DETERMINATION OF OPTIMAL CONDITIONS FOR THE ENHANCEMENT IN SAPONINS EXTRACTION FROM DANG SAM (*CODONOPSIS PILOSULA* NANNF.)

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ABSTRACT

The demand for improvements in saponin extraction has recently been high for industrial application. The objective of this study was to identify and evaluate the extraction of saponins from Dang Sam (*Codonopsis pilosula*) using optimal conditions. The methods used were subsequent determination of conditions for solvent extraction, i.e. ethanol concentration, extraction duration, and sample to solvent ratio, followed by the evaluation for possible enhancement in saponin yield. Results showed that the best conditions were identified as: i) 50% of ethanol concentration; ii) 6 hours of extraction duration; and iii) 1:40 sample to solvent ratio. And applying those optimizations for extraction brought statistically higher saponins yield (45.82 ± 0.26 mg.g^{-1}) ($P < 0.05$) in comparison with treatments with unoptimized conditions (30.48 ± 0.50 mg.g^{-1}). The contribution of this study was a fundamental procedure for further scientific research and industrial application of saponin extraction from the potential *C. pilosula* herb.

Keywords: Codonopsis pilosula, Dang Sam, saponins, solvent extraction.

INTRODUCTION

Codonopsis pilosula (Franch.) Nannf, locally known as Dang sam, is a medicinal herb found in Vietnam and other Asian countries. *C. pilosula* has been proven to possess many beneficial properties, including spleen strengthening, lung moisturizing, blood nourishing, liquid engendering, immune function enhancing and modulating, and anti-tumor effects (Guo *et al.*, 2024). The scientific foundation for *C. pilosula*'s extensive use in the treatment of numerous diseases is provided by its abundant active components, one of which is saponins. *C. pilosula*'s saponins have been found to have pharmaceutical properties of hemolytic, molluscicidal, anti-inflammatory, antimicrobial, and crucial in the treatment of digestive disorders, leading to a high demand in its extraction and application in the pharmaceutical industry (Cheok *et al.*, 2014).

The development of sufficient extracting techniques has drawn huge attention in order to maximize the yield of saponins from herbal plants for industrial uses. Thus, a variety of methods have been applied recently, such as physical, chemical, and biochemical methods, including certain advanced green extractions with positive outcomes (Azmir *et al*., 2013). In a number of common industrial-based methods, the extraction stage with ethanol as solvent has been considered crucial as an important baseline for improvement before any further assisting/recovery steps can be considered. However, the lack of thorough optimization of these specific conditions in the ethanol extraction for saponins from *C. pilosula* certainly creates significant drawbacks to the recovery efficiency.

Therefore, in this study, optimal conditions for saponin extraction from Dang Sam including solvent concentration, extraction duration, and sample to solvent ratio were determined, and the enhancement in saponin yield applying all optimal conditions was evaluated.

MATERIALS AND METHODS

Materials

Dang Sam (C. pilosula):

Dried *C. pilosula* (moisture: < 10%) in good conditions from Tu Mơ Rông District, Kon Tum Province, was ground and sieved to the particle size of 500 µm, and stored in the desiccator for later use.

Chemicals and reagents:

Chemicals and reagents for analysis, such as oleanolic acid, vanillin and sulfuric acid were purchased from Sigma-Aldrich (Dorset, UK).

Determination of optimal conditions for saponin extraction from Dang Sam

Determination of solvent concentration:

The optimal solvent concentration for the extraction of saponins from *C. pilosula* was determined (in triplicate) from a range of ethanol concentrations, i.e. 30%, 50%, 70%, 90% (a), via the conventional solvent extraction method (Mai *et al.*, 2018). Briefly, 1 g of *C. pilosula* powder was extracted with 30 mL of the respective ethanol concentration in (a) at 55^{o}C for 2 hours. Ethanol was removed from the extract via an 8-hour evaporation at 60^{o}C. The obtained extract was either used straight away, or fridge-stored at 0^{o}C until further analysis. The total saponin content was determined to identify the best concentration for the next experiments.

Determination of duration for extraction:

The optimal duration for the extraction of saponins from *C. pilosula* was determined (in triplicate) from a range of duration, i.e. 0, 2, 4, 6, 8, 12, 24 hours (b), via the conventional solvent extraction method (Mai *et al.*, 2018). Briefly, 1 g of *C. pilosula* powder was extracted with 30 mL of the optimal ethanol concentration from the previous experiment (a) at 55^{o}C for the respective duration of (b). Ethanol was removed from the extract via an 8-hour evaporation at 60^{o}C. The total saponins content was determined to identify the best duration for next experiments.

Determination of sample:solvent ratio:

The optimal sample:solvent ratio for the extraction of saponins from *C. pilosula* was determined (in triplicate) from a range of ratio, i.e. 1:10, 1:20, 1:30, 1:40 (c), via the conventional solvent extraction method (Mai *et al.*, 2018). Briefly, the sample:solvent ratios in (c) were set up between 1 g of *C. pilosula* powder and the respective volume of ethanol of the optimal concentration from the previous experiment (a) at 55^{o}C for optimal duration from the previous experiment (b). Ethanol was removed from the extract via an 8-hour evaporation at 60^{o}C. The total saponin content was determined to identify the best duration for the final experiment.

Evaluation of the enhancement in saponin yield from Dang Sam using optimal extracting conditions

The enhancement in saponin yield from the extraction process was evaluated with three treatments in triplicate: i) E50: ethanol extraction with optimal conditions from (a), (b) and (c); ii) E70: ethanol extraction with previously reported conditions (1g sample:30mL of 70% ethanol, extracted in 3 hours at 55^{o}C) (Mai *et al.*, 2018); and iii) W: extraction with water solvent (1 g sample:40 mL H_2O, extracted in 6 hours at 55^{o}C) (Mac, Ngo, 2020). The total saponin content was subsequently identified.

Data collection for total saponins content

The total saponin contents (in mg saponins per 1 g of the initial *C. pilosula*'s powder) from all extracts were determined by colorimetric assays consisting of the color development reaction with 8% (w/v) vanillin reagent and 72% (v/v) sulfuric acid, followed by the optical density measurement at 560 nm of absorbance (BioTek Epoch 2 Microplate Spectrophotometer, Agilent, USA) and quantification against the respective oleanolic acid standard curve (Hiai *et al.*, 1976).

For the construction of standard curve, oleanolic acid was used as a replacement for the extract sample (Lim *et al.*, 2019). Standard solutions of oleanolic acid (ranging from 0.2 – 0.8 mg.mL^{-1}) were preprared and processed via a similar procedure to the colorimetric determination of saponin as described earlier.

Data analysis

Data were calculated by Excel (Microsoft 365) and statistically analyzed by SPSS 27.0. One-way analysis of variance (ANOVA) and Duncan's test were used to determine the significant difference between treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Determination of optimal conditions for saponin extraction from Dang Sam

Determination of the optimal solvent concentration:

Results indicated that the lower the ethanol concentration (30% and 50%) used for extraction, the higher the saponin yield (45.65 ± 1.37 mg.g^{-1} and 38.62 ± 2.70 mg.g^{-1}, respectively) in comparison with the lower concentration (70% ethanol: 29.35 ± 3.08 mg.g^{-1}; 90% ethanol: 28.22 ± 0.50 mg.g^{-1}). The differences were significant ($P < 0.05$). (Figure 1)

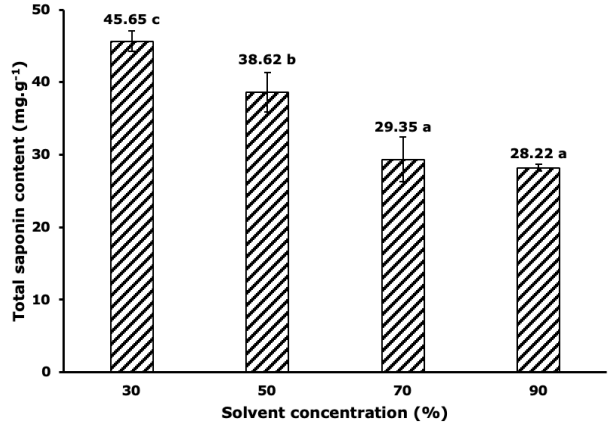

Figure 1. Optimal solvent concentration for the extraction of saponins from Dang Sam

Data were presented as mean ± standard deviation (n = 3). Different alphabetical letters next to the data values on each column indicated significant differences between treatments (P < 0.05).

Determination of the optimal duration for extraction:

Overall, the longer the extraction time, the higher the saponin yield. These differences were significant ($P < 0.05$). The obtained saponin content peaked with the 6-hour extraction duration (45.80 ± 1.55 $mg.g^{-1}$) ($P < 0.05$) when compared to the 2-hour and 4-hour durations (35.26 ± 1.13 $mg.g^{-1}$ and 39.17 ± 1.40 $mg.g^{-1}$, respectively). However, beyond six hours, there was then no significant difference ($P > 0.05$) for any longer extraction durations in saponin yield (8-hour: 45.80 ± 0.77 $mg.g^{-1}$, 12-hour: 43.21 ± 1.20 $mg.g^{-1}$, and 24-hour: 44.13 ± 0.58 $mg.g^{-1}$, respectively). The control with 0-hour of solvent treatment had the significantly lowest saponin yield (20.72 ± 1.05 $mg.g^{-1}$). (Figure 2)

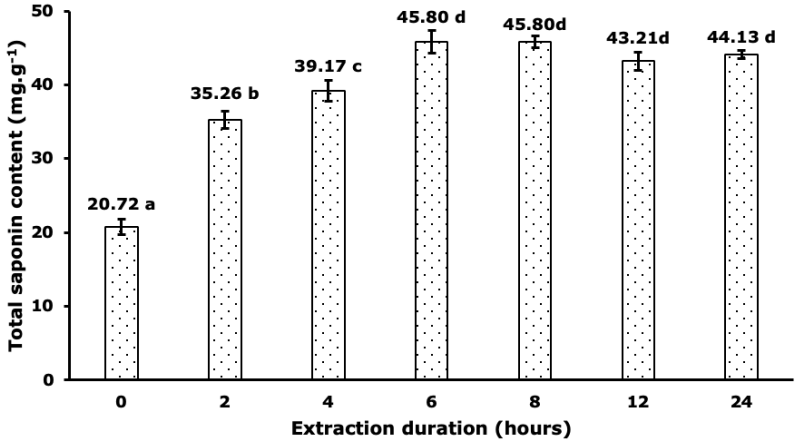

Figure 2. Optimal duration for the extraction of saponins from Dang Sam

Data were was presented as mean ± standard deviation (n = 3). Different alphabetical letters next to the data values on each column indicated significant differences between treatments (P < 0.05).

Determination of the optimal sample:solvent ratio:

The change in sample to solvent ratio with increasing solvent volume resulted in statistically difference ($P < 0.05$) in saponin content. The ratio of 1:40 yielded the highest saponin content (41.37 ± 0.66 $mg.g^{-1}$) ($P < 0.05$). The 1:30 ratio produced the second highest yield (37.47 ± 1.40 $mg.g^{-1}$) ($P < 0.05$). There were no statistically significant differences between the 1:10 and 1:20 ratio (33.32 ± 0.69 $mg.g^{-1}$ and 35.12 ± 2.30 $mg.g^{-1}$, respectively) ($P > 0.05$). (Figure 3)

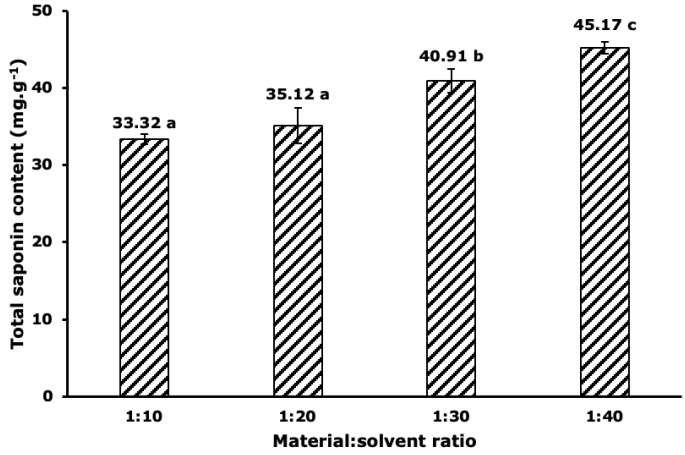

Figure 3. Optimal sample:solvent ratio for the extraction of saponins from Dang Sam

Data were presented as mean ± standard deviation (n = 3). Different alphabetical letters next to the data values on each column indicated significant differences between treatments (P < 0.05).

Evaluation of the enhancement in saponin yield from Dang Sam using optimal extracting conditions

In the evaluation experiment, the E50 treatment using all optimal extracting conditions brought a statistically higher saponins yield (45.82 ± 0.26 $mg.g^{-1}$) ($P < 0.05$) compared to the E70 treatment using previously reported extracting conditions (30.48 ± 0.50 $mg.g^{-1}$). The outcome was also significantly better ($P < 0.05$) than another conventional extracting method using water as solvent (44.86 ± 0.05 $mg.g^{-1}$) (Figure 4). Our results are consistent with the similar study on the extraction of saponins from *Pouteria cambodiana* (Sanneur *et al.*, 2023). Those authors also reported a significant increase in saponins yield (36.04 $mg.g^{-1}$) ($P < 0.05$) using 50% ethanol in comparison with 60% and 70% ethanol. In contrast, 70% ethanol extraction in *Panax ginseng* resulted in statistically higher yield of saponins (5.4%) ($P < 0.05$) compared with any lower ethanol concentration (Kim *et al.*, 2007).

Solvent extraction techniques have been widely employed and optimized to identify the ideal conditions in a few recent investigations, both international and domestic, concerning saponin extraction (Pham *et al.*, 2020; Nguyen *et al.*, 2023). A total saponin of 23.7 $mg.g^{-1}$ from *Polyscias fruticosa* (L.) Harms was extracted under comparable extraction circumstances and results with E70 treatment (ethanol concentration: 74%, extraction duration: 3.32 hours, extraction temperature: 80^{o}C, solvent:material ratio: 1:16.5) (Pham *et al.*, 2020). In addition, applying 70% ethanol concentration yielded a similar result, 24.27 mg/g of total saponin content, with a solvent-to-material ratio of 1:25 (Huang, 2018). Furthermore, after 3 hours of extraction at 70°C and a 70% ethanol concentration (ratio of 1:14), 23.85 $mg.g^{-1}$ of total saponin content of *Eclipta prostrasta* L. was recovered (Hu *et al.*, 2012). However, using methanol as solvent (ratio of 1:12) and extracting saponin from *Celastrus hindsii* Benth over the course of 4 hours at 70°C produced a noticeably lower result (8.12 ± 0.59 $mg.mL^{-1}$) (Nguyen *et al.*, 2023).

From previous experiments, the optimal conditions were identified as: i) ethanol concentration: 50%; ii) duration for extraction: 6 hours; and iii) sample to solvent ratio: 1:40. Those conditions also agreed with recent research in the same field: Sanneur *et al.* (2023) for ethanol concentration; Kim *et al.* (2007) for extracting duration; and Chuyen (2023) for sample:solvent ratio. In this study, using 30% ethanol as solvent produced the significantly highest saponin yield. However, it is strongly recommended that the 50% ethanol should be preferred as the optimal concentration for applicable reasons. Firstly, although saponin was the main parameter in this study, medicinal extracts also consist of a variety of bioactive compounds, e.g. polysaccharides, other phenolic and flavonoid contents, etc. Hence, their recovery efficiency is also varied according to different ethanol concentrations. And as herbal extracts are always preferably used as the whole instead of any single components, the choice of solvent concentration is extremely important (Zhu *et al.*, 2019). For instance, besides saponins, polysaccharides are currently another main concern in both research and application. And polysaccharides were reported to be obtained more significant in ethanol in comparison with water (Zhao *et al.*, 2017).

Secondly, although saponin extraction from medicinal tincture was proven to be highly effective with water, ethanol expressed an effective capability in the removal of the interfering fat-soluble compounds, thus, enhanced the extraction process for valuable compounds (Zhu *et al.*, 2019). This matter was strongly reported through the forming of thick gum consisting of impurities that significantly lower the extraction efficiency of saponins (Love, Simons, 2020), which was also observed in our study for the 30% ethanol treatment. And using 50% ethanol as a solvent effectively solved this issue in this study. Therefore, the further application of 50% ethanol is recommended for the context of future industrial application.

In addition, the chosen extraction conditions: i) particle size of 500 µm for Dang Sam's powder, ii) 55^{o}C of extraction temperature for 2 hours, and iii) 8-hour of ethanol evaporation at 60^{o}C, were reported be optimal for the

extraction of valuable compounds from Dang sam in a recent study using both conventional and modeling optimizations (Tran *et al.*, 2024). The testing range of ethanol concentrations in this study (30%, 50%, 70%, 90%) was also proved to bring in positive result for saponin extraction from Korean ginseng (*Panax ginseng* Meyer) (Kwon *et al.*, 2003)

This study's limitations were the lack in accessing of different extraction methods, including high performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC-MS), which will be fulfilled in future research for bringing more in-depth knowledge. However, this study aims for providing fundamental extraction conditions so that further scientific research and especially industrial applications and commercialization can rely on to develop. Hence, this conventional and classic approach is also informative and cost effective.

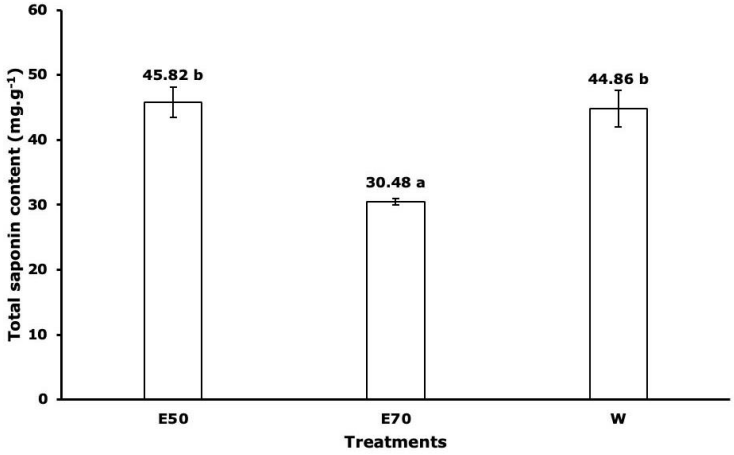

Figure 4. Saponin yields from Dang Sam applying: i) E50: ethanol extraction with optimal conditions; ii) E70: ethanol extraction with previously reported conditions; and iii) W: extraction with water solvent method

Data were presented as mean ± standard deviation (n = 3). Different alphabetical letters next to the data values on each column indicated significant differences between treatments (P < 0.05).

CONCLUSION

In conclusion, the optimal conditions for saponin extraction from Dang Sam were successfully determined (50% ethanol concentration, 6-hours duration, and 1:40 sample:solvent ratio) and evaluated with significantly improved outcome in saponin yield (45.82 ± 0.26 $mg.g^{-1}$) ($P < 0.05$). The results set an insight for further research and applications in saponin recovery from Dang Sam. Future studies should focus on optimizing more thorough conditions not only for the recovery of saponins but also for other important bioactive compounds, such as polysaccharides.

Competing interest

The authors declare no conflicts of interest.

Author contributions

Phu H. Le conceived the research idea, provided administrative and material support, and critically revised the manuscript. Phuc N.T. Le designed the experiments, provided technical support, interpreted the data and revised the manuscript. An D.X. Nguyen collected and statistically analyzed the data. Uyen P. Le and An D.X. Nguyen wrote the manuscript. An D.X. Nguyen, Uyen P. Le and Nghi B.P. Nguyen performed all experiments. All authors read and approved the final manuscript.

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XÁC ĐỊNH ĐIỀU KIỆN CHIẾT TỐI ƯU NHẰM NÂNG CAO HIỆU QUẢ CHIẾT XUẤT SAPONINS TỪ CÂY ĐẢNG SÂM (*CODONOPSIS PILOSULA* NANNF.)

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TÓM TẮT

Nhu cầu nâng cao hiệu quả chiết xuất saponins từ cây dược liệu đang rất được quan tâm trong nghiên cứu lẫn sản xuất trong công nghiệp thực phẩm và dược phẩm. Nghiên cứu này được tiến hành với mục tiêu xác định và đánh giá hiệu quả việc thu hồi saponin từ cây Đảng Sâm (*Codonopsis pilosula*) bằng cách tối ưu hóa các điều kiện chiết xuất. Các điều kiện trong phương pháp chiết xuất bằng dung môi cồn lần lượt được tối ưu bao gồm: nồng độ cồn, thời gian chiết, tỷ lệ bột sâm và dung môi, và sau đó áp dụng tổng thể các điều kiện để đánh giá hiệu quả nâng cao về lượng saponin thu được. Kết quả nghiên cứu đã chỉ ra điều kiện chiết tối ưu: i) nồng độ cồn 50%; ii) thời gian chiết 6 giờ và iii) tỷ lệ Đảng sâm:dung môi là 1:40. Và chiết xuất với cả 3 điều kiện đã tối ưu giúp thu được lượng saponins cao nhất (45,82 ± 0,26 $mg.g^{-1}$) ($P < 0.05$) khi so sánh với nghiệm thực sử dụng điều kiện chiết chưa tối ưu (30,48 ± 0,50 $mg.g^{-1}$). Nghiên cứu này góp phần thiết lập quy trình chiết hiệu quả cho việc thu hồi saponin từ Đảng Sâm phục vụ cho nghiên cứu và sản xuất trong tương lai gần.

Từ khóa: Codonopsis pilosula, Đảng Sâm, saponin chiết xuất.

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