Antioxidant activity, total phenolics and flavonoids contents of
Pandanus amaryllifolius (Roxb.)

N T C Quyen1,2, N T N Quyen1,2, L T H Nhan3 and T Q Toan3,4,*

1NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
2Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
3Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam
4Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
5Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
*Corresponding author: tranquoctoan2010@gmail.com

Abstract. Pandanus amaryllifolius Roxb. was commonly used in Vietnam as a fragrance because of its aroma. These aromatic plants are the primary source of medicinal herbs because of their valuable biological activities. This study was aimed at determining the phytochemical content, Total flavonoids contents (TFC), total phenolic content (TPC) and antioxidants were analyzed by Folin-Ciocalteu and DPPH scavenging assays, respectively. The total flavonoid content is measured by the aluminum chloride method. The qualitative phytochemical analysis confirmed the presence of flavonoids, terpenoids, coumarin, and reducing sugar, which is chemical components in Pandanus amaryllifolius ext rac. Results showed that ethanol extract exhibited higher DPPH (129.327 ug/ml) and TPC (38.12 mgGAE/DW) activity than water extraction (265.738 ug/ml and 10.97 mgGAE/DW). Research shows that using this plant more in pharmaceuticals or food should be focused and developed.

1. Introduction
Recently, utilizing plants as a means of food and medicine has reawakened global awareness [1-5]. Most plants are the primary source of raw materials for pharmaceutical products, agrochemicals, aromas, fragrances, food additives, and pesticides [6-10]. Depending on the different plants, there will be distinct secondary compounds and their respective characteristics [11-15]. Phenolic and flavonoid compounds are a common secondary metabolite group with broad pharmacological activity and are source of natural antioxidants in plants [16-19]. They are referred to as a biologically produced substance with antioxidant effects and the potential to drive out free radicals by reducing the risk of human diseases, including degeneration, cardiovascular or cancer [20-22]. Previous reports show that the activity of inhibiting cancer cells or creating mechanisms that can destroy cancer cells while preventing tumor growth and invasion is expressed by flavonoid components effective [23,24]. Flavonoids also have the ability to boost the immune system in humans, while helping to reduce blood glucose and lipids [25].

Pandan (Pandan amaryllifolius Roxb.) is a tropical species in the screw pine genus belonging to the Pandanaceae family. Although the Pandanaceae has about 600 species, only Pandanus
amaryllifolius Roxb and another species with fragrant leaves are primarily found in the tropical and subtropical regions [26]. Pandan is an evergreen herb grown to harvest the leafy part. They are commonly used in food in Asia. In Vietnam, due to the sweet taste and the fresh scent of this plant, it is used to create flavors for the processing of dishes such as rice, juice, desserts, candy, ice cream. Besides, Pandan leaves are also used as natural colorings due to their abundant chlorophyll [27]. In recent years, knowledge of the use of nutrient-rich organic plants has been increasing (especially in developing countries). It is believed that people can make the most of the ingredients. However, previous reports have not exploited the diverse data of typical Pandan materials in different growing areas [28-30]. Therefore, this study conducted a phytochemical assessment of Pandan species in Vietnam. Moreover, it also performed the quality of phenolic compounds and their antioxidant behavior to provide practical knowledge for the dataset.

2. Materials and method

2.1. Chemicals and plant materials

Pandanus amaryllifolius leaves are randomly collected in Go Vap District, Ho Chi Minh City, Vietnam. Conducting preliminary classification, washed, dried completely at 60°C and finely ground into powder. 10g Pandanus amaryllifolius leaf powder was extracted with two different solvents, ethanol and water respectively, at temperatures of 70°C for 1 hour. The solution obtained after the extract was filtered and evaporated to obtain the dry material. Store the dried ingredients in an airtight container at 40°C until the next test was carried out. Chemicals used for analysis such as: Folin-Ciocalteu, absolute ethanol and other chemicals are purchased in Merk (Germany) or Xilong (China) based on standards for each experiment.

![Raw materials Pandanus amaryllifolius](image)

Figure 1. Raw materials Pandanus amaryllifolius a) before grinding and b) after processing and pulverizing

2.2. Phytochemical screening

Crude ethanolic extracts of bark and leaves have been tested for alkaloid, tannin, anthraquinon, anthraquinon, flavonoid, terpenoid, coumarin, saponin and reducing sugar. Since ethanol is a polar solvent (as opposed to butane), it blends readily with water, breaking down water-soluble molecules like chlorophyll. The numerical tests for the inclusion are represented as (+), and (-) for the absence of phytochemicals. Alkaloid test with Mayer, Bouchardat, Dragendorff test. Flavonoid test with H₂SO₄ and Wilstatter test. Testing anthraquinones and tannins with color transfer reaction. Coumarin test with fluorescence reaction. Terpenoid test with Liebermann- Burchard and Salkowski test. Saponin test with foam test. Sugar reduction test with precipitation test.
2.3. Total Phenolic Contents (TPC)
The method was conducted based on previous research [13]. Continue with the extraction process to achieve the concentration needed. Afterward, 0.5 ml of diluted sample solution was drawn into a test tube. It was then added 2.5 ml of 10% Folin-Ciocalteu solution and homogenized using a Vortex machine and left to react for 5 minutes in the dark. Next, add 2 ml of 7.5% Na₂CO₃ solution and shake well, put in the dark for 1 hour. Finally, measure the optical absorbance at 756 nm on the UV-Vis spectrophotometer. Use gallic acid as a standard and express polyphenol content in micrograms of gallic acid equivalent per 1 mg of extract (µgGAE/mg extract).

2.4. Total flavonoids contents (TFC)
The method was conducted based on previous research [31]. 0.5 ml of the diluted sample solution was put into a test tube. It was then added 0.1 ml of 10% AlCl₃. 0.1 ml of CH₃COOK 1M was mixed with 4.3 ml of distilled water. Next, the solution was put at RT for 30 minutes. Then measure the optical absorbance at 415 nm on the UV-Vis spectrophotometer. Quercetin is appropriate for usage. The total flavonoids is contained in quercetin equivalent micrograms in 1 mg of extract (µgQE/mg extract) [31].

2.5. Qualitative analysis of antioxidant activity of Pandanus amaryllifolius Free radical removal method DPPH (1,1-diphenyl-2-picrylhydrazyl)
The extraction obtained from the previous extraction was diluted to a reasonable concentration. 0.5 ml of diluted sample was then put into a test tube. Control sample was ethanol extract (99.5%). Then, add a tube of 1.5 ml DPPH solution (OD517 nm = 1.1 ± 0.02) to a test tube and leave in the dark for 30 minutes. Measure optical absorbance at 517 nm on UV-Vis spectrophotometer. Vitamin C (ascorbic acid) is used as the reference standard. The following formula is used to determine DPPH free radical scavenging operation (IC%):

\[
IC(\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100
\]

Inside:
Absₐ: Optical absorbance of the control sample
Absₜ: Optical absorbance of the sample
The result is reported based on the IC₅₀ value, which is the concentration at which the sample removes 50% of DPPH free radicals.

2.6 Free radical removal method ABTS (2,2'-azino-bis)
The free radical solution ABTS was prepared by adding 10 ml of ABTS solution of 7.4 mM into 10 ml of K₂S₂O₈ solution of concentration of 2.6 mM and incubating in the dark for 24 h, then diluting with ethanol and then adjusting the absorbance of the solution at a wavelength of 734 nm to 1.1 ± 0.02. Dilute the extract to the appropriate concentration, collecting 0.5 ml of diluted sample extract into a test tube. Control sample was ethanol (99.5%). Afterward, add 1.5 ml ABTS solution (OD517 nm = 1.1 ± 0.02) to a test tube and leave in the dark for 30 minutes. Measure optical absorbance at 734 nm on UV-Vis spectrophotometer. Vitamin The reference standard has been used for C (ascorbic acid). The ABTS (IC%) is carried out by the following formula:

\[
IC(\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100
\]

Inside:
Absₐ: Optical absorbance of the control sample
Absₜ: Optical absorbance of the sample
The result is stated based on the IC₅₀ value, which is the sample concentration, which removes 50% of the free ABTS radicals.
3. Results and discussion

3.1. Phytochemical screening

Table 1 illustrated the phytochemical component evaluation of Pandanus amaryllifolius leaf extract, including alkaloid, flavonoid, terpenoid, coumarin, saponin, and reducing sugar. This result shows the presence of most valuable secondary metabolic compounds in Pandanus amaryllifolius leaves. The phytochemical properties of the samples are correlated with their biological activity properties. The bulk of plant antioxidant production is attributed to the action of phenolic compounds [32]. Flavonoids are widely available in plants. Moreover, they have a different correlation between reducing the risk of cancer and heart disease when increasing flavonoid intake [33]. Because of their popular biological activities, they are also much applied in medicines. Besides, terpenoids are also known as antibiotics, used as pesticides, and disinfectants in the medical industry [34].

| Chemical composition group | Alcohol extract | Water extract |
|----------------------------|-----------------|--------------|
| Alkaloid                   | -               | +            |
| Tannin                     | -               | -            |
| Anthraquinon               | +               | -            |
| Flavonoid                  | +               | +            |
| Terpenoid                  | +               | +            |
| Coumarin                   | +               | +            |
| Saponin                    | +               | -            |
| Reducing sugar             | +               | +            |

Phenolic and polyphenol compounds form a massive family of important secondary metabolic compounds in plants. Previous studies have reported that the Folin-Ciocalteu method allows accurate measurement of TPC. The yellow to green color shift of the Folin reagent shows that the Folin reagent reacts similarly between groups of different phenolic compounds [35].

In parallel, the total content of flavonoids is measured using the aluminum chloride method. Aluminum chloride will form a stable complex with carbonyl groups in C4 and hydroxyl in C3 (flavonol) and C5 in flavonols and flavones. It may also form an unstable acid complex with hydroxyl at the ortho position within B of the flavonoid [36],[37]. Calibration curve for gallic acid at concentrations of 0.2022; 0.4103; 0.5974; 0.7732; 0.9413 mg/ml and calibration curve for quercetin at concentrations of 0.1305; 0.2720; 0.4243; 0.5720 0.7149 was presented in Figure 2. Based on previous reports, the higher TPC and TFC, the greater the antioxidant activity, and the correlation line was linear. The TPC and TFC results of P. amaryllifolius extract were calculated, and the results are shown in Table 2. Analysis of polyphenol content in both extracts showed the TPC content of ethanolic extract (38.12 ± 1.49 mg GAE/g) 3.47 times greater than extract with water (10.97 ± 0.29 mg GAE/g). Similarly, ethanolic (11.79 ± 0.44 mg QE/g) was found to be an effective solvent for extracting TFC higher than water (3.56 ± 0.14 mg QE/g).
Figure 2. Standard calibration curve of (a) gallic acid and (b) quercetin

Table 2. TPC and TFC and antioxidant activities (IC$_{50}$ values) of CA extract

| Sample          | Total polyphenol content (mg GAE/g) | Total flavonoid content (mg QE/g) | IC$_{50}$ value (µg/mL) | DPPH   | ABTS   |
|-----------------|------------------------------------|----------------------------------|-------------------------|--------|--------|
| Ethanolic extract | 38.12 ± 1.49                      | 11.79 ± 0.44                     | 129.32                  | 104.31 |
| Aqueous extract  | 10.97 ± 0.29                       | 3.56 ± 0.14                      | 265.73                  | 204.99 |
| Ascorbic acid   | -                                  | -                                | 3.05                    | 2.51   |

Statistically irrelevant percentages with similar letters in a column (p < 0.05)

Quantitative analysis of DPPH and ABTS free radical capture ability was used to assess the antioxidant capacity of P. Amaryllifolius leaf. The criterion used to measure an extract’s antioxidant function is vitamin C. Sample concentration at which the inhibition level is 50% is its IC$_{50}$ value. IC$_{50}$ is negatively correlated with antioxidant activity as it describes the amount of antioxidant required to reduce its radical concentration by 50%. The lower IC$_{50}$ amount, the higher the test sample’s antioxidant content. Figure 3 and Table 2 display the findings obtained from the antioxidant activity. It shows that the antioxidant activity of the ethanolic extract using ethanolic solvent requires 129.32 µg/mL to capture 50% of the radicals by DPPH. In contrast, aqueous extract showed a 2.05 times less effective radical reduction (265.73 µg/mL). ABTS testing depends on the ability of the ABTS radical cleaning antioxidant compound. The outcome for ABTS determined from the calibration graph was linear with an R$^2$ value of 0.9999 in the calibration range. The IC$_{50}$ value of the CA extract from alcohol presents that the ability to remove free radicals is almost two times higher than that extracted from water. While the ability to remove free radicals is less than vitamin C, it still demonstrates the tremendous potential to harness and utilize this product’s antioxidant capability.
Figure 3. DPPH free radical scavenging activity from *Pandanus amaryllifolius*; a) ethanolic extract, b) water extract and c) ascorbic acid.
Figure 4. ABTS free radical scavenging activity from *Pandanus amaryllifolius*; a) ethanolic extract, b) water extract and c) ascorbic acid.

4. Conclusion
This study demonstrated the presence of secondary plant chemicals like alkaloids, flavonoids, terpenoids, coumarins, saponins, and sugar reduction in *Pandanus amaryllifolius extraction*. The results also illustrated a high phenolic and flavonoid content in the extract with ethanolic solvent, achieved 38.12 ± 1.49 mgGAE/g and 11.79 ± 0.44 mgQE/g, respectively. Moreover, results presented that ethanol extract exhibited DPPH (129.327 ug/ml) and TPC (38.12 mgGAE/DW) activity, which was higher than that of extraction by water extraction (265.738 ug/ml and 10.97 mgGAE/DW). Together with the DPPH and ABTS free radical scaffolding, confirms the correlation between the concentration of phenolic compounds and their antioxidant activity.

Acknowledgment
This work was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

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