Microwave-assisted extraction of chlorophyll and polyphenol with antioxidant activity from *Pandanus amaryllifolius* Roxb. in Vietnam

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Abstract. *Pandanus amaryllifolius* Roxb., also called as pandan leaf, is distributed mainly in hot and humid climate areas. Pandan leaf, grown very popular in Vietnam, contains many biological value compounds such as chlorophyll and polyphenol with oxidation prevention activity. Microwave-assisted extraction (MAE) has advantages such as reduced extraction time and high extraction efficiency in comparison to other extraction technologies. The aim of this study was to assess the effect of microwave-assisted extraction conditions on chlorophyll and polyphenol content as well as antioxidant activity of pandan leaf. Research results show that acetone solvents of 90°, pandan powder : acetone ratio of 1:30, microwave capacity of 300W, microwave-assisted time of 2 minutes are parameters for the highest extraction efficiency. With these conditions, chlorophyll a, b and total chlorophyll content are respectively 9.4278 μg/mL, 4.2460 μg/mL, 13.6738 μg/mL, polyphenol content is 2.7577 g/L and DPPH radical collection activity 1409.51 μmol/L.

Keywords: microwave-assisted extraction, *Pandanus amaryllifolius* Roxb., chlorophyll, polyphenol, DPPH, radical-scavenging activity

1. Introduction

*Pandanus amaryllifolius* Roxb., also known as pandan leaf [6], belongs to the Pandanaceae family, Pandanus genus. There are about 700 species of which 52 species are found in the Philippines but only *Pandanus amaryllifolius* Roxb. and *Pandanus odoratissimus* Linn. are scented species [7], [8]. *Pandanus* genus is grown primarily in hot and humid climate areas such as Africa and the Pacific. *Pandanus* genus leaves are called with different names such as pandan wangi (Malaysia), Toei hom (Thailand), daun pandan (Indonesia), pandan mabango (Philippines), and sticky leaves (Vietnam)[7]. *Pandanaceae* family trees are tall or brush-like plants [9]. The *pandanus* genera are dust-sized trees that are 1 m to 20 m high, with broad foliage and moderately developed [9]. Pandan leaves are pale green to dark green, with a leaf's length of about 30 cm and a width of about 6 cm [9]. Pandan leaves (*Pandanus amaryllifolius* Roxb.) do not produce fruit because they undergo vegetative propagation [10]. Since their sufficient chlorophyll content, pandan leaves that are also a common green color is used in foods [11].
The important chemical component to create the fragrance of pandan leaves is 2-acetyl-1-pyrroline, the volatile flavoring components include hexanone, 2-hexanone, 3-methyl pyridine, 2-penten-1-ol, nonanone, benzaldehyde and linalool [12]. In some other studies, the chemical composition of pandan leaves also contains maltodextrin [10]. In addition, pandan leaves also contain quercetin [13], carotenoids, tocopherols, tocotrienols, and essential oil content [14]. Besides, pandan leaves comprise phytochemicals such as steroids, carbohydrates, polyphenols, alkaloids, flavonoids, saponins, tannins [15].

Chlorophyll is discriminated by a small reversal of substituents on the B ring from methyl to formyl. Chlorophyll a and chlorophyll b coexist in plants in a 3 : 1 ratio with the dominant methyl (chlorophyll a) variant. Chlorophyll a contains methyl substituents -CH3 which are less stable and have greener properties than chlorophyll b containing formyl substituents -CHO [2]. According to some other studies, plants in shade have a higher proportion of chlorophyll b compared to chlorophyll a. This difference was observed in sunlight of some other types of leaves, as well as when a single species was grown under different light intensities [3].

Polyphenols are a large family of naturally occurring organic compounds that are abundant in plants [4]. Polyphenols are polyhydroxylated phytochemicals and they represent a range of similarly structural compounds. Polyphenols are synthetic by shikimate and acetate/polyketide pathways, and they are plant secondary metabolites. Glucose metabolism produces acetic acid and shikimic acid [5]. The physical and chemical properties of secondary metabolites are distinct so the polyphenol structure is very diverse. Soluble phenolic is present in the vacuoles of plant cells, while insoluble phenolic is found in the cell walls, but the outer layers of plant cells contain higher levels of phenolic than their inner layers [4].

2. Materials and methods

2.1. Material preparation

Pandanus amaryllifolius Roxb. leaves were harvested after 3 months of planting in Long An, Vietnam. Pandan leaves were washed, dried, powdered, sieved and refrigerated at 4℃ during the study. Acetone, ethanol, methanol and Na2CO3 were originated from China; Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox were bought from Sigma Aldrich, USA; second distilled water was prepared before performing experiments in the laboratory.

2.2. Procedure

To evaluate the effect of the extraction conditions, 2 g of pandan leaf powder (Pandanus amaryllifolius Roxb.) was extracted under the investigating conditions - solvents (acetone 90°, methanol 100°, ethanol 96°); microwave powers (100 W, 300 W, 400 W, 800 W); pandan powder-to-solvent ratios (1:15, 1:30, 1:45) and microwave-assisted extraction times (30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes). The extract was descaled by filtering with Whatman No.1 filter paper and then diluted with suitable solvent used in extraction process to 100 mL. The obtained filtrate is used for further analysis of chlorophyll, polyphenol content and DPPH radical-scavenging activity.

2.3. Analytical method

2.3.1. Determination of the method of chlorophyll. Chlorophyll a and b are absorbed with maximum light at 662 - 665 nm, minimum at 645 - 652 nm, which represents the intensity of pigment or amount of chlorophyll in extract. Based on this property, chlorophyll can be quantified by spectrometric method [1]. For each solvent, the chlorophyll content was calculated by the formula:

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\text{Chlorophyll a content} (\mu g/mL) \approx 11.93 \times A_{664} - 1.93 \times A_{647}
\]

\[
\text{Chlorophyll b content} (\mu g/mL) \approx -5.5 \times A_{664} + 20.36 \times A_{647}
\]
Total chlorophyll ($\mu g/mL$) $\approx 6.43 \times A_{664} + 18.43 \times A_{647}$

Calculation formula (using methanol 100°) [29]
- Chlorophyll a content ($\mu g/mL$) $\approx 16.29 \times A_{665} - 8.54 \times A_{652}$
- Chlorophyll b content ($\mu g/mL$) $\approx -13.58 \times A_{665} + 30.66 \times A_{652}$
- Total chlorophyll ($\mu g/mL$) $\approx 2.71 \times A_{665} + 22.12 \times A_{652}$

Calculation formula (using ethanol 96°) [30]
- Chlorophyll a content ($\mu g/mL$) $\approx 11.75 \times A_{662} - 2.53 \times A_{645}$
- Chlorophyll b content ($\mu g/mL$) $\approx -18.61 \times A_{662} - 3.96 \times A_{645}$
- Total chlorophyll ($\mu g/mL$) $\approx 7.79 \times A_{662} + 16.08 \times A_{645}$

2.3.2. Determination of polyphenol content. The total polyphenol content (TPC) was determined by the Folin-Ciocalteu method, the reaction in blue [28]. The obtained extract was diluted with distilled water, 1 mL of diluted solution was drawn into a test tube, 1 ml of Folin-Ciocalteu reagent was added, waited for 5 minutes, then 1 mL of Na$_2$CO$_3$ was added and put in dark place for 30 minutes. Then poured into the cuvette and optically measured at a wavelength of 765 nm.

2.3.3. Determination of the antioxidant activity (DPPH). DPPH (2,2-Diphenyl-picrylhydrazyl) method is used to find antioxidant activity of panda leaf extract. The purple color of DPPH free radicals is changed depending on the antioxidant capacity of the extract, the higher the antioxidant capacity of the extract, the more purple color is lost [19]. The standard curve is contructed by using Trolox. The operation consists of 4 steps as follows. First, extracted solution was diluted with methanol with the appropriate dilution factor. Second, 0.2 mL of diluted solution and 3 mL of DPPH reagent were drawn into a test tube. Third, the reaction was taken place for 30 minutes in the absence of light. Finally, the post-reaction solution was poured into the cuvette for photometric measurement at 515 nm.

2.3.4. Data analysis. All results of the experiment were performed in triplicates. The results are calculated as the average of 3 runs, the values are shown with the standard deviation. IBM SPSS Statistics 20 were used to get data analysis. One-factor variance analysis ANOVA was used to test the mean hypotheses of the sample groups with the probability of error of only 5%.

3. Results and discussion

3.1. Effect of acetone 90°, methanol 100° and ethanol 96° on the extraction process
The solvent kinds have a significant effect on the extracted substance content and antioxidant activity of the extract.

![Figure 1. Effect of solvent kinds on chlorophyll, polyphenol content and DPPH radical-scavenging activity.](image-url)
Figure 1 shows different solvents resulting in different chlorophyll, polyphenol content and DPPH free radical-scavenging activity in the extracts. The chlorophyll a (9.995 ± 0.07 μg/mL), chlorophyll b (5.603 ± 0.15 μg/mL); total chlorophyll (15.53 ± 0.21 μg/mL); polyphenol content (2.6655 ± 0.04 g/L) and DPPH radical removal activity (1456.81 ± 26.62 μmol/L) were the highest values when extracted with acetone 90° and the lowest ones when extracted with ethanol 96°. The relative static permittivity or the dielectric constant of a solvent is a relative measure of its chemical polarity. The difference of the relative static permittivity will lead to the variance polarization of the extraction solvent. The strongly polarized solvent has a large dielectric constant; the less polar the solvent, the smaller the dielectric constant. When the solvents are combined, the relative static permittivity and the polarity of the solvent mixture change, the solubility for the solutes is much greater than that of the individual solvents [20]. Therefore, when combining water with different organic solvents, it is possible to increase the efficiency of extraction of solvents on some extracts. This outcome is similar to the recognized result that the acetone-water mixture is the extraction solvent that get the highest yield with a number of polyphenols, as well as flavonoids and anthocyanins [21]. The research of Ritchie, 2008 on extracting chlorophyll from spinach and some algae by acetone, methanol, ethanol with concentrations respectively of 90°, 100° and 100°, acetone 90° also gives the highest extraction efficiency [30]. The study of chlorophyll extraction on the ingredients Lemna minor (duckweed), Potamogeton crispus, and Egeria densa with acetone 80°, acetone 90° and ethanol 95° also shows that acetone 90° for the highest extraction yield [22].

3.2. Effect of microwave power on the extraction process

The microwave power produces the necessary heat to be applied to the extracted sample, so the microwave power significantly affects the extraction process.

![Figure 2](image)

**Figure 2.** Effect of microwave power on chlorophyll a, b; total chlorophyll; polyphenol content and DPPH radical-scavenging activity.

Figure 2 shows that at 300W the chlorophyll a (9.9944 ± 0.41 μg/mL), chlorophyll b (4.3882 ± 0.162 μg/mL); total chlorophyll (14.3826 ± 0.566 μg/mL); polyphenol content (2.796 ± 0.07 g/L) and DPPH radical removal activity (1390.06 ± 54.19 μmol/L) are the highest values. Each power of microwave oven generates a certain temperature impacting on the sample in the microwave oven. Microwave radiation causes interruption of plant tissue, releasing compounds having in the materials into solvents [23], [24]. Microwave power affects the interaction between particles and solvents, the rate of solvent diffusion into solid samples [18]. Acetone 90° has a boiling point of 57.4°C [25], so at a power of 100 W, the temperature is not sufficient to penetrate all the molecular particles resulting in an extraction efficiency not as high as at 300 W. At the higher microwave power,(400, 600, and 800W) the higher thermal energy causes thermal degradation, reducing chlorophyll, polyphenol content and DPPH radical-scavenging activity. In the study of (Nguyen Thi Hanh, 2016), effect of thermal processing on chlorophyll and vitamin C content in peas (*Pisum sativum*) also shows that when blanching temperature increases to 90°C, chlorophyll content reduces. According to another
research, Mustapa N A also uses the microwave power of 300W to extract chlorophyll from Clinacanthus nutans Lindau (C. nutans) [27].

3.3. Effect of pandan powder : solvent ratio on the extraction process

The ratio of pandan powder : solvent affects the difference in solute concentration inside the material and outside the environment.

**Figure 3.** Effect of pandan powder : solvent ratio on chlorophyll a, b; total chlorophyll; polyphenol content and DPPH radical-scavenging activity.

Figure 3 shows that chlorophyll a (9.7403 ± 0.37 μg/mL), chlorophyll b (4.5026 ± 0.19 μg/mL); total chlorophyll (14.2429 ± 0.46 μg/mL); polyphenol content (3.015 ± 0.096 g/L) and DPPH radical removal activity (1374.03 ± 25.26 μmol/L) are the highest values at pandan powder : solvent ratio of 1:30 in the extraction. In microwave-assisted extraction, the material-to-solvent ratio determines extraction effectiveness. If the volume of acetone is less than the number of solid sample, the solvent does not penetrate into the material, microwave radiation cannot affect the molecular particles much, resulting in these particles being limited to rotate, reduces extraction efficiency. If the volume of the solvent is greater than the number of solid samples, the solvent will fully absorb into the particles, microwave radiation will better affect the particles, making the particles move more strongly, the extraction efficiency should be higher. But if the solvent volume is too high, the extracted mixture will be diluted, leading to a longer thermal action time, reducing the chlorophyll, polyphenol content and the DPPH free radical scanning ability [16].

3.4. Effect of microwave-assisted time on the extraction process

Microwave-assisted extraction time generates heat that acts directly on material molecules, so microwave-assisted extraction time is one of the important factors affecting the extraction process.

**Figure 4.** Effect of microwave-assisted time on chlorophyll a, b; total chlorophyll; polyphenol content and DPPH radical-scavenging activity.

Figure 4 shows that chlorophyll a (9.4278 ± 0.13 μg/mL), chlorophyll b (4.246 ± 0.07 μg/mL); total chlorophyll (13.6738 ± 0.15 μg/mL); polyphenol content (2.7577 ± 0.04 g/L) and DPPH radical removal activity (1409.51 ± 55.8 μmol/L) are the highest values obtained in 2 minutes of microwave-
assisted extraction time. The power level and extraction time are the main parameters affecting the efficiency of the extraction process [26]. At the time of microwave-assisted extraction of 30 seconds, due to the short time, the microwave energy is low. At microwave-assisted time of 1 minute, radiation is more active than 30 seconds, so the extracted substance content is a bit higher. At the microwave-assisted time of 2 minutes, the extraction time is longer than 30 seconds and 1 minute, so the microwave radiation has a positive impact on the particles, causing friction and breaking down the cell walls, diffusing many soluble substances into solvent. At 3 minutes and 4 minutes, microwave radiation strongly affects the particles, causing more friction, resulting in more impact temperatures, which degrades some extracted compounds. Prolonged microwave support can lead to the decomposition of target compounds along with the overheating of soluble substances [17]. Microwave-assisted extraction time of 2 minutes is also the result of the study of F. Dahmoune when extracting phenolic compounds from pistachio leaves (Pistacia lentiscus) [31].

4. Conclusion
Pandan leaf is a very popular plant grown in Vietnam. Factors affecting chlorophyll, and polyphenol extraction, as well as DPPH antioxidant activity of extract were investigated, they are solvent kinds, microwave capacity, pandan powder : acetone ratio, and extraction time used with microwave. The research resulted that the used solvent was acetone 90°C; microwave capacity was 300W; pandan powder : acetone ratio was 1:30 and used time with microwave was 2 minutes. These parameters brought highest amounts of chlorophyll a, b; total chlorophyll; polyphenol content and DPPH radical-scavenging activity.

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