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Codonopsis javanica Root Extraction with Enzyme Support

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Codonopsis javanica (CJ) contains various valuable bioactive compounds and is a cheaper medicinal herb than other ginsengs. In this study, *Codonopsis javanica* roots (CJR) were soaked in hot water in a thermostatic bath for extraction. Experiments were performed based on a change in the amount of enzyme α-amylase, extraction temperature and investigation time. Polyphenol, saponin content and antioxidant capacity by DPPH free radical scavenging were determined spectrophotometrically. The results showed that the optimal extraction conditions were obtained when adding 1% enzyme α-amylase at 90^0C in 3.5 hours. The extract of CJR obtained from experiments using enzyme α-amylase showed higher levels of TPC, saponin, antioxidant capacity by DPPH and ^{0}Brix compared to ones without enzymes. The aqueous enzymatic extraction method can be seen as a potential alternative to conventional solvent extraction methods and becomes more popular as an efficient, sustainable, non-toxic and environmentally friendly extraction technique.

1. Introduction

Codonopsis javanica (CJ) is a species in the Campanulaceae family, mainly found in mountainous provinces of Northern and Central Vietnam. It is a plant with high medicinal and economic value but more affordable than Korean ginseng. Its roots contain important bioactive compounds such as saponins, phytosteroids, alkaloids and polysaccharides (Chen et al., 2013). It can be used as an ingredient in production of high value products such as functional foods, tea (Thuong et al., 2020), cosmetic material, lotion, etc. (Meng et al., 2022). Proven health benefits of this plant include antioxidant, anti-inflammatory (Do et al., 2022), antibacterial, hypoglycemic, anti-arthritic and anti-diabetic activities, treatment of cough, pulmonary tuberculosis, yellow skin, anaemia and neurasthenia (Dictionary, 2006), reduction of insulin, high blood (Chen et al., 2013) and cancer cells, and body strengthening (Jie et al., 2015) . In 2021, Wu el at studied the active ingredients in ginseng root by water (80^0C) for 2.2 hours as extraction condition.

Figure 1: Dried Codonopsis javanica root in Kon Tum

In recent years, most studies have focused on isolating and determining the structure of secondary metabolites in CJR (Phan et al., 2020), polysaccharides purification (Long et al., 2020) or basic extraction process of CJR

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(Phan et al., 2020; Tri et al., 2020) in Vietnam. There was little scientific information about biological activities of CJR derived from Kon Tum. Food, pharmaceutical and some other related industries have used enzymes in production to increase technological and economic efficiency and improve productivity (Marathe et al., 2017). Enzyme α- amylase helps hydrolyze alpha bonds of polysaccharides such as starch and glycogen, yielding simple substrates such as glucose and maltose. Therefore, in this study, we continued to investigate the water extraction of CJ with the addition of enzyme α- amylase to find the optimal extraction conditions of the water extraction process from roots. Research could be a potential for the development of wellness drinks from CJR grown in Kon Tum, Vietnam.

2. Materials and methods

2.1. Material, chemicals and instrucments

Dried *Codonopsis javanica* root (CJR) (moisture <10%) was purchased from Kon Tum province, Vietnam (Figure 1). Samples were milled, smoothed and stored in dark zip bags (Figure 2).

Figure 2: Powder of Codonopsis javanica root

Applied chemicals include Enzyme α-amylase from China; Folin (> 95%, Himedia India. DPPH (>96%, China); Methanol, Na_2CO_3, Vanilin (>99%, Xilong Scientific Co., Ltd) from Germany, distilled water.
Experimental tools include Convection dryer (Vietnam), Analytical balance (Model: PA2102, China); Thermostat tank (HH-4, China); UV-VIS spectrophotometer (UV-2602, China).

2.2. Extraction of CJR

Take 2g CJR powder, add 40mL water and α-amylase enzyme to the beaker, soak the mixture in a thermostatic bath, then filter with 110 mm diameter filter paper. Finally, the solution was obtained to determine its TPC, antioxidant activity by DPPH, saponin, and ^{0}Brix. The survey parameters include: enzyme α-amylase: 0; 0.25; 0.5; 0.75; 1; 1.25; 1.5% (compared to dried material weight); survey temperature from 80, 85, 90, 95℃ during 2.5h; 3h; 3.5h; 4 h.

2.3. Methodological analysis of saponins, total polyphenols (TPC) and antioxidant activity by DPPH free radical scavenging

The total polyphenol content in the sample was determined by the Folin Ciocalteu method with some variations. Put 0.5 ml of sample into a centrifuge tube, then add 2 ml of 10% Folin (v/v), shake well (wait 2-3 minutes). Then add 2 ml of 0.7 M Na_2CO_3 and continue to wait for 60 min, avoiding direct exposure to lights. TPC was spectrophotometrically measured at 765 nm (Sánchez-Rangel et al., 2013).The DPPH free radical scavenging antioxidant effect was obtained by mixing 3.5 mL of methanol-corrected DPPH with 0.5 mL of the sample solution. After 30 min reaction, determination of antioxidant content was measured at 517 nm in a UV-Vis spectrophotometer (Sharma and Bhat, 2009). Saponin content was determined according to the method described by Hiai et al. (1976). Add 0.5 ml of 8% vanillin to 5 ml of H_2SO_4 72%. Then add 0.5 ml of the extract, soak the new mixture for about 5 min in cold water (10°C). Then, the mixture was soaked at 60℃ in 15 min for it to rapidly cool again. Saponin content was determined spectrophotometrically at 525 nm.

2.4. Statistical analysis

Each experiment was performed 3 times and the collected results were analyzed by descriptive statistics on mean, standard deviation and processed by Excel software.

3. Results and discussion

3.1. Effect of enzymes on saponin content, total polyphenol content and antioxidant capacity by DPPH of CJR extract

The influence of α-amylase enzyme on biological activities of ginseng root extract is shown in Figure 3. Research conducted to investigate the α-amylase enzyme changed from 0; 0.25; 0.5; 0.75; 1; 1.25; 1.5% (compared to dry material weight) at fixed condition of material/water ratio 1/20 g/mL for 3 hours. According to Figure 3, when changing the enzyme α-amylase, the content of saponin has a slight increase (Figure 3a). After saponin reached the highest value at 1.25% α-amylase enzyme, the saponin content did not change significantly. As for TPC, the graph tends to rise to a peak and then falls (Figure 3b). Specifically, TPC reached the highest value at α-amylase enzyme 0.75%. This could be explained that α-amylase degraded polyphenol into smaller compounds under high enzyme concentrations as stated by Aline et al. (2021). As for the DPPH free radical scavenging antioxidant capacity, it was in the range of 52.3 – 66.9% (Figure 3c).

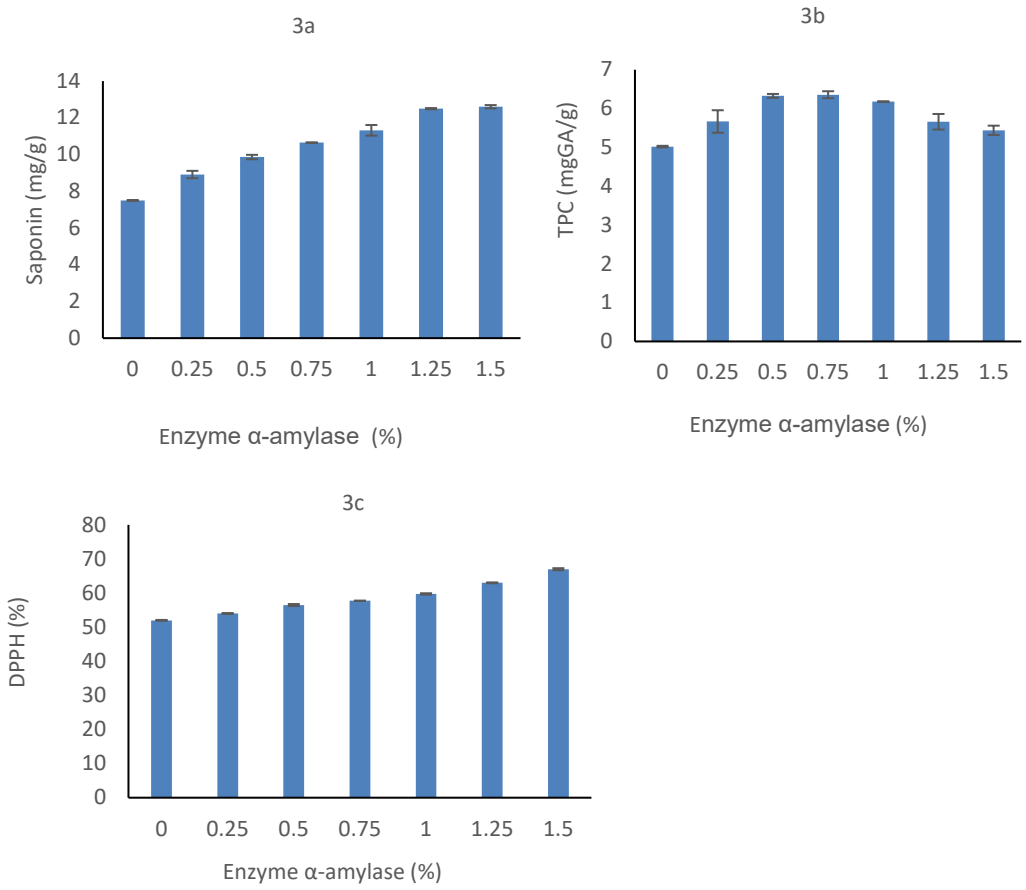

Figure 3: Effect of enzymes on saponin content, total polyphenol content and antioxidant capacity of CJR extract: (a) Saponin content; (b) TPC; (c) DPPH

The enzyme α-amylase acted as a catalyst for the hydrolysis reaction. When using the enzyme, the active ingredients of the raw materials were hydrolyzed and released inside the cells (Puri et al., 2012).

Thus, in the extraction process with enzymes, biological compounds were generated more. With an aim to increase the amount of active ingredients extracted from the root of the CJ, the survey results showed that when enzyme α-amylase was added to the extraction process, the amount of saponin, TPC and antioxidant capacity by DPPH all gave a higher value than the sample without enzyme α-amylase. The appropriate amount of α-amylase enzyme selected for the following experiments was 1%.

3.2. Effect of extraction time on saponin content, total polyphenol content and antioxidant capacity by DPPH of CJR extract

The study carried out at temperature range from 80 to 95^{0}C at fixed condition of material/water ratio of 1/20 g/mL during 2.5h; 3h; 3.5h; 4h (Figure 4). When increasing the extraction time from 2.5h to 3.5h, the content of

polyphenols and saponins increased slightly. After reaching the highest value at 3.5h, the content of polyphenols and saponins tended to decrease. Increasing the extraction time would facilitate the dissolution of the material and the rapid breakdown of the cell structure. But if the extraction time were too long, it would destroy the unstable active ingredients in the raw materials. Figure 4 showed that the DPPH free radical scavenging ability at 3.5h gave a high value, if the time continued to increase to 4h, the DPPH free radical scavenging ability did not change significantly. To ensure that the content of polyphenols, saponins and high DPPH free radical scavenging values were obtained, the optimal extraction time was selected as 3.5h. The study also gave similar results as the study on the anti-cancer effects of Panax ginseng Berry Polysaccharides (Jie et al., 2015).

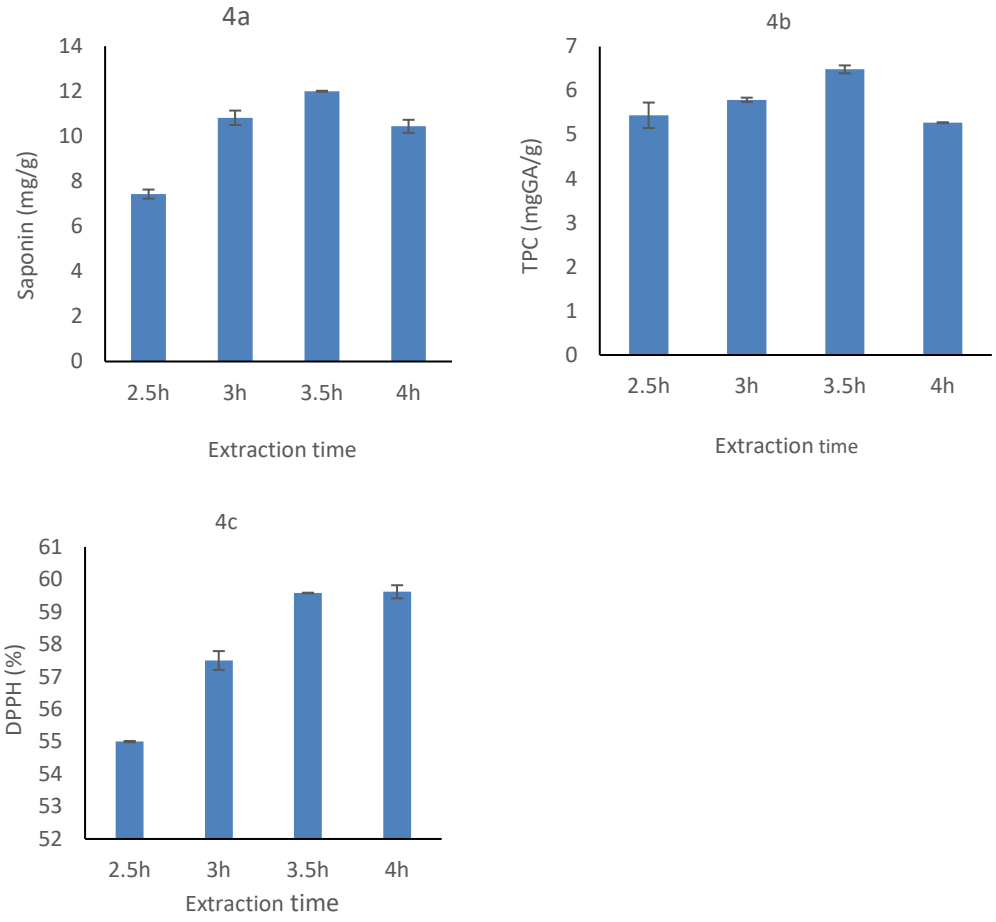

Figure 4: Effect of extraction time on saponin content, total polyphenol content and DPPH antioxidant capacity of the extracts: (a) Saponin content; (b) TPC; (c) DPPH

3.3. Effect of extraction temperature on saponin content, total polyphenol content and DPPH antioxidant capacity of the extracts

Experiments to investigate the effect of extraction temperature on biological activities of extracts were conducted at the following conditions: ratio 1/20g/mL, 1% enzyme, extraction time 3.5h. Figure 5 showed the change of the survey parameters when changing the extraction temperature from 80, 85, 90, 95^{0}C. Specifically, when increasing the soaking temperature, the TPC, saponin values and DPPH free radical scavenging ability also increased. All three analytical parameters reached the maximum value at the temperature of 90^{0}C and then decreased accordingly. The right soaking temperature would help active ingredients in the raw materials be released to the outside environment. But if the temperature were too high, it denatured those biologically active substances. The research results were higher than those reported by Lee et al. (2008) when performing saponin extraction from Korean red ginseng (80^{0}C). This difference could result from different sources of local materials and survey methods.

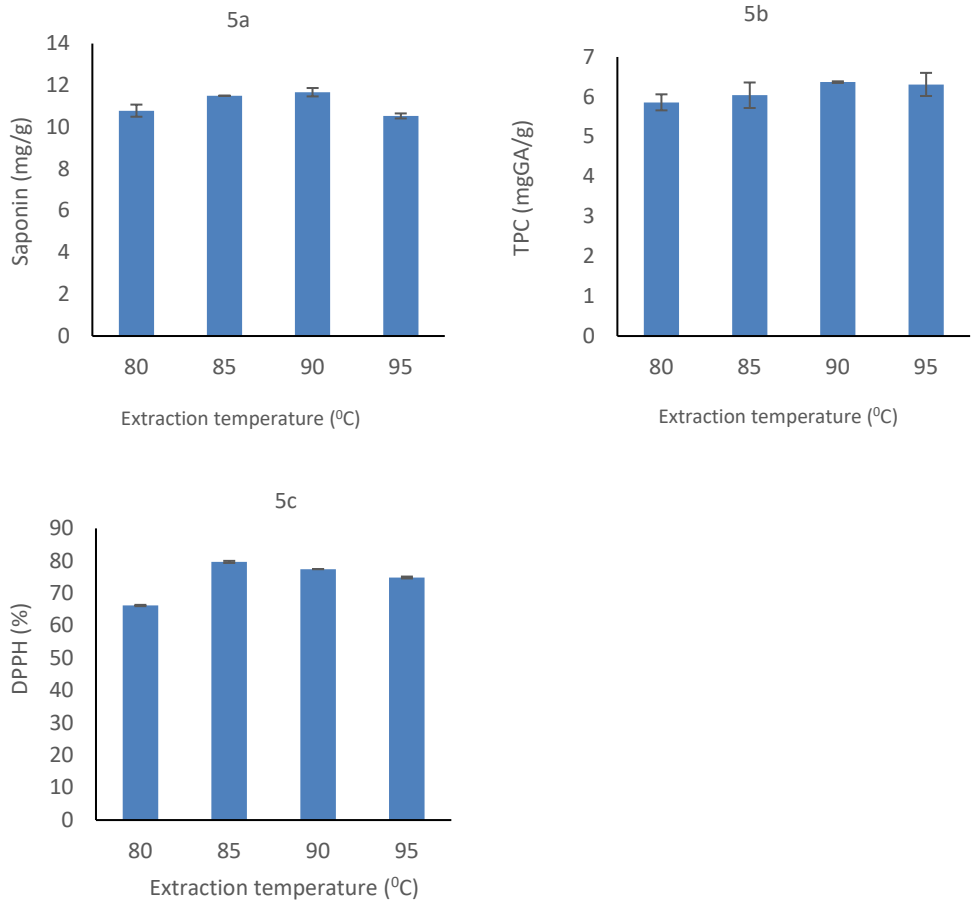

Figure 5: Effect of extraction temperature on saponin content, total polyphenol content and DPPH antioxidant capacity of the extracts: (a) Saponin content; (b) TPC; (c) DPPH

Table 1 showed that the CJR extract obtained pleasant characteristics with light yellow, aromatic, sweet and sour in sense.

Table 1: Biological properties of CJR extract with 1% enzyme α-amylase treatment

№	Target	Result
1	Sense	gold; light aroma; sweet, sour
2	^{0}Brix	4.2%
3	pH	5.5
4	Saponin	11.67 mg/g
5	DPPH free radical scavenging ability	79%
6	TPC	6.48 mgGA/g
7	Moisture	<10%

4. Conclusion

Codonopsis javanica root from Kon Tum, Vietnam has long been regarded as a valuable source of biologically active ingredients widely used in food, cosmetic, oriental and modern medicine. The research results showed that the optimal conditions for extraction of CJR by immersion in thermostatic bath were found when using 1% enzyme α-amylase at 90^{0}C, 3.5h and 1/20 g/mL. The total phenol content of the aqueous extract was 6.48 mgGA/g, saponin content 11.67 mg/g and DPPH free radical scavenging ability 79%. *Codonopsis javanica* root extracted with enzyme had ^{0}Brix value (4.2%) higher than without-enzyme treatment (2.2%).

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References

Cascaes-Teles A.S., Chavez D.W.H., Coelho M.A.Z., Rosenthal A., Gottschalk L.M.F., Tonon R.V., 2021, Combination of enzyme-assisted extraction and high hydrostatic pressure for phenolic compounds recovery from grape pomace, Journal of Food Engineering 288, 110128.

Chen K.N., Peng W.H., Hou C.W., Chen C.Y., Chen H.H., Kuo C.H., Korivi M., 2013, Codonopsis Javanica Root Extracts Attenuate Hyperinsulinemia and Lipid Peroxidation in Fructose-Fed Insulin Resistant Rats, Journal of Food and Drug Analysis 21 (4), 347–55.

Dictionary Chinese Herbal, 2006, Nanjing University of Traditional Chinese Medicine, Dictionary of Traditional Chinese Medicine.

Do T.H., Tan D.H., Kiet A.N., Tan S.C., Kim N.N., Ho A.H.N., Thuy T.N., Le N.P.N., Truong D.H, 2022, Saponin-rich Fractions from Codonopsis Javanica Root Extract and Their in Vitro Antioxidant and Anti-enzymatic Efficacy, Journal of Food Processing and Preservation 46 (1), e16113.

Hiai S., Oura H., Nakajima T., 1976, Color Reaction of Some Sapogenins and Saponins with Vanillin and Sulfuric Acid, Planta Medica 29 (02), 116–22.

Jie Z., Li C.Y., Li J.P., Guo R., Wang H., Pan J., Liu S.L., 2015, Immunoregulation on Mice of Low Immunity and Effects on Five Kinds of Human Cancer Cells of Panax Japonicus Polysaccharide, Evidence-Based Complementary and Alternative Medicine 2015.

Lee S.H., Kang J.I., Lee S.Y., 2008, Saponin Composition and Physico-Chemical Properties of Korean Red Ginseng Extract as Affected by Extracting Conditions, Journal of the Korean Society of Food Science and Nutrition 37 (2), 256–60.

Long T.T.N., Boi N.V., Cuong X.D., 2020, The Content, Purification Degree, and Molecular Weight of Inulin of Natural Dangshen Roots (Codonopsis javanica) in Highland Lam Vien, Vietnam, Journal of Pharmaceutical Research International, 32(24), 83-92.

Marathe S.J, Swati B.J., Sandip B.B., Rekha S.S., 2017, Enzyme-Assisted Extraction of Bioactives, Food Bioactives, 171–201, Springer.

Meng H., Liu X.K., Li J.R., Bao T.Y., Fan Y., 2022, Bibliometric Analysis of the Effects of Ginseng on Skin, Journal of Cosmetic Dermatology 21 (1), 99–107.

Phan H.T.N., Thuan T.D.N., Hien T.T.N., Huyen V.P., Duyen H.H.N., Hanh T.H. T., Cuong X.N., Quang H.T., Nam H.N., Minh V.C., 2020, Codojavanosides A-C, three new sesquiterpenoid glycosides from the roots of Codonopsis javanica, Phytochemistry Letters 40, 166–170.

Phan H.T.N., Thuan T.D.N., Hien T.T.N., Huyen V.P., Duyen H.H.N., Hanh T.H. T., Cuong X.N., Quang H.T., Nam H.N., Minh V.C., 2020, Polyacetylene and phenolic constituents from the roots of Codonopsis javanica, Natural Product Research, Vol.36, Issue 9.

Puri M., Deepika S., Colin J.B., 2012, Enzyme-Assisted Extraction of Bioactives from Plants, Trends in Biotechnology 30 (1), 37–44.

Sánchez R., Juan C., Jorge B., Heredia J.B., Luis C.Z., Daniel A.J.V., 2013, The Folin–Ciocalteu Assay Revisited: Improvement of Its Specificity for Total Phenolic Content Determination, Analytical Methods 5 (21), 5990–99.

Sharma O.P. and Tej K.B., 2009, DPPH Antioxidant Assay Revisited, Food Chemistry 113 (4), 1202–5.

Shin B.K., Sung W.K., Jeong H.P., 2015, Chemical Diversity of Ginseng Saponins from Panax Ginseng, Journal of Ginseng Research 39 (4), 287–98.

Thuong N.N.P., Vu N.D., Thanh L.V., Phuong T.T.M., Bach L.G., Toan T.Q., 2020, Instant Tea from Condonopsis Javanica L. Root Extract via Spray Drying, Foods and Raw Materials 8 (2), 385–91.

Tri N. P., Van T.N., Tran Q.T., Mai H.C., Long G.B., Nguyen V.M., 2020, Effects of Various Processing Parameters on Polyphenols, Flavonoids, and Antioxidant Activities of Codonopsis javanica Root Extract, Natural Product Communications, Vol.15(9), 1–12.

Wu L., Mei P., Yongshuai J., Xiaosheng Y., Juan Y., 2021, Immunomodulatory Effect of Polysaccharides from the Extraction of Codonopsis Javanica (Blume) Hook. f. et Thomson (Campanulaceae) Roots in Female Rats, Natural Product Research 35 (24), 5883–87.