Study to Evaluate the Effectiveness and Quality of Products of The Process of Extracting *Codonopsis javanica* Extract at Pilot Scale

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Abstract. The *Codonopsis javanica*, also known as “Đãng sâm” in Vietnamese, is a medicinal plant of high economic value. In this study, we further extended the previously reported process to produce *C. javanica* root extract by examining the concentrating stage to produce dried extract. Afterwards, effects of storage conditions on were evaluated and the pilot scale extraction process was compared with the lab-scale process. These processes were investigated with respect to different extract quality parameters including total polyphenol content (TPC), total flavonoid content (TFC), DPPH and ABTS scavenging activity. Best conditions for the concentration process included time of 120 minutes, temperature of 60°C. The efficiency of extracting *C. javanica* extract at pilot scale shows a difference of about 20% and this dried extract will retain the best quality when stored at 5°.

1. Introduction

Since ancient times, *Codonopsis javanica* (Blume) Hook.f. & Thomson is a valuable herb commonly used in Asian folk medicine such as India, China, Indonesia, Laos and Vietnam. In Vietnam, they are known as "Đãng sâm" [1–5]. This is a perennial herbaceous plant in the form of branched vines, often scattered in the northern mountainous provinces (such as Lai Chau, Lao Cai) and in the south (Kon Tum, Dak Lak, Gia Lai) [6–8]. The root portion of the "Đãng sâm" is edible. It has a long cylindrical shape, with an overall light-yellow color and white core. According to previous studies, *C. javanica* roots have been reported to have ingredients such as polyacetylenes and phenolics, while phytochemical evaluation results also demonstrate that it has a diverse group of self-compounds such as flavonoids, alkaloids, tannins, coumarin and triterpenoids [9–12]. Such diversity in useful compounds establishes the use of this raw material in traditional medicine and explains the pharmacological effects as anti-oxidant, anti-fatigue, anti-tumor, antibacterial and immune system enhancement of *C. javanica* [13–15].

In a previous study, we examined the technological parameters of lab-scale production process of *C. javanica* root extract [15]. A wide range of conditions in some main production steps including drying, pre-treatment and extraction were taken into account for optimization. The obtained extract
also showed good antioxidant activity and had high flavonoid content [15]. Continuing this research pathway, this study aimed to justify the scalability of the process by evaluating some quality parameters, including total polyphenol content (TPC), total flavonoid content (TFC), DPPH and ABTS scavenging activity of the *C. javanica* root extract obtained at later stages. We first contemplating the concentrating process and determine drying conditions that afford the dried extract with good quality parameters. The dried extract was then preserved at different storage conditions and the trend of quality parameters were evaluated. Lastly, we compared the quality outcomes of *C. javanica* wet extract obtained at pilot scale and those obtained at lab scale. The results are expected to suggest the direction of production scale development and commercialization in the future [7], [16], [17].

2. Materials and methods

2.1. Materials and procedures

The material for the study is the tuberous root of the *C. javanica* collected in January 2020 in Kontum province, Vietnam. The extraction process to afford the extract followed our previous study [15]. Briefly, the ingredients were first dried in the OVEN JSOF-150 convection oven at 70°C for 8 hours, then broken down by a medicinal flour mill (Super Blender, QE-500, China) and sifted through an eye sieve grid (θ = 0.5 mm) to obtain a sample of uniform size (Figure 1).

![Figure 1](image_url)

**Figure 1.** Process of preliminary processing and processing of *C. javanica* materials

Reflux extraction was adopted to afford the extract from the ground material. Following conditions were used: material size <= 0.5mm; 55% ethanol extraction solvent; extraction temperature 60°C; extraction time 67 minutes; solvent material rate 1/38 (g / ml); and stirring speed of 300rpm. The experiments were replicated in lab-scale, medium-scale (100g or 500g of material) and pilot instruments (1,000g or 2,000 g material) with identical conditions. The obtained extract was then concentrated with a rotary evaporator (WEV-1005, Daihan Scientific, South Korea) to afford the dried *C. javanica* extract.

2.2. Total polyphenol content determination (TPC)

The method of Zivic et al. (2019) with some modification was used to determine the total phenolic content in the extract [10]. First, the 0.5 mL ethanol extract was pipetted into a test tube containing 2.5 mL Folin–Ciocalteu reagent 10% (v/v). After 5 minutes, 2 mL Na₂CO₃ 7.5% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 60 minutes in the dark. Finally, the absorbance was measured at 765 nm and the results were shown in µg of gallic acid equivalents per dry weight of the sample (µgGAE/mg).
2.3. Total flavonoid content (TFC)
Base on the aluminum chloride colorimetric method, the total flavonoid content was determined. The content of total flavonoid was estimated by Ebrahimzadeh et al. (2018) with a little modification [11]. After 5 minutes, 0.5 mL of the extract and 0.1 mL of AlCl$_3$ 10% were mixed, vigorously shaken with 0.1 mL of CH$_3$COOK 1M and 4.3 ml distilled water. Then, the mixture was incubated for 30 minutes in the dark. The absorbance was measured at 415 nm with quercetin was a standard. The TFC results were shown in µg of quercetin equivalents per dry weight of the sample (µgQE/mg).

2.4. Evaluation of antioxidant potential
The antioxidant activity of the extract was evaluated by IC$_{50}$ values of DPPH and ABTS scavenging activity assay by Islam et al. with some modification [12]. Stock solutions of DPPH and ABTS were diluted in order to have working solutions, its absorbance 1.1 ± 0.02 at 517 nm and 714 nm, respectively. Then, the 1 mL ethanol extraction was pipetted into 3 mL working solution and the mixture was stable at room temperature in the dark within 30 minutes. The optical measurement of mixture was using UV/VIS–1800 Shimadzu Spectrometer. In blank sample, 0.5 mL sample was replaced ethanol 96%. Standard sample: Vitamin C (0.1g ± 0.01) was dissolved ethanol 96% into volume flask 100mL, in the dark (C = 100 µL/mL). The percent DPPH and ABTS scavenging effect were calculated by using following equation: Scavening effect (%) or percent inhibition (%I).

$$\%I = \frac{Ab - As}{Ab} \times 100$$ (1)

Where: Ab–Absorbance of blank sample, As–Absorbance of sample, %I–Percent inhibition.

The IC$_{50}$ value was the concentration of the sample which inhibited percentage reaches 50%. Therefore, IC$_{50}$ values are negatively correlated to the antioxidant activity, the lower IC50 value means the highest antioxidant activity of the tested sample.

2.5. Statistical analysis
All experiments were carried out in triplicate and the results were expressed as mean values and standard deviation. One–way analysis of variance (ANOVA) was performed using SPSS (version 23, IBM, USA) and differences between samples were compared using Tukey’s test (P <0.05)

3. Results and discussion

3.1. Effects of concentration temperature and time on quality parameters of dried extract
Concentration time of extract determines the content of active ingredients (TPC, TFC, DPPH, ABTS) and is shown in Figure 3. The results showed that the concentration of TPC, TFC, DPPH and ABTS tended to increase when the time for the concentration process increased from 60 minutes to 120 minutes. For TPC, the concentration increased 1.6 times at 120 minutes compared with concentration at 60 minutes. Similarly, for TFC, the content increased slightly when changing the time from 60 minutes to 90 minutes (increased 5%) and increased rapidly when the time reached 120 minutes (increased 20%). Besides, for the antioxidant value, the content increased by 4.7 and 1.4 times, respectively, corresponding to DPPH value (from 0.212 mgAAE / g DW up to 1.015 mgAAE / g DW) and ABTS (from 2.324 mgAAE / g DW up to 3.422 mgAAE / g DW). The fact that the content of active ingredients tends to increase gradually when increasing the concentration time is explained by the fact that when at a high humidity level, the content will be directly diluted by the water component, so when conducting the assessment, it will be obtained. the results were not high. The removal of water will simultaneously increase the purity of the active substance and along with that, it will help support the better preservation of the extract in the future. Therefore, the concentration time selected at 120 minutes is appropriate to conduct the survey of concentration temperature of C. Javanica extract. The effect of concentration temperature of C. Javanica extract was evaluated and shown in figure 3. In general, when the temperature of the concentration process changes from 40 °C to 60 °C, the content of active ingredients tends to increase and decrease after 60 °C. The TPC and TFC values increased from 3.264 mgGAE / g to 3.782 mgGAE / g (1.15 times) and 3.396 mgQE / g to
3.733 mgQE / g (1.09 times). According to Marja et al., Plants with a greater concentration of polyphenols and flavonoids can be considered to have strong antioxidant activity [10]. Results of assessment of antioxidant content of C. Javanica extract showed that DPPH and ABTS values increased corresponding to the increase of TPC and TFC content. Specifically, the highest increase in DPPH value was 1.7 times (from 1.123 mgAAE / gDW to 1.987 mgAAE / gDW) and ABTS value also increased the most at 60 °C (reaching 4.344 mgAAE / gDW compared to 3.467 mgAAE / gDW at 40 °C). The tendency to increase the concentration is shown by an increase in the temperature where the concentrate is present. However, when conducting a reduction in the hydration of the extract at a high temperature (80 °C), the content of bioactive substances decreases gradually. This is consistent with previous studies on the temperature sensitivity of TPC and TFC in nature. Long-term concentration of the extract at high temperature will degrade these compounds, thereby reducing the quality of the final product. Therefore, the temperature of 60 °C is suitable for use in concentrating C. Javanica extract.

![Figure 3](image1.png) **Figure 3.** Effects of drying time on the content of bioactive substances of dried C. javanica extract

![Figure 4](image2.png) **Figure 4.** Effects of drying temperature on the content of bioactive substances of dried C. javanica extract

### 3.2. Comparison between quality parameters of wet C. javanica extract obtained by pilot scale and by laboratory scale

The results of the extraction process of C. javanica extract at pilot scale are shown in Figure 5. The results showed that, when extracting in batch of 100g, the values of TPC, TFC, DPPH and ABTS reached 2.763 mgGAE / gDW, 2.304 mgQE / gDW, 0.059 mgAAE / gDW and 2.804 mgAAE / gDW, respectively. Compared with the laboratory scale, the extraction results in batch 100g did not change much. For 500g batch, the values of bioactive contents tended to decrease compared to batch 100g. From the 1kg batch onward, the values were similar in trend to the 500g batch. Specifically, the TPC and TFC values of the 2kg batch decreased by 1.4 and 1.2 times, respectively, for 500g and 2kg batch. This decrease in concentration can be due to a change in the extraction parameters. However, when it comes to economic efficiency and reduction of emissions to the environment, it is appropriate to reduce the ratio of solvents used for extraction. At the same time, the content of the extract of C. Javanica extract in 2kg batch was not too low (only about 20% compared to the extraction at laboratory scale), which can be improved through adjustment. relevant parameters during extraction such as temperature, time, equipment
3.3. Effects of temperature and storage time on quality parameters of dried C. javanica extract

*C. javanica* was extracted at a scale of 100g / batch based on the optimized extraction conditions in the previous study of the research group Nguyen Van Thuy et al (2020). The extract after concentration was stored under different conditions of temperature and time (5 °C, 30 °C, 45 °C and 0 day, 10 days, 20 days, 30 days). Besides, the results of the variation of content of biological activities in extracts are shown in figure 6, 7 and 8, respectively.

The results of sensory evaluation of the dried extract after 30 days showed that storage time and temperature did not significantly affect the sensory parameters. At different values of storage temperature and time, *C. Javanica* extract showed no changes in color or general condition of the product compared to the first day. No change in taste or appearance of mildew was noted.

*C. Javanica* extract after concentration is stored at different temperature and time conditions. Results of the change of content of biological activities in extracts are shown in figure 7. First, for extracts stored at 5°C, the results showed that the content of bioactive activities decreased gradually. TPC, TFC, DPPH and ABTS values decreased slightly by about 5% on the 30th day of storage compared to the first day. Overall, this degradation is minor and insignificant. At the same time, for extracts stored at 30 °C, it is easy to see the change in content at day 30 compared to the first day. Specifically, the values of TPC and TFC decreased by 1.8 and 2 times at the high and low peaks of the experimental results, respectively. In terms of antioxidant activity, the DPPH value decreased significantly after 10 days of storage (decreased from 0.51 mgAAE / gDW to only 0.05 mgAAE / gDW) and completely lost its effectiveness after 10 days onwards. Along with that, ABTS values were
also decreased by 2.6 times when stored on day 30 compared to the beginning. This shows that, at the temperature of 30°C, the content of bioactive substances is still partially stored (TPC, TFC and ABTS), however the degradation of the antioxidant values is one point. care should be taken when storing in this condition. Finally, for the temperature of 45°C, the content fluctuated strongly after only 10 days. TPC and TFC values decreased by 1.3 times on day 10, 2.4 times on day 20 and 3 times on day 10 and day 20. The same variation occurred in DPPH and ABTS values (DPPH value is invalid. purely on the 10th day of the survey). ABTS value decreased to 7.7 times (from 4.344 mgAAE / gDW to 0.562 mgAAE / gDW). The above results have shown that the temperature of 5oC is the best condition to help preserve almost completely the biological value of C. Javanica extract. However, if the above storage conditions are not available, extracts can be used at temperatures below 30°C and need to be used within 20 days for the product to achieve the best effect.

![Figure 7. Effects of storage time at 5°C, 30°C and 45°C on quality of C. javanica extract](image-url)

4. Conclusion
Codonopsis javanica is a medicinal herb with high biological value, enhances resistance, increases fitness, replenishes the body, reduces stress. This study has evaluated the extraction efficiency C. Javanica extract at pilot scale, extract concentration conditions and microbiological safety of the product. The concentration time and temperature of C. Javanica extract were 120 minutes and 60°C respectively, resulting in TPC, TFC, DPPH and ABTS values of 3.782 mgGAE/gDW, 3.733 mgQE/gDW, 1.987 mgAAE/gDW and 4.344 mgAAE/gDW, respectively. The efficiency of extracting C. Javanica extract at pilot scale shows a difference of about 20% and this dried extract will retain the best quality when stored at 5°C.

Acknowledgements: This research is funded by Nguyen Tat Thanh University under grant number 2020.01.140/HD-KHCN. We would like to thank Nguyen Tat Thanh University, Ho Chi Minh City,
Vietnam and Kon Tum Department of Science and Technology, Kon Tum Province, Vietnam for the support of time and facilities for this study.

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