MINISTRY OF EDUCATION AND TRAINING
**NHA TRANG UNIVERSITY**

**ĐANG BUU TUNG THIEN**

# RESEARCH ON OBTAINING POLYPHENOLS, CHLOROPHYLL FROM PENNYWORT *(Centella asiatica (L.) Urb)*, AND APPLICATION IN BEVERAGES

**SUMMARY Ph.D. THESIS**

**KHANH HOA – 2023**

**The thesis is completed at the Nha Trang University**

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**The thesis is defended at the Nha Trang University Council, meeting at the Nha Trang University at the hour…..Date… ….Month……., 2023**

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# THE SUMMMARY OF THE THESIS

## 1. The necessary of the thesis

Bioactive herbs and natural compounds are currently being used in medicine, food, cosmetics, agriculture... Pennywort (*Centella Asiatica* (L.) Urb) belongs to the Umbelliferae family and is a vegetable or a herb. It has known to have the effect of enhancing memory, supporting the treatment of skin diseases, detoxifying the liver, and having high antioxidant activity... It has many good attributes because it contains chemical compounds that have good effects on the body; including polyphenols, chlorophylls, and their derivatives.

In many studies, the use of foods and herbs rich in polyphenols and chlorophyll is good for health, has the effect of reducing the risk of disease, preventing cancer, prolonging life... The biological properties and benefits of polyphenols and chlorophyll for human health have attracted much attention in research and production of products with antioxidant activity for health. Research in the food industry for the production of products rich in polyphenols and chlorophylls with antioxidant activity to benefit human health is encouraging and has great potential.

For the above reasons, the thesis topic is: "Research on obtaining polyphenols, chlorophyll from pennywort *(Centella Asiatica (L.) Urb)*, and application in beverages".

## 2. The goal of the thesis

Developing a process for obtaining polyphenols, chlorophylls with antioxidant activity from pennywort (*Centella asiatica* (L.) Urb)) grown in Nha Trang - Khanh Hoa and creating pennywort powder, orienting its application in beverages.

## 3. Research subjects

The research materials for the extraction of polyphenols, and chlorophyll with antioxidant activity are fresh pennywort, a mixture randomly collected from the field of pennywort at the age of 75 days.

Pennywort extract obtained under optimal conditions is the raw material used for spray-drying process research to create pennywort powder containing polyphenols, and chlorophyll with antioxidant activity.

## 4. Research scopes

Research to determine the optimal conditions for the extraction of polyphenols, chlorophylls with antioxidant activity from pennywort grown in Nha Trang - Khanh Hoa;

Research to determine optimal conditions for spray drying process to create pennywort powder containing polyphenols, chlorophylls with antioxidant activity;

Study on preservation of pennywort powder containing polyphenol, chlorophylls with antioxidant activity.

## 5. Research Methodologys

In the study of the collection of polyphenols, and chlorophyll with antioxidant activity from pennywort (*Centella asiatica* (L.) Urb), the thesis used standard research methods in the world and Vietnam. Data processing software and optimized software are used for highly reliable results.

## 6. The structure of the thesis

The thesis consists of 165 pages, of which 25 pages of overview, 28 pages of research methods, 91 pages of research results, 2 pages of conclusions, 20 tables of data, 88 pictures, 184 references (20 documents in Vietnamese, 164 documents in English) and 10 pages of appendices.

# CHAPTER 1: OVERVIEW

## 1.1. Introduction

Pennywort with the scientific name is Centella Asiatica (L.) Urb. (Or Hydrocotyle Asiatica (L.) Urb.). This species has many active chemical compounds, with the polyphenols mainly existing in the two groups of flavonoids and phenolic acids, and also the chlorophyll A and chlorophyll B. So, pennywort has many activities such as: protecting skin cells, stomach, and nerve cells; reducing diabetes, liver cancer, and lung cancer; antimicrobial activity, and antioxidant activity...

The study to obtain polyphenols, and chlorophyll with antioxidant activity from pennywort to use it in food technology is suitable for today's life needs.

## 1.2. Extraction of polyphenols, chlorophyll

The Solid-Liquid extraction method can be used to extract polyphenols and chlorophylls. In the process, it is necessary to consider the solvent polarity and chemical characteristics of the substance to choose the appropriate extraction solvent. Studies show that solvents with high polarity are often used to extract polyphenols, and chlorophylls such as methanol, ethanol, acetone...

The extraction process also considers influencing factors, which are temperature, time, solvent-to-powder ratio, pH... Besides, the extraction process can also use physical factors to support such as: ultrasonic waves, microwaves, electric fields, hydrostatic pressure...

The extraction and acquisition of polyphenols and chlorophyll from pennywort are 02 processes: Extraction and spray drying.

## 1.3. Spray drying process to pennywort powder

Spray drying is the process of dehydrating liquid droplets that have been dispersed to very small sizes in hot air to obtain a fine granules dry powder. To implement this technique, it is required to make a solution form, spray the raw materials to form small particles in high-temperature air, converting them to powder form quickly by a spray dryer. The spray drying technique creates very small, fine liquid droplets by modularizing the solution in the drying chamber of spray dryer and obtaining a spherical, powdery granular product.

To obtain quality pennywort powder with good physical and chemical properties, the spray drying equipment must ensure the technical characteristics and the appropriate spray drying conditions must be selected. The quality of powder products of spray drying depends on the carrier material, drying conditions such as: drying fluid concentration, inlet and outlet air temperature, feed flow rate, compressed air flow rate, type of spray mechanism, and spray speed...

### 1.4. Studies on the application of polyphenols, chlorophylls with antioxidant activity

Polyphenols can be used in the food industry because they give flavor, color, aroma and create new aromatic components to food products. It is also used in the production of plant-based beverages due to their antioxidant activity, of which phenolics are considered powerful antioxidants. In addition, polyphenols are also used in traditional and modern medicine to prevent and treat diseases.

Besides, in Vietnam chlorophyll is also a food additive with code E140 with the use of creating natural green color for foods such as cakes, candies, soft drinks... It is also used to produce cosmetics and functional foods with antioxidant effects, improving body health.

# CHAPTER 2: METHODS

## 2.1. MATERIALS

Pennywort (*Centella asiatica* (L.), Urban) belongs to the kingdom Plantae, order Apiales, family Apiaceae and genus Centella. It was collected in Nha Trang, Khanh Hoa.

## 2.2. RESEARCH METHODS

### 2.2.1. General research scheme

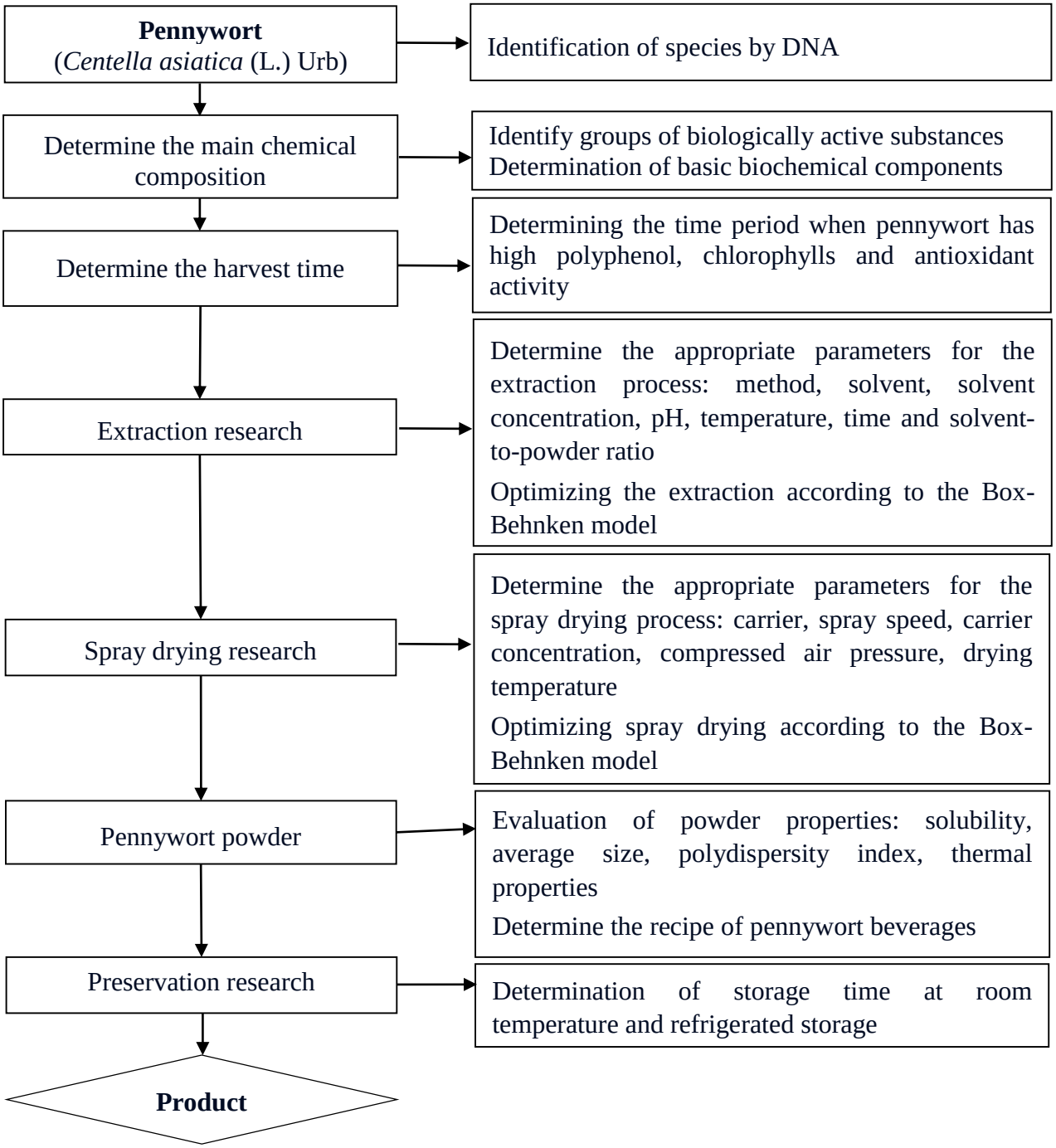



**Fig 2.1. General experiment**

Pennywort samples were treated, dried, ground into powder, and stored. Then the sample was analyzed, evaluating the basic nutritional composition, polyphenol content, chlorophyll, and antioxidant activity.

During the extraction process, the factors affecting the recovery of polyphenol and chlorophyll compounds with antioxidant activity were investigated from Pennywort, including the following factors: extraction method, extraction solvent, extraction temperature, extraction time, solvent-to-powder ratio, solvent concentration, extraction solution pH. The extract was then studied by spray drying process to create powder, determining the characteristics and quality of pennywort powder.

**2.2.2. Methods for identification, sample handling and chemical analysis**

- Pennywort was identified by total DNA separation based on the basic CTAB procedure of Kurt Weising, gene sequencing by Sanger method and the sequencing results were corrected by BioEdit software version 7.2.6.1.

- Sample processing was performed according to Gunathilake and et al. (2002).

- Determination of moisture content of pennywort according to Vietnam Pharmacopoeia V, volume 2 (Appendix 9.6).

- Determination of total ash content of pennywort according to Vietnam Pharmacopoeia V, volume 2 (Appendix 9.8).

- Quantification of total antioxidant activity (TAA) according to Prieto et al. (1999) with ascorbic acid as standard and measurement of absorbance by UV-VIS spectrophotometer at 695 nm.

- Quantification of ferric reducing antioxidant power (FRAP) according to Zhu et al. with the standard is iron(II) sulphate FeSO4 (2002) and measure the absorbance by UV-VIS spectrophotometer at 655 nm..

- Quantification of DPPH free radical scavenging activity according to Brand-Williams et al. (1995) and measurement of absorbance on a UV-VIS spectrophotometer at 517 nm.

- Determination of polyphenols according to Folin - Ciocalteu (Singleton et al., 1999) with gallic acid as standard and absorbance measurements on a UV-VIS spectrophotometer at 755 nm.

- Determination of chlorophyll according to Lichtenthaler H. K. (1987) in 95% ethanol, and the absorbance of the extract was measured by UV-VIS spectrophotometer at 664 nm and 648 nm.

- The solubility of powder was determined according to Vietnam Pharmacopoeia V, (2017).

- Determination of mean particle diameter and polydispersity index (PDI) was measured by Dynamic Light Scattering (DLS) technique according to ISO 22412 (2008) (Technical Committee ISO/TC 24, 2017).

- Differential scanning calorimetry (DSC) was performed using a thermal analyzer DSC-60, Shimadzu, Japan..

- Determination of metal: Mercury (Hg) according to Vietnam standard QCVN 8-2: 2011/BYT; Cadmium (Cd) according to Vietnam standard QCVN 8-2: 2011/BYT; Lead (Pb) according to Vietnam standard QCVN 8-2: 2011/BYT and Arsenic (As) according to Vietnam standard QCVN 8-2: 2011/BYT.

### 2.2.3. Methods of microbiological analysis

- Determine the total number of E.coli bacteria (CFU/g) according to Vietnam standard 8-3: 2012/BYT.

- Determination of Salmonella spp (yes or KPH/25g) according to Vietnam standard 8-3: 2012/BYT.

## 2.3. DATA PROCESSING METHODS

The experimental data were obtained by statistical method. Each experiment was carried out 3 times, with 3 samples each time and the results were the average of the times. Calculation, graphing, result processing, analysis of variance ANOVA, difference check with Minitab 18.0 software and data analysis, optimization using Design Expert 12.0 software.

# CHAPTER 3: RESEARCH RESULTS

## 3.1. INDICATION AND ANALYSIS OF MAJOR CHEMICAL COMPOSITION OF PENNYWORT

### 3.1.1. Identifier of pennywort

Pennywort sample was extracted DNA according to CTAB I procedure. Two genes matK and ITS1–5.8S–ITS2 were isolated and enriched.

Perform PCR reaction with specific primers obtained from the separated DNA, then sequence the genes of the matK fragment and the 5.8S rDNA fragment. The results showed that the sequences of the studied sample had high similarity with the sequences of matK (98.44%-98.76%) and ITS (98.21-100%) of species Centella asiatica on NCBI. Therefore, the research sample belongs to the species of pennywort (*Centella asiatica* (L.) Urban).

### 3.1.2. Quantification of some main chemical components of pennywort

The content of polyphenols, chlorophyll and triterpenoids in pennywort grown in Khanh Hoa is higher than that of the same species in some countries such as Malaysia and

India. It is also higher than some species of the genus Centella growing in Khanh Hoa such as: Hydrocotyle verticillata Thunb., Hydrocotyle javanica Thunb. In addition, pennywort growing in Khanh Hoa also contains a lot of nutritional ingredients such as protein, fat, carbohydrates, vitamins and minerals...

The results of analysis of the photochemical and nutritional components of pennywort are shown in Table 3.1.

**Table 3.1. Photochemical and nutritional components of pennywort**

| Chemical composition | Unit | Content |
|---|---|---|
| Polyphenol | mg acid galic/g DW | 13,56 ± 0,20 |
| Chlorophyll | mg/g DW | 4,73 ± 0,03 |
| Triterpenoid | mg acid oleanolic/g DW | 5,90 ± 0,02 |
| Protein | g/100g DW | 11,32 ±0,12 |
| Fat | g/100g DW | 3,15 ±0,01 |
| Carbohydrate | g/100g DW | 28,42 ±0,02 |
| Fiber | g/100g DW | 42,80 ±0,12 |
| Total mineral | g/100g DW | 3,62 ±0,09 |

### 3.1.3. Determining the time to harvest pennywort

The growth stage of pennywort affects its polyphenol and chlorophyll content. Moreover, there is also a strong correlation between polyphenol and chlorophyll content and antioxidant activity.

The influence of the growth stage on polyphenol, chlorophyll, and antioxidant activities of pennywort is shown in Fig 3.1 ÷3.3.

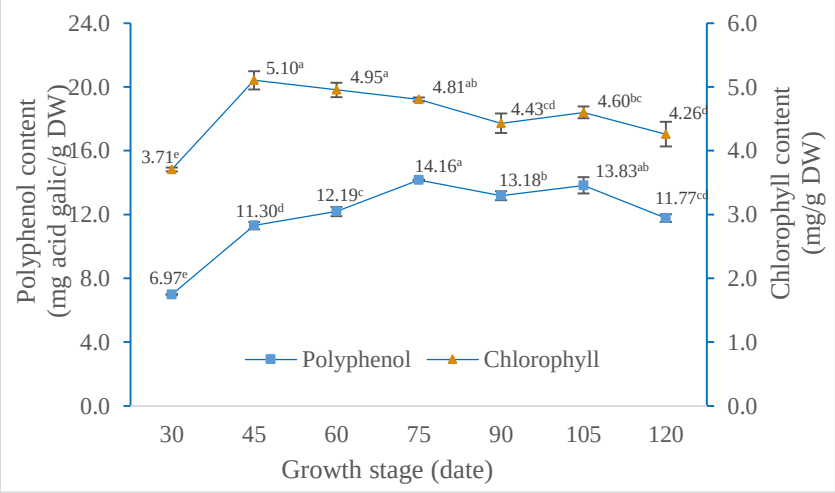



**Fig 3.1. Effect of harvest time on polyphenol and chlorophyll content in pennywort extract**

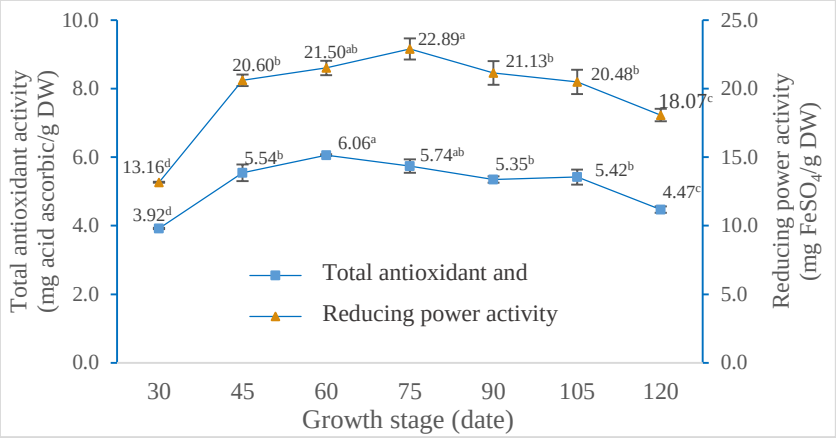



**Fig 3.2. Effect of harvest time on total antioxidant and reducing power activity of pennywort extract**

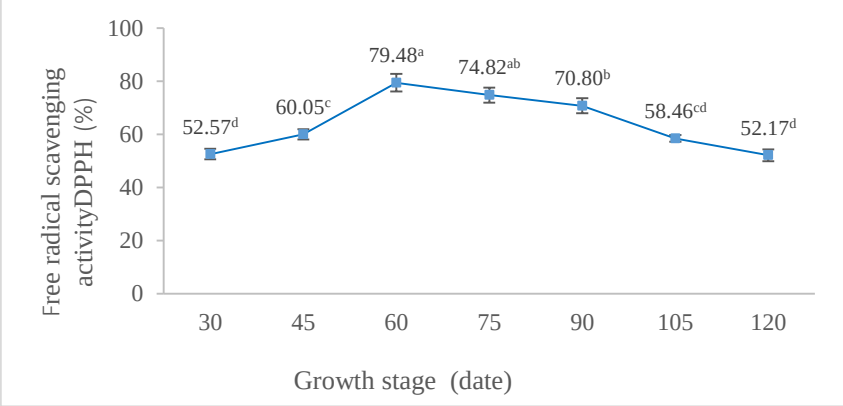



**Fig 3.3. Effect of harvesting time on DPPH free radical scavenging activity of pennywort extract**

The analysis showed that the suitable harvesting time for pennywort used as raw materials to obtain polyphenols, chlorophyll with biological activity is 75 days.

3.2. RESEARCH FOR EXTRACT OF POLYPHENOL AND CHLOROPHYLL FROM PENNYWORT

**3.2.1. Factors affecting the extraction of polyphenols, chlorophyll with antioxidant activity from pennywort**

- *Extraction method and solvent:* Research results show that ultrasonic-assisted extraction has a higher content of polyphenols, and chlorophyll with antioxidant activities than reflux extraction and immersion extraction. Moreover, when extracted with solvents of different polarities, ethanol showed high content of polyphenols, chlorophyll, and antioxidant activities. Therefore, ethanol is suitable for extracting polyphenol and chlorophyll compounds with antioxidant activity from pennywort.

- *Solvent concentration:* When changing the ethanol concentration from 75% to 99,5%, the content of polyphenols, chlorophyll, and antioxidant activities of the extract changed nonlinearly. The ethanol concentration for the highest polyphenol and chlorophyll

content was 95% and 85%, and the total antioxidant activity and the highest iron reduction activity were 95% and 90%.

Therefore, 95% ethanol is suitable for extracting polyphenols, and chlorophyll with antioxidant activity from pennywort.

- *Extraction temperature:* Extraction temperature strongly affects polyphenol, and chlorophyll content with antioxidant activity. It is nonlinear when varying the temperature from 30$^o$C to 80$^o$C;

The suitable temperature for extraction of polyphenols and chlorophyll compounds with antioxidant activity from pennywort is 60$^o$C.

- *Extraction time:* The content of polyphenols, chlorophyll, and antioxidant activity at different extraction temperatures had statistically significant differences ($p<0.05$), it changed nonlinearly when changing extraction time from 30 to 180 minutes.

The suitable extraction time to extract polyphenol, chlorophyll compounds with antioxidant activity from pennywort is 90 minutes.

- *Solvent-to-powder ratio:* The research results show, when increasing the solvent-to-powder ratio from 10/1 to 50/1 (ml/g), it is increase the polyphenol and chlorophyll content with a statistically significant difference ($p<0.05$). Increasing the solvent-to-powder ratio higher 50/1 is also increased the content of polyphenols, and chlorophyll but the difference was not statistically significant.

The solvent-to-powder ratio is 50/1 (ml/g) that is suitable for extracting polyphenol, and chlorophyll compounds with antioxidant activity from pennywort.

**3.2.2. Optimization of extraction of polyphenols and chlorophyll**

- *Regression model:* Select the range of variation: Temperature ($X_1$: 40-80$^o$C, $\delta_1$=20), time ($X_2$: 30-150 minutes, $\delta_2$=60) and solvent-to-powder ratio ($X_3$: 30/1-70/1, $\delta_3$=20). The optimization is arranged according to the Box-Behnken surface response model with the objective function: Polyphenol content ($Y_1$) (mg acid galic/g DW), chlorophyll content ($Y_2$) (mg/g DW) and total antioxidant activity ($Y_3$) (mg acid ascorbic/g DW).

Regression equation for polyphenol content: $Y_1 = 19,74 + 1,57X_1 + 1,53X_2 + 1,19X_3 + 0,69X_1X_2 + 1,1X_1X_3 – 0,69X_2X_3 – 6,83X_1^2 – 6,46X_2^2 – 6,48X_3^2$

Regression equation for chlorophyll content: $Y_2 = 7,66 + 0,94X_1 + 0,43X_2 + 0,61X_3 + 0,38X_1X_2 + 0,14X_1X_3 – 0,11X_2X_3 – 2,05X_1^2 – 2,09X_2^2 – 2,68X_3^2$

Regression equation of total antioxidant activity: $Y_3 = 14,45 + 0,39X_1 + 0,88X_2 + 0,70X_3 + 0,81X_1X_2 + 1,48X_1X_3 - 0,45X_2X_3 – 4,28X_1^2 – 4,69X_2^2 – 5,48X_3^2$

- *Determine the optimal value:* Perform regression equations to determine the optimal values of the variables ($X_1$, $X_2$ and $X_3$). The process is calculated on Design Expert 12 software and overlaps the response surfaces to find the optimal point of prediction with temperature $X_1$ (62,92$^o$C), time $X_2$ (96,74 minutes) and solvent-to-powder ratio $X_3$ (51,95/1 ml/g) and predictive objective functions with polyphenol content $Y_1$ (19,99 mg gallic acid/g DW), chlorophyll content $Y_2$ (7,81 mg/g DW) and antioxidant activity total $Y_3$ (14,50 mg ascorbic acid/g DW).

The experiment was repeated 3 times at the condition of temperature $X_1$ (63$^o$C), time $X_2$ (97 minutes) and solvent-to-powder ratio $X_3$ (52/1 ml/g) and determined the objective functions as the polyphenol content $Y_1$ (19,18±0,72 mg gallic acid/g DW), chlorophyll content $Y_2$ content (7,45±0,16 mg/g DW) and total antioxidant activity $Y_3$ (13,81±0,31 mg ascorbic acid/g DW).

## 3.3. RESEARCH FOR SPRAY DRYING PROCESS TO MAKE PENNYWORT POWDER

### 3.3.1. Determine the parameters of the spray drying process

- *Carrier material:*

The drying process uses carrier materials (maltodextrin, dextrin, and gum arabic). The content of polyphenols, chlorophyll, antioxidant activities, solubility, size, characteristics, and thermal properties of pennywort powder are summarized in tables 3.2, 3.3, and 3.4.

**Table 3.2. Solubility and sensation of pennywort powder**

| Powder type | Solubility | Sensation | Coverage of the carrier materials |
|---|---|---|---|
| Pennywort powder with gum arabic carrier | Dissolve easily | Bright green color, fine powder, spongy | The active substance is covered in the middle of the granules |
| Pennywort powder with dextrin carrier | Dissolve easily | Dark green color, coarse powder, spongy | The active substance is covered and located in the granule wall |
| Pennywort powder with maltodextrin carrier | Dissolve easily | Dark green color, coarse powder, slight lump | Active substance bind on the surface of the granules |

**Table 3.3. Content of polyphenols, chlorophyll, antioxidant activities and particle size, polydispersity index**

| Powder type | Content of polyphenols (mg acid galic/g DW) | Content of chlorophyll (mg/g DW) | Total antioxidant activity (mg acid ascorbic/g DW) | Ferric reducing power (mg FeSO4/g DW) | Average particle size (nm) | Polydis-persity index (PDI) |
|---|---|---|---|---|---|---|
| Pennywort powder with gum arabic carrier | 13.72±0.31 | 4.14 ±0,14 | 10.62 ±0,38 | 32.96±1.16 | 157,8 | 0,517 |
| Pennywort powder with dextrin carrier | 9.21±0.24 | 3.46 ±0,09 | 8.07 ±0,13 | 26.37±0.28 | 318,5 | 0,662 |
| Pennywort powder with maltodextrin carrier | 10.35±0.39 | 2.87 ±0,11 | 8.98 ±0,07 | 18.29±0.22 | 278,5 | 0,756 |

**Table 3.4. Thermal properties**

| Carrier materials | Temperature (°C) | | | |
|---|---|---|---|---|
| | Dehydration heat ($T_m$) | The bonds are broken ($T_đ$) | Decomposition of functional groups ($T_n$) | Completely decomposed ($T_p$) |
| Powder type | 132,94 | 180,11 | 256,85 v à 288,80 | 422 |
| Pennywort powder with gum arabic carrier | 94,05 | - | 317,48 v à 375,35 | 549,33 |
| Pennywort powder with dextrin carrier | 86,14 | - | 289,44 v à 415,68 | 549,01 |
| Pennywort powder with maltodextrin carrier | 69,60 | 128,03 | 282,99 v à 417,08 | - |

Compared with maltodextrin and dextrin, pennywort powder has a high content of polyphenols, chlorophyll, and antioxidant activities when using gum arabic. About the general characteristics of the pennywort powder, it has good solubility, small average particle size, bright green color, fine powder, spongy, and large particle dispersion. In addition, its thermal properties are good, such as heat of decomposition of functional groups (Tn), and complete decomposition heat (Tp).

Therefore, gum arabic is a suitable carrier for spray drying process to create pennywort powder containing polyphenols and chlorophylls with antioxidant activity.

- *Spray speed:*

The drying process uses a gum arabic with 100 g/l carrier, 2 kg/cm$^2$ compressed air pressure, 140°C drying chamber air temperature and 15 ml/p of input flow. The speed of the ejector disc fluctuates at 10,000, 15,000, 20,000 and 25,000 (rpm). Quantification of polyphenols, chlorophyll content, and antioxidant activities of pennywort powder, results are presented in Fig 3.4 and 3.5

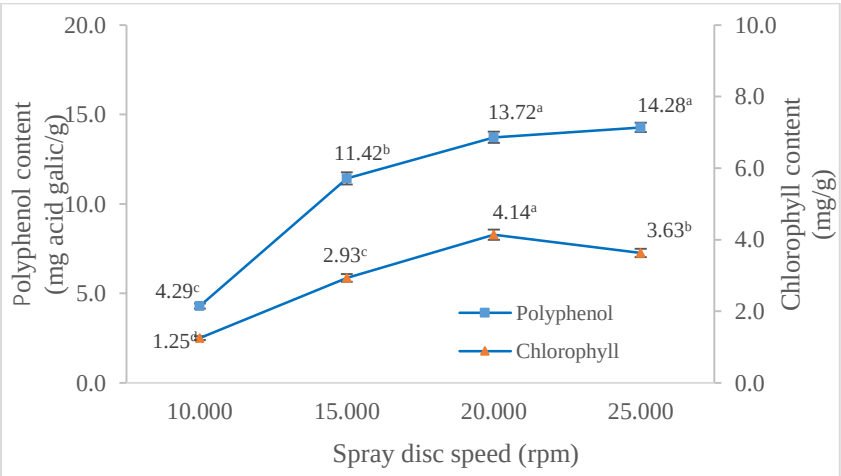



**Fig 3.4. Effect of spray disc speed on polyphenol and chlorophyll content in pennywort powder**

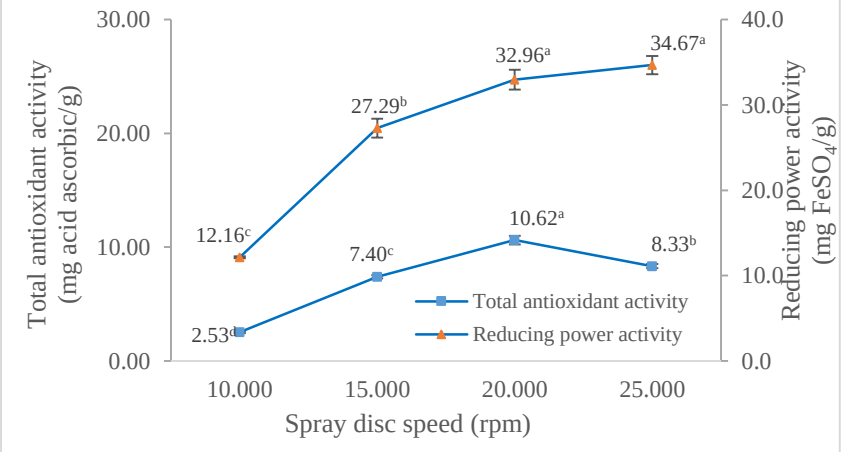



**Fig 3.5. Effect of spray disc speed on total antioxidant activity and reducing power activity of pennywort powder**

To obtain pennywort powder, spray speed of 20,000 rpm is suitable for polyphenols, and chlorophyll with antioxidant activity. At that conditions, pennywort powder was easily soluble, the average particle size (Z) was 133.5 nm, the polydispersity index (PDI) was 0.851.

Furthermore, the dehydration temperature at 137.07ºC, the breaking temperature of the bonds at 153.06ºC, the decomposition temperature of functional groups in the molecule at 314.21ºC and 433.27ºC and complete decomposition temperature 540.43ºC.

- *Gum arabic concentration*

The drying process uses gum arabic content of 50, 75, 100, 125, 150 and 175 (g/l), 2 kg/cm$^2$ compressed air pressure, 140ºC drying temperature, 20,000 rpm spray disc speed, and 15 ml/m input flow rate. Quantification of polyphenols, chlorophyll content, and antioxidant activities of pennywort powder, results are presented in Fig 3.6 and 3.7.

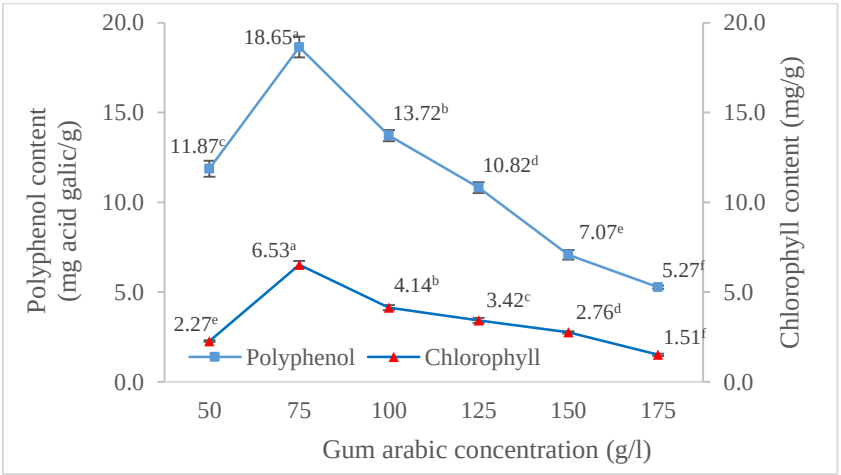



**Fig 3.6. Effect of gum arabic concentration on polyphenol and chlorophyll content in pennywort powder**

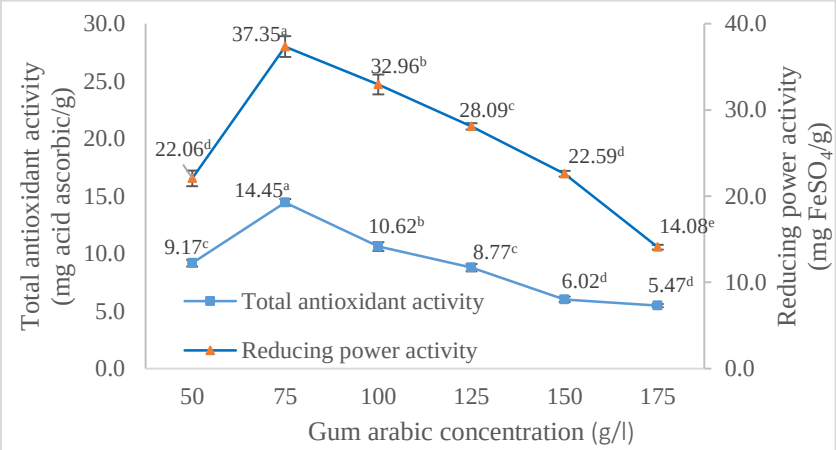



**Fig 3.7. Effect of gum arabic concentration on total antioxidant activity and reducing power activity of pennywort powder**

The content of gum arabic suitable for spray drying process to obtain polyphenols, and chlorophyll with antioxidant activity from pennywort is 75 g/l. Under that conditions, pennywort powder was easily soluble, the average particle size (Z) was 135.7 nm, and the polydispersity index (PDI) was 0.657;

Furthermore, the dehydration temperature is at 166.63ºC, the decomposition temperature of functional group is 309.24ºC and the complete decomposition temperature is 427.35ºC.

- *Drying temperature*

Using gum arabic with the content of 75 g/l and 2 kg/cm$^2$ compressed air pressure, 20,000 rpm spray disc speed, 15 ml/m inlet flow rate, and drying chamber temperature is 120, 140, 160, 180, 200 and 200 (ºC). Quantification of polyphenols, chlorophyll, and antioxidant activities of pennywort powder, results are shown in Fig 3.8 and 3.9.

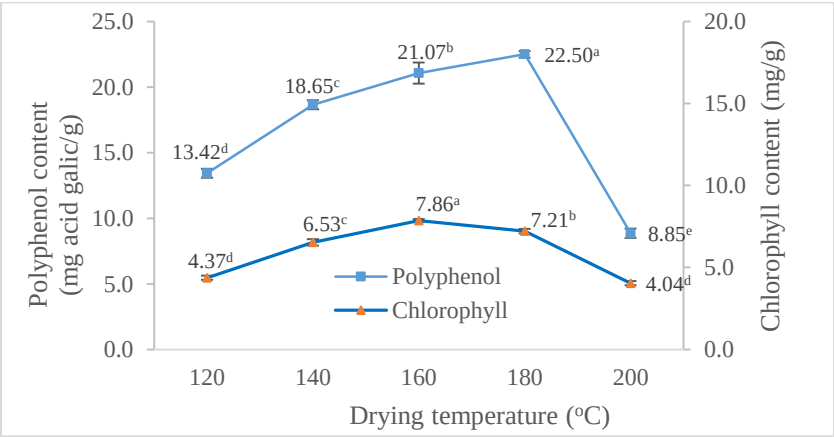



**Fig 3.8. Effect of drying temperature on polyphenol and chlorophyll content in pennywort powder**

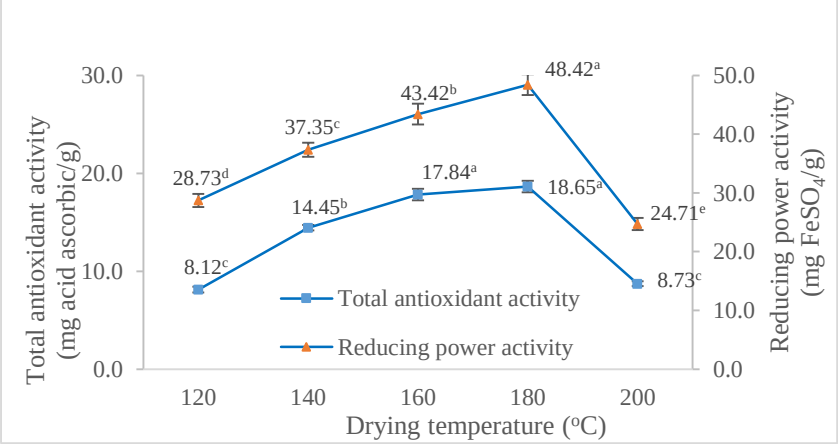



**Fig 3.9. Effect of drying temperature on total antioxidant activity and reducing power activity of pennywort powder**

The suitable drying temperature to obtain pennywort powder is 180ºC. At that condition, pennywort powder contains polyphenols, chlorophyll with soluble antioxidant activity, the average particle size (Z) is 173.9 nm and the polydispersity index of the powder (PDI) is 0.432.

Thermal properties, the dehydration temperature is 164.89ºC, the decomposition temperature functional group is 264.94ºC and 309.34ºC and the complete decomposition temperature is 433,03ºC.

- *Compressed air pressure*

Using gum arabic with the content of 75 g/l, 180ºC the temperature, 20,000 rpm, the spray disc speed 15 ml/m the input flow rate and the compressed air pressure is 1, 2, 3, 4 and 5 (kg/cm$^2$).

Quantification of polyphenols, chlorophyll, and antioxidant activities of pennywort powder, results are presented in Fig 3.10 and 3.11.

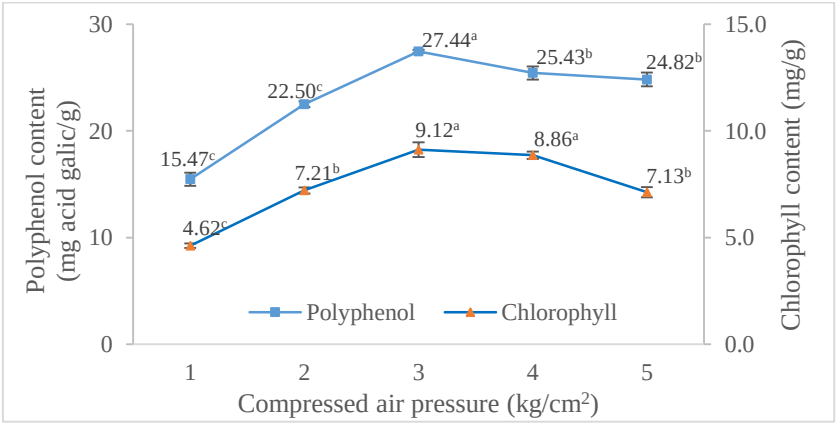



**Fig 3.10. Effect of air pressure on polyphenol and chlorophyll content in pennywort powder**

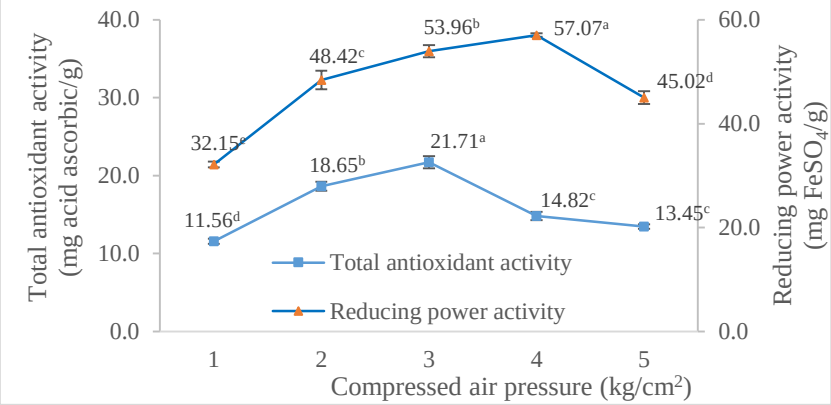



**Fig 3.11. Effect of air pressure on total antioxidant activity and reducing power activity of pennywort powder**

Compressed air pressure is suitable for spray drying to obtain pennywort powder containing polyphenols, and chlorophyll with antioxidant activity is 3 kg/cm$^2$. With that condition, pennywort powder is easily soluble, the average particle size (Z) is 131.5 nm and the polydispersity index (PDI) is 0.437.

Furthermore, the dehydration temperature is 156.75ºC, the decomposition temperature functional group is 306.78ºC and the complete decomposition temperature is 424.62ºC.

### 3.3.2. Optimization of spray drying process to create gotu kola powder containing polyphenols and chlorophyll with antioxidant activity

- *Regression model:* Select the range of variation: Gum arabic ($X_1$: 50÷100 g/l, $\delta 1$=25), $\delta_1$=25), temperature ($X_2$: 160÷200ºC, $\delta_2$=20) and compressed air pressure ($X_3$: 2÷4 kg/cm$^2$, $\delta_3$=1,0). The optimization was arranged according to the Box-Behnken surface response model with the objective function: polyphenol content ($Y_1$) (mg gallic acid/g), chlorophyll content ($Y_2$) (mg/g) and total antioxidant activity ($Y_3$) (mg ascorbic acid/g).

Regression equation for polyphenol content: $Y_1 = 28,49 – 0,83X_1 – 1,68X_2 +1,06X_3 - 1,68X_1X_2 -0,76X_1X_3 – 2,18X_2X_3 – 7,35X_1^2 – 7,65X_2^2 – 5,33X_3^2$

Regression equation for chlorophyll content: $Y_2 = 10,61 + 0,57X_1 – 0,74X_2 – 0,32X_3 + 0,05X_1X_2 - 0,16X_1X_3 + 0,13X_2X_3 – 3,21X_1^2 – 3,02X_2^2 – 1,64X_3^2$

Regression equation for total antioxidant activity: $Y_3 = 19,42 – 2,38X_1 – 1,00X_2 + 0,68X_3 +1,16X_1X_2 + 1,17X_1X_3 - 0,67X_2X_3 – 5,43X_1^2 – 3,26X_2^2 – 1,96X_3^2$

- *Determine the optimal value:* Perform regression equations to determine the optimal values of the variables ($X_1$, $X_2$ and $X_3$). The process is calculated on Design Expert 12 software and overlaps the response surfaces to find the optimal point predicted with gum arabic content $X_1$ (73,34 g/l), drying temperature $X_2$ (177,35ºC) and compressed air pressure $X_3$ (3,07 kg/cm$^2$) and predictive objective functions with polyphenol content $Y_1$ (28,66 mg

acid galic/g), chlorophyll content $Y_2$ (10,57 mg/g) and antioxidant activity total $Y_3$ (19,68 mg ascorbic acid/g).

The experiment was repeated 3 times at the condition of gum arabic content $X_1$ (73.0 g/l), drying temperature $X_2$ (177.0$^o$C), and compressed air pressure $X_3$ (3.1 kg/cm2) and determined the objective functions as the polyphenol content $Y_1$ (28,15 ±1,13 mg gallic acid/g), the chlorophyll content $Y_2$ (10,62 ±0,27 mg/g) và the total antioxidant activity $Y_3$ (19,47 ±0,42 mg ascorbic acid)/g).

### 3.3.3. Spray drying process to create pennywort powder containing polyphenols, chlorophyll

#### 3.3.3.1. Proposed spray drying process to create pennywort powder

From the research results, a spray-drying process is proposed to create pennywort powder containing polyphenols and chlorophyll with antioxidant activity with the parameters shown in Fig 3.12.

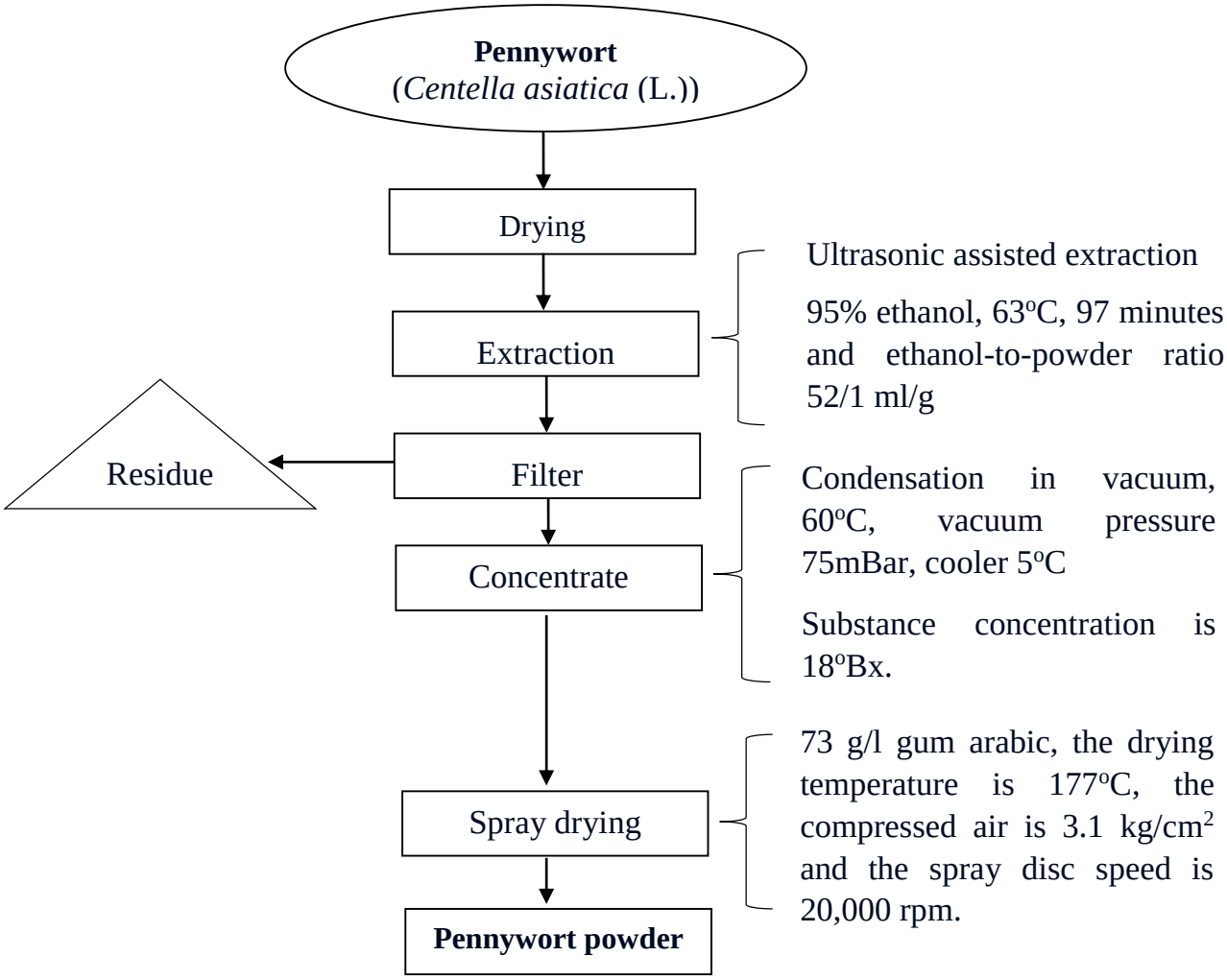
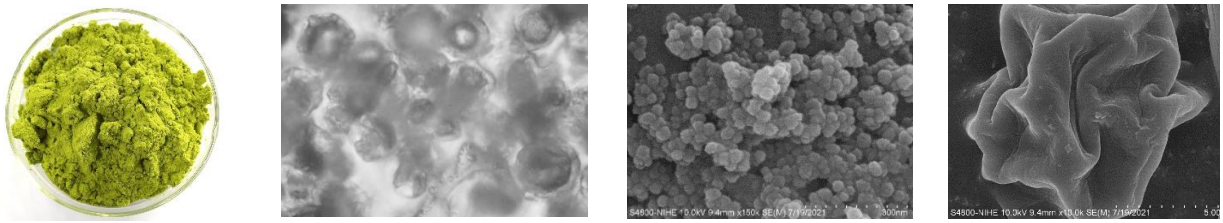



**Fig 3.12. Spray drying process to create pennywort powder**

### 3.3.3.2. Evaluation of the quality of pennywort powder

Drying under optimal conditions, pennywort powder containing polyphenols, and chlorophyll with antioxidant activity has bright green color; high fine powder, and high porosity. Observing the shape of the powder with a microscope, we see that the active substance is covered in the middle of the granules by gum arabic.



(a) (b) (c) (d)

**Fig 3.13. Observational image of pennywort powder**

(a) Photo of pennywort powder, (b) image viewed using a microscope, and (c, d) images viewed with a scanning electron microscope (SEM)

At optimum drying conditions, pennywort powder containing polyphenols, chlorophyll with antioxidant activity is good solubility, the average particle size (Z) is 137.6 nm, the polydispersity index is 0.627, the degree of density is 0.627, and humidity is 5.25%.

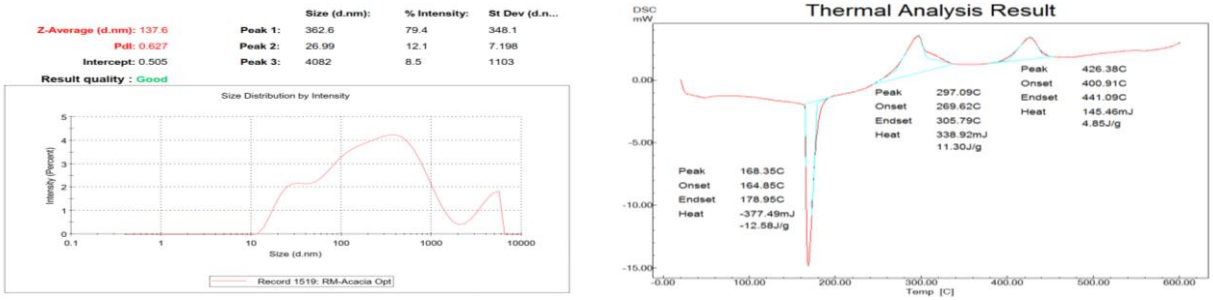



**Fig 3.14. Average particle size distribution spectrum, the polydispersity index (PDI), and thermal characteristic spectrum at optimum conditions**

Moreover, pennywort powder has a dehydration temperature of 168.35$^{o}$C, no endothermic peak indicating bond breaking temperature, functional group decomposition temperature is 297.09$^{o}$C and temperature complete decomposition is 426.38$^{o}$C.

Analysis of chemical and microbiological parameters for pennywort powder containing polyphenols, and chlorophyll with antioxidant activity were obtained by spray drying technique at optimal conditions. The analytical results were satisfactory according to regulations of the Ministry of Health: "Maximum Limits of Biological and Chemical Contamination in Food".

**Table 3.5. Chemical and microbiological composition of pennywort powder**

| **Composition** | **Unit** | **Result** |
|---|---|---|
| **Chemical composition** | | |
| Asen (As) | mg/kg | 0,04 |

| | | |
|---|---|---|
| Cadimi (Cd) | mg/kg | Not detected (MDL=0,2) |
| Mercury (Hg) | mg/kg | Not detected (MDL=0,2) |
| Plumbum (Pb) | mg/kg | Not detected (MDL=0,2) |
| **Microbiological composition** | | |
| *Salmonella* | CFU/gam | Not detected |
| *Escherichia coli* | CFU/gam | Not detected |

### 3.3.3.3. Mix to create pennywort juice from pennywort powder

To mix and create pennywort juice, experiment with each sample is 50ml of purified water with different concentrations of pennywort powder 1, 2, 3, 4, 5 and 6 (%) and sucrose 2, 4, 6 and 8 (%). Pennywort juice samples were evaluated for sensory evaluation. The results are presented in Fig 3.15.

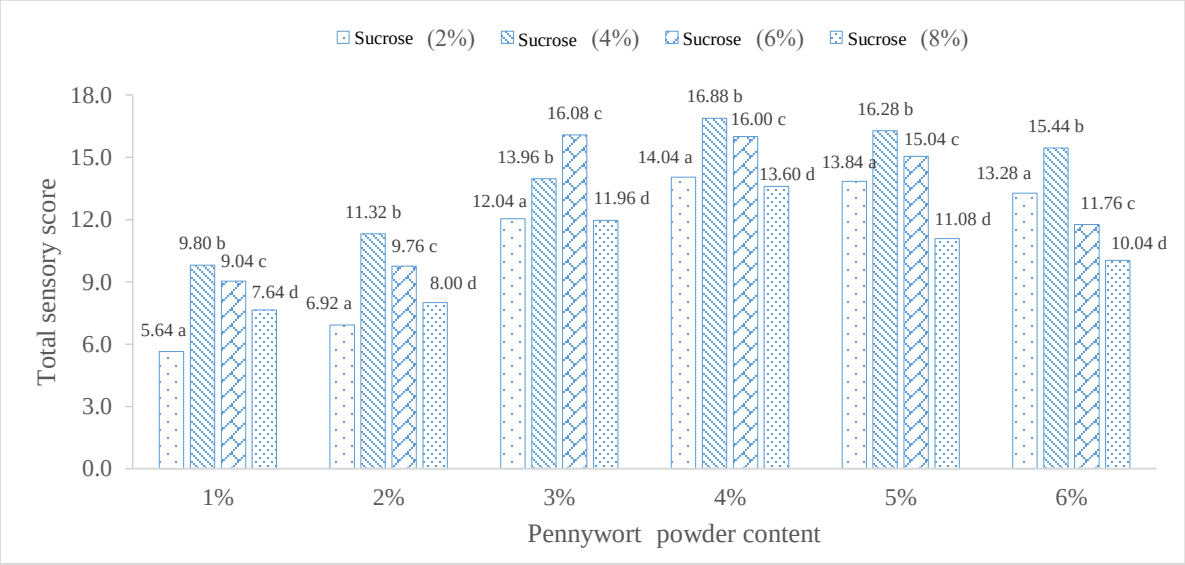



**Fig 3.15. Effect of pennywort powder and sucrose on sensory scores**

Pennywort juice with 4% centella asiatica powder and 4% sugar will give a total sensory score of 16.88 points and the highest. Therefore, pennywort juice with 4% pennywort powder and 4% sugar content was selected to be mixed to create the pennywort beverages and it has total antioxidant activity was 67.23±1.44 mg ascorbic acid/100 ml, reducing power activity was 195.63±2.99 mg FeSO4/100 ml and DPPH free radical scavenging activity was 64.73±1.47%.

## 3.3.4. Evaluation of changes in content of polyphenols, chlorophyll and activities of pennywort powder during storage

Pennywort powder was obtained from the drying process under optimal conditions. It is stored at room temperature 25ºC and cold temperature 5ºC, storage time from 0÷12

months, and evaluation of the reduction of polyphenol, and chlorophyll with antioxidant activity in pennywort powder every 2 months. The results are presented in Fig 3.16÷3.19.

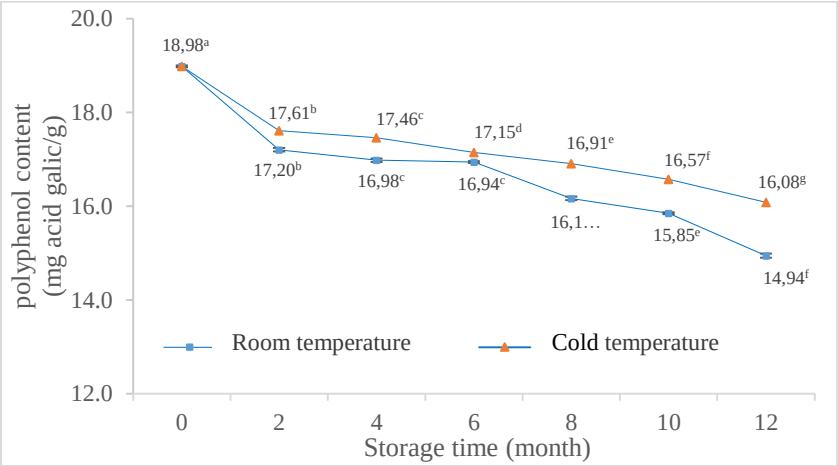



**Fig 3.16. Changes in polyphenol content in pennywort powder during storage**

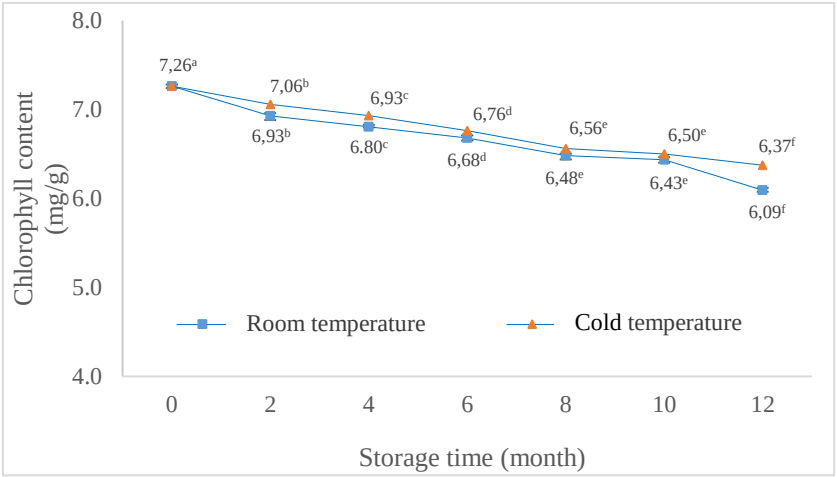



**Fig 3.17. Changes in chlorophyll content in pennywort powder during storage**

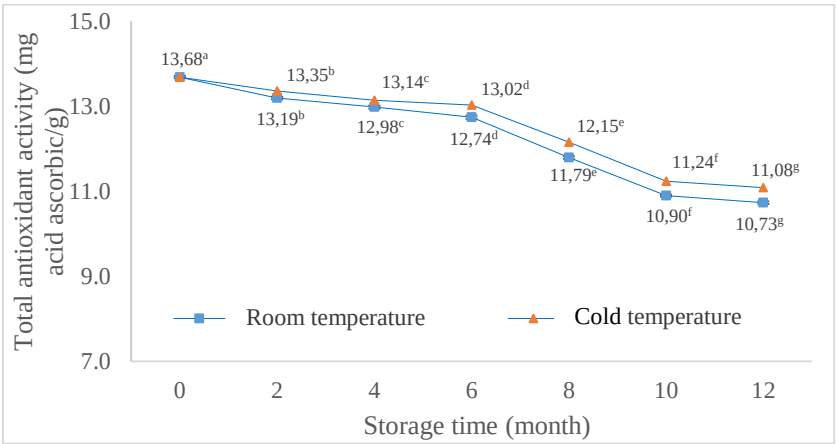



**Fig 3.18. Changes in total antioxidant activity of pennywort powder during storage**

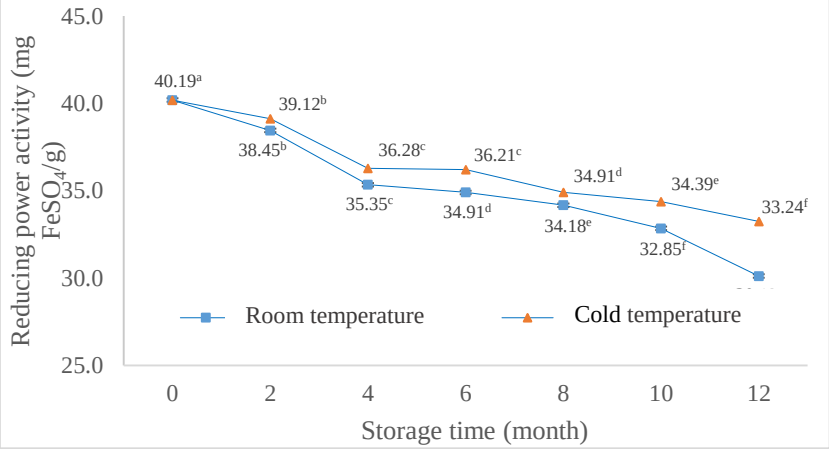



**Fig 3.19. Changes in reducimg power activity of pennywort powder during storage**

The polyphenols content, chlorophylls content and antioxidant activities of pennywort powder decreased when stored at both room (30ºC) and cold temperatures (5ºC). However, storage at room temperature resulted in a higher loss compared to cold storage.

Specifically, the content of polyphenols, chlorophyll, total antioxidant activity and ferric reducing antioxidant power stored at room temperature after 12 months will be reduced more than those stored at cold temperature are 5%, 3.9%, 2.6% and 7.8%, respectively.

# CONCLUSIONS AND RECOMMENDATIONS

## 1. CONCLUSION

From the above research results, it shows that the thesis has completed all the research contents. Conclusions drawn from the research process of the thesis:

1) Identification of pennywort species used in the thesis by DNA gene analysis. The results show that the DNA of pennywort used in the thesis has high similarity (98.44% - 98.76%) with the DNA gene of matK and high similarity (98.21-100%) with the DNA gene of ITS of the species Centella asiatica is available on NCBI. This result confirms that the pennywort sample collected by the thesis in Nha Trang - Khanh Hoa belongs to *Centella asiatica* (L.) Urban species.

2) The main chemical composition of pennywort has been evaluated. The results showed that pennywort collected in Nha Trang - Khanh Hoa had a polyphenol content of 13.56 mg of gallic acid/g dry, chlorophyll content of 4.73 mg/g dry, and a total antioxidant activity of 6.06 mg of ascorbic acid/g dry, reducing power activity was 22.89 mg FeSO4/g dry and DPPH free radical scavenging activity was 79.48%. In addition, pennywort contains a number of biologically active compounds such as triterpenoid, asiaticoside, quercetin, rosmarinic acid.

3) Researched on extraction of polyphenols, chlorophylls with antioxidant activity from pennywort by ultrasonic assisted extraction using 95% ethanol. The result was optimal conditions extraction process: temperature is 63oC, time is 97 minutes and ethanol-to-powder ratio is 52 ml/g. At optimal extraction conditions, the extracts of pennywort had polyphenols, chlorophylls, total antioxidant activity, reducing power activity and DPPH free radical scavenging activity of 19.18±0.72 (mg gallic acid/g dry), 7.45±0.16 (mg/g dry), 13.81±0.31 (mg ascorbic acid/g dry), 37.31±1.32 (mg FeSO4/ g dry) and 81.66±3.4%, respectively.

4) Researched on spray drying process to create pennywort powder containing polyphenols, chlorophyll with antioxidant activity. As a result, some parameters are suitable for spray drying: the carrier is gum arabic, the spray disc speed is 20,000 rpm and the spray drying conditions: gum arabic concentration is 73 g/l, the drying temperature is 177oC, the air pressure is 3.1 kg/cm2. Pennywort powder spray-dried according to optimal parameters has the content of polyphenols, chlorophyll, total antioxidant activity and iron reduction activity is 28.15±1.13 mg gallic acid/g, 10.62± respectively. 0.27 mg/g, 19.47±0.42 mg ascorbic acid/g and 59.50±2.08 mg FeSO4/g, respectively. The obtained respectively powder has a low moisture content of 5.25%, the average particle size is 137.6 nm and the respectively powder has good thermal properties such as: temperature of dehydration absorption, decomposition temperature of groups. function, the complete decomposition

temperature of molecules is 168.35 oC, 297.09 oC, 426.38oC, respectively.

5) Research on storage of pennywort powder for 12 months at 30ºC and 5ºC shows that pennywort powder stored at 5ºC has a slower degree of compound changes when stored at 30ºC. After 12 months of storage at 30ºC and 5ºC, pennywort powder had a decrease in polyphenol, chlorophyll, total antioxidant activity, and iron reduction activity (21.3%; 16.1%; 21.6%, 25.1%) and (15.3%; 12.2%; 19.0%; 17.3%), respectively.

6) Developed a mixing formula to create 100 ml of pennywort juice with the ratio of pennywort powder 4g and saccharose sugar 4g. The obtained got pennywort juice with good sensory quality. With the above mixed formula, pennywort juice has total antioxidant activity of 67.23±1.44 mg ascorbic acid/100ml, reducing power activity is 195.63±2.99 mg FeSO4/100ml and DPPH free radical scavenging activity was 64.73±1.47%.

## 2. RECOMMENDATIONS

From the research process of the thesis, provide some recommendations:

- It is necessary to continue to study and evaluate some biological activities such as: antifungal, antibacterial, antiviral, immune enhancing activity. Thereby researching to produce products on a large scale to calculate production costs and proceed to commercial development of pennywort powder in the future.

- It is necessary to continue to study and evaluate some other biologically active compounds in pennywort powder such as asiaticoside, quercetin, rosmarinic acid.