Chlorophyll content and antioxidant activity from folium sauropi (Sauropus androgynus (L.) Merr) with microwave-assisted extraction

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Abstract. Folium sauropi is grown commonly in Vietnam, it is a vegetable that contains many bio-active compounds such as chlorophyll, polyphenol, and antioxidant activity. The use of natural pigments having clear origin is a trend in food processing nowadays. The objective of this study was to find suitable conditions for extracting chlorophyll with antioxidant activity (DPPH) in folium sauropi leaves by microwave-assisted extraction. When extracted with acetone of 90%, microwave power of 300W, solid to solvent ratio of 1:30, microwave-assisted extraction time of 120 seconds; chlorophyll content (14.43± 0.16 μg/mL), and DPPH radical-scavenging activity (886.64± 15.89 μmol/L) obtained highest.

1. Introduction
Scientific name of Folium sauropi is Sauropus androgynus (L.) Merr, it is a native Southeast Asian vegetable [1]. Folium sauropi is an erect shrub found in Southeast Asia [3], widely distributed in humid highlands such as Malaysia, Indonesia, Southwest China and Vietnam [2], with a height of about 0.5 m. Folium sauropi is a soft trunk tree, has many cylindrical or angular branches. The length of leaf is about 2.0 - 7.5 cm, it has oval shape. Male flowers and female flowers differently, one grows at the bottom at the leaf edge, another fowers above. The capsule are white, spherical and 1.5 cm in diameter [4]. Humid places with moderate temperatures and heavy rainfall are good conditions for plant growth [3].

Steroids, resins, tannins, saponins, alkaloids, flavonoids, terpenoids, glycosides, phenols, catechols, glycosides and acid compounds are bioactive compounds on Folium sauropi leaves [4]. They have the content of 142.64 mg / 100g fresh weight, with quercetin, myricetin, luteolin, apigenin and kaempferol when analyzed by HPLC method, they have the highest content of flavonoids and bioactive compounds among the 11 kinds of Indonesian vegetables [5]. Folium sauropi is a leafy vegetable with high antioxidant and phenolic activity [6].

Chlorophyll is present in all plants, most commonly in eubacteria. The chemical structure of chlorophyll, chlorophyll has a porphyrin-like ring structure called the phorin ring in chlorophyll. As a group of compounds with four pyrole rings linked by central magnesium ions and phytol chains [7]. Macrocycl porphyrins are esterified with diterpene alcohol, phytol to form chlorophyll [8].
a, b, c and d are the forms of chlorophyll. Plants contain more chlorophyll a and b, in contrast photosynthetic algae and diatoms contain more chlorophyll c and d. Chlorophyll is widely distributed in fruits and vegetables such as primary photosynthetic iron, the ratio of chlorophyll a : chlorophyll b is often found in a ratio 3 : 1 [9]. The difference between chl a and chl b originates from a single group attached to the basic chlorophyll structure at position C7. Chlorophyll contains one methyl group (CH$_3$), whereas chlorophyll b contains an aldehyde group (CHO) [9]. Chlorophyll structure is destroyed by the high intensity of light. Oxidation is the decomposition of chlorophyll. Therefore, a light-restricted medium is suitable for chlorophyll extraction [10].

Microwave-assisted extraction (MAE), also known as microwave extraction, is an extraction technique that combines a traditional microwave extraction and traditional solvent to extract dissolved solid or liquid substances from many materials using microwave energy [11], [12]. Microwave-assisted extraction (MAE), known as an environmentally friendly process with economic advantages compared to the current extraction methods that used to extract active compounds from many different materials [13]. Therefore, microwave-assisted extraction (MAE) is an excellent alternative to conventional extraction methods especially in plant material extraction [14]. The main advantages of the microwave-assisted extraction method (MAE) compared to conventional extraction methods are the reduction of the amount of solvent, shortening of extraction time, high extraction efficiency, simple operation and reduced power, amount of inputs without reducing extraction yield and target functions [15]–[17].

The main objective of this study was to investigate the appropriate conditions such as type of solvent, microwave power, material to solvent ratio, microwave-assisted time to obtain highest chlorophyll content and antioxidant activity.

2. Materials and methods

2.1 Materials
Folium sauropi was bought in Coopmart supermarket in Vietnam. The leaves were plucked, washed and dried, crushed and screened by sieve, particles that were smaller than 30 mesh in diameter were selected. The powder was preserved in the refrigerator at 5°C in sealed plastic bag prior to analysis. Food grade ethanol (99%), acetone (98%), methanol (98% Shanghai Dingsheng Chemical Technology Co., Ltd.) were obtained from a local distributor. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox were purchased from Sigma–Aldrich Co., and imported by Hoa Nam Company.

2.2 Extraction procedure
Chlorophyll and polyphenolic compounds were extracted form folium sauropi powder using a LG MS2324D microwave extraction system that was redesigned with Allihn Condenser tuber to condense solvent. The system supplies 800W of microwave energy at 100% power. The factors that affect the extraction process were investigated, microwave power (100W, 300W, 400W, 600W, 800W), irradiation time (30, 60, 120, 180, 240s) and material/solvent ratio (1/20, 1/30, 1/40). The extract was then diluted to 100mL and filtered through a cloth before filtration through Whatman No.1 filter paper. The obtained filtrate was used for further analysis chlorophyll, polyphenol and antioxidant activity (DPPH).

2.3 Chlorophyll determination
The maximum light absorption spectrum of chlorophyll in the blue-ray region (λ: 430 - 460 nm) and the red light region (λ: 620 - 700 nm) [18], the absorbance at these wavelengths represents intensity degree of pigment or amount of chlorophyll in solution. Based on this property, chlorophyll values are measured. The extract of the sample after extraction is diluted 7 times with acetone 90°, methanol 100° and ethanol 96°. Then measured at wavelengths 664 nm and 647 nm (acetone 90°), 665 nm and 652 nm (methanol 100°); 662 nm and 645 nm (ethanol 96°) [19].
2.4 Antioxidant activity (DPPH) determination
The total antioxidant content was measured by the DPPH method described previously by Brand-Williams et al in 1995. Antioxidant compounds could scavenge free radicals so these compounds discolor purple in DPPH solution. 0.2ml of the diluted extract was mixed with 3ml DPPH solution. The samples were placed in the dark for 30 minutes and then sample absorbance was measured at 515 nm. The results were expressed as μmol Trolox equivalent per volume of the sample (μmol/L) [20].

3. Results and discussion

3.1. Effects of solvent on the extraction process
Solvents are divided into two categories, polar and non-polar solvents [21]. The degree of polarization of the solvent depends on the dielectric constant. Combining water and different polar solvents will yield different amounts of extracts. The chlorophyll content and antioxidant activity of folium sauropi extract with different solvents were shown in Figure 1.

![Figure 1](image-url)

**Figure 1.** Effect of solvent on chlorophyll content and antioxidant activity in the extract from folium sauropi (Note: a, b, c show significant differences at significance levels P <0.05)

The dielectric constant and the polarity of the solvent mixture increase rapidly with the addition of a suitable amount of water to absorb microwave energy, thereby increasing the disruption of the cell membrane of plant materials, increasing the area surface contact between material and solvent, increasing extraction efficiency [22]. According to the author Serban Moldoveanu, the solvent mixture system used for extraction was more effective than the use of individual solvent [23]. The author (Lu and Foo, 2000), also mentioned that acetone/water mixture was a good extraction solvent for polarizing antioxidants and has more benefits when used for phenolic extraction [24]. This result was similar to the assumption that acetone mixture with water is the best extraction solvent with some polar polyphenols, especially the most effective extract for flavonoid and anthocyanin compounds [25]. This result also coincided with a study of (RJ Ritchie, 2008) when extracting chlorophyll from spinacia with 3 solvents: acetone 90°, methanol 100° and ethanol 100°; similar results also showed the extract with acetone 90° had the highest chlorophyll content [26].
3.1. Effects of microwave power on the extraction process

Microwave power is one of the factors that significantly affect the extraction process, microwave power determines the microwave energy and temperature of extraction mixture.

Figure 2. Effect of microwave power on chlorophyll content and antioxidant activity in the extract from folium sauropi (Note: a, b, c show significant differences at significance levels P <0.05)

Microwave-assisted extraction (MAE), microwave radiation causes disruption of hydrogen bonds and the movement of dissolved ions, leading to disruption of plant tissue, releasing compounds present in their material into the solvent [27], [28]. Low microwave power (100W) generated low temperature, and low molecular diffusion rate, so it reduced extraction efficiency. When the microwave power was 300W, cell membrane temperature, the interaction between molecules in the solvent increased, resulting in increased extraction efficiency [29], [30]. Microwave power were 400W, 600W and 800W, the extraction efficiency tends to decrease gradually because the active compounds in the extract were decomposed by high temperature. In addition, chlorophyll has poor thermal stability and easy oxidation. This result was similar to the result of microwave-assisted chlorophyll extraction (MAE) from Bim Bip of the authors (AN Mustapa et al.) [31] Therefore, microwave power (300W) was chosen for the next experiment.

3.3. Effects of microwave-assisted time on the extraction process

The extraction time directly affects the temperature of the material and solvent mixture during extraction. The relationship between extraction time and chlorophyll content and antioxidant capacity of the extract was shown in Figure 3.

The microwave-assisted time was 30 seconds and 60 seconds, the temperature of extraction mixture was not enough, only some cell membranes were affected, so the compounds extracted in the original material into the solvent were not much. When the microwave-assisted time increased to 120 seconds, the temperature of mixture increased, broken cells also increased, and the content of the extracted substances in the extract was higher. The microwave-assisted time continuously increased to 180s and 240s that made the extraction temperature significantly increase, causing thermal degradation of bio-active compounds in the extract. Long extraction time may have no effects or negative effects due to the unstable compound resulting in degradation or conversion of the analyzed compounds [32]. Temperature increase will generate some undesirable compounds, this result was consistent with a study of (G. Spigno and DM De Faveri), polyphenols was extracted from tea at intervals of 30 seconds, 60
seconds, 90 seconds, 120 seconds, 150 seconds, 180 seconds and 210 seconds, the microwave-assisted extraction time for highest polyphenol content was also 120 seconds. [33]

![Figure 3. Effect of microwave-assisted time on chlorophyll content and antioxidant activity in the extract from folium sauropi (Note: a, b, c show significant differences at significance levels P <0.05)](image)

3.4. Effects of solid to solvent ratio on the extraction process

The solid to solvent ratio is an important factor that affects extraction process. The solid to solvent ratio of 1:15, 1:30 and 1:45 in this experiment was investigated, the differences in the contents of extraction compounds were shown in Figure 4.

![Figure 4. Effect of solid to solvent ratio on chlorophyll content and antioxidant activity in the extract from folium sauropi (Note: a, b, c show significant differences at significance levels P <0.05)](image)
When the liquid volume varies and the solid mass remains constant, the solvent volume must be sufficient to ensure that the entire sample is immersed, so that the material can swell during extraction [35]. With the ratio of 1:30, the recovery extraction was low, the material can not swell enough during extraction; when the solvent volume was low, it made the temperature of the mixture increased significantly, so bio-active compounds in the extract were decomposed. In the MAE, a higher solid/solvent may give lower recoveries due to inadequate stirring of the solvent by microwave and excessive swelling of the plant material. Moreover, a higher solvent volume requires higher power and more time to achieve the temperature required. Excessive solvent may also cause the dissolution of other undesirable compounds, lowering the extraction selectivity towards target compounds [34].

4. Conclusion
In this study, we investigated the chlorophyll extraction conditions and the antioxidant activity of the extract on the folium sauropi leaves grown in Vietnam by microwave-assisted extraction. The results showed that acetone 90° obtained the highest chlorophyll content and antioxidant activity compared with 100° methanol and ethanol 96°. Microwave power, extraction time and the solid to solvent ratio that got the highest extraction efficiency were respectively 300W, 120s, and 1/30. This remarkable results also give basic parameters for optimizing extraction process with microwave-assisted extraction on folium sauropi leaves.

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