPC                         | TFC                         |
| TPC                                | –                           | –                           |
| TFC                                | –0.786                      | –                           |
| DPPH radical scavenging activity   | 0.972*                      | –0.761                      |
| FRAP assay                         | 0.964*                      | –0.689                      |
| Metal chelating activity           | 0.382                       | –0.350                      |
| Hydrogen peroxide scavenging activity | 0.898†                     | –0.691                      |
| *In vitro* xanthine oxidase inhibitory activity | 0.809                     | –0.684                      |

*Correlation is significant at the 0.01 level (two-tailed). †Correlation is significant at the 0.05 level (two-tailed).
DPPH, this result associated with TPC as FRAP assay showed significant correlation with TPC. This result is in agreement with other studies that discovered positive significant correlation between TPC and FRAP assay (Gan et al., 2013; Ku et al., 2014; Hanis Mastura et al., 2017). Study by Kabouche et al. (2007) stated that a few terpenoids displayed effective reducing power in FRAP assay. As terpenoids were detected in all five extracts during TLC experiment, this advocates the role of terpenoids in Pandanus amaryllifolius Roxb. as reductants that donates an electron, transforming Fe³⁺ to Fe²⁺.

Metal chelating activity evaluates the ability of antioxidant in chelating ferrous ion. The system is dose dependent whereby higher sample concentration results in higher chelating activity. In our body, ferrous ion (Fe²⁺) leads to the formation of reactive oxygen species which can harm body cells (Yamaguchi et al., 1988). The metal chelating mechanism involves the formation of ferrous-ferrozine complex from the chelating activity of Fe²⁺ by extracts. In this assay, P. amaryllifolius Roxb. extracts were studied for its ability to compete with ferrozine for ferrous ion and the chelating ability was measured via absorbance detected at 562 nm with the formation of Fe²⁺–ferrozine complex. Andjelković et al. (2006) stated that phenolics in plants may not be able to chelate iron as good as EDTA. The order of ferrous ion chelating activity is PA-W > PA-H > PA-M > PA-PE > PA-C. The result from this assay showed no correlation with TPC. This implies that the amount of phenolics in Pandanus amaryllifolius Roxb. extracts did not affect metal chelating activity. Study by Taherkhani (2016) also found that TPC and metal chelation activity possessed no correlation. On the other hand, metal chelating activity showed negative correlation with TFC. This suggests that flavonoids do not appear to be effective ferrous ion chelators.

### Table 3 Serum uric acid level in hypouricemic study

| Group | Condition | Treatment | Serum uric acid level (mg dL⁻¹) |
|-------|-----------|-----------|---------------------------------|
| 1     | Normal rats | Control   | 1.00 ± 0.00                     |
| 2     | Normal rats | High dose of PA-W [1000 mg kg⁻¹] | 1.80 ± 0.13                     |
| 3     | Hyperuricemic rats | Control | 16.57 ± 2.98*                  |
| 4     | Hyperuricemic rats | High dose of PA-W [1000 mg kg⁻¹] | 2.55 ± 0.23b                  |
| 5     | Hyperuricemic rats | Low dose of PA-W [500 mg kg⁻¹]  | 6.08 ± 1.00b                   |
| 6     | Hyperuricemic rats | Allopurinol [10 mg kg⁻¹]    | 1.72 ± 1.01b                  |

Values are expressed as mean ± SEM (n = 6).

*P < 0.001 significant when compared to normal control.

**P < 0.001 significant when compared to hyperuricemic control.
Hydrogen peroxide is nonreactive; however, it may possess toxic effect to cells by transforming into hydroxyl radicals that react with biomolecules. This results in the destruction of body tissues as well as the death of cells (Khan et al., 2012). Result from this assay proposed that there is a significant correlation between TPC and hydrogen peroxide scavenging activity, similar to DPPH assay. This indicates that high phenolic content lead to high scavenging activity of hydroxyl radicals. A number of studies also revealed the presence of strong correlation between TPC and hydrogen peroxide scavenging activity (Chakraborty et al., 2013; Khan et al., 2012; Kumar et al., 2017). Meanwhile, TFC exhibited negative correlation with H$_2$O$_2$ scavenging activity. Tiveron et al. (2012) stated that each phenolic and nonphenolic compounds possessed varying antioxidant potential by which high amount of particular compounds will not necessarily assure potent antioxidant effects.

*In vitro* study was conducted to determine the ability of *P. amaryllifolius* Roxb. extracts to retard the activity of xanthine oxidase enzyme. According to Umamaheswari et al. (2009), phenols and flavonoids present in extracts have the ability to express inhibitory activity of xanthine oxidase. The highest xanthine oxidase inhibitory activity was shown by PA-W, followed by PA-M and lastly PA-H. Two other extracts which are PA-PE and PA-C have shown no inhibition activity. This is possibly because of inadequate specific compounds that possess xanthine oxidase inhibition capability in the extracts (Azmi et al., 2012).

The PA-W was selected and screened for *in vivo* hypouricemic activity. Azmi et al. (2012) stated that in a large scale, high amount of solvents is needed and it is wiser to use the most economical solvent in the extraction process while considering its safety measure. As methanol is toxic to the body as it can interfere the physiological acid–base balance, solvents with lower toxicity with same potential should be utilised wherever possible. Water as the world’s universal solvent is crucial for every living thing. It is easily obtainable, pretty well significant and cost-effective.

Based on the results obtained, PA-W exhibited significant hypouricemic activity for both high (1000 mg kg$^{-1}$) and low (500 mg kg$^{-1}$) dosage. The allopurinol still possessed the most outstanding effect on both *in vitro* xanthine oxidase inhibitory activity and *in vivo* hypouricemic activity. Allopurinol is widely used and easily available to treat hyperuricemia, gout and other inflammatory ailments. However, due to hypersensitivity, intolerance and failure of the treatment on certain patients (Lü et al., 2013), alternative therapeutic agents especially xanthine inhibitors in natural compounds are recommended as it displays fewer side effects (Wong et al., 2014). In this experiment, dose-dependent PA-W possessed practically comparable effect with allopurinol in reducing serum uric acid level and XO activity. Umamaheswari et al. (2007) stated that the presence of phytoconstituents in extracts along with their anti-inflammatory and antioxidant activities manifests a notable XO inhibitory activity and hypouricemic activity. However, TPC showed no correlation with *in vitro* xanthine oxidase inhibitory activity in this study. According to Kostić et al. (2015), the way to treat gout and hyperuricemia is by elevating the uric acid excretion or minimising the production of uric acid. The antioxidant and XO inhibitory activities of the PA-W leaf extract were due to the presence high amount of total phenol and flavonoids in the *Pandanus amaryllifolius*. Ghasemzadeh & Jaafar (2013) have reported high total phenols and flavonoids in the *Pandanus amaryllifolius*. Similarly, the total phenol contents were reported high amount of *Pandanus amaryllifolius* (Sheila et al. 2011; Singh & Parle, 2015). As PA-W has significantly and successfully reduced the uric acid level and inhibited XO activity in hyperuricemic rats serum, this indicates that the use of PA-W may be beneficial in gout and hyperuricemia therapy. This contributes to the beginning of further examination on this species to isolate its active constituents and invention of drug.

**Conclusions**

*Pandanus amaryllifolius* Roxb. extracts contain a number of phytochemicals which include phenolic and flavonoid compounds. These bioactive compounds possess antioxidant activities and display an

| Group      | Condition       | Treatment                     | XO activity (µ mL$^{-1}$) |
|------------|-----------------|-------------------------------|---------------------------|
| 1          | Normal rats     | Control                       | 1.77 ± 0.43               |
| 2          | Normal rats     | High dose of PA-W [1000 mg kg$^{-1}$] | 1.46 ± 0.41               |
| 3          | Hyperuricemic   | Control                       | 10.72 ± 1.17$^a$          |
| 4          | Hyperuricemic   | High dose of PA-W [1000 mg kg$^{-1}$] | 3.84 ± 0.68$^b$          |
| 5          | Hyperuricemic   | Low dose of PA-W [500 mg kg$^{-1}$] | 6.35 ± 0.87$^b$          |
| 6          | Hyperuricemic   | Allopurinol [10 mg kg$^{-1}$]  | 1.06 ± 0.21$^b$          |

Values are expressed as mean ± SEM ($n = 6$).  
$^aP < 0.001$ significant when compared to normal control.  
$^bP < 0.001$ significant when compared to hyperuricemic control.
outstanding impact in healing numerous diseases. In antioxidant assays, extracts that were extracted using different solvent polarity displayed different level of antioxidant capability. PA-W acts as the most potent candidate in this research study. It exhibited the highest effectiveness in both antioxidant activity and xanthine oxidase inhibitory activity compared to other extracts. PA-W and PA-PE contributed the highest TPC and TFC, respectively. PA-W possessed the highest inhibition in DPPH radical scavenging activity, metal chelating activity and hydrogen peroxide scavenging activity. PA-M showed the most potent reducing power in FRAP assay. *In vitro* xanthine oxidase inhibitory activity displayed PA-W as the strongest xanthine oxidase inhibitor. PA-W also had significantly reduced serum uric acid level and inhibited xanthine oxidase activity in *in vivo* xanthine oxidase inhibitory. The results obtained from this study suggest *P. amaryllifolius* Roxb. as a novel xanthine oxidase inhibitor derived from natural sources shows potent activity and low toxicity in the future.

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**Conflicts of interest**

The authors declare no conflict of interest.

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