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In vitro bioactivities of *Codonopsis javanica* root extract from Kon Tum province, Vietnam

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Abstract. Dangshen *Codonopsis javanica* exhibits invaluable medicinal properties in herbal remedies; however, there has currently not been much specific analysis of the phytochemicals and bioactivities of this plant. The root ethanol extract of *C. javanica* contains substances such as saponins, phenolic acids, terpenoids, and alkaloids. It displays an antibacterial effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus* with the IC_{50} values of 150, 100, 150, and 90 μg/mL, respectively. The antioxidant capacity of the root extract was also observed with an IC_{50} value of 46.8 ± 6.8 μg/mL. Furthermore, the extract exhibits activity on human cancer cell lines HepG2 (IC_{50} = 83.6 ± 2.7 μg/mL) and MCF-7 (IC_{50} = 95.3 ± 2.3 μg/mL). Hence, this study provides the basic data for further research on the bioactivities of natural compounds of Dangshen *C. javanica* for the first time.

Keywords: antibacterial, anticancer, antioxidant, bioactive compounds, Dangshen *Codonopsis javanica*

1 Introduction

Dangshen *Codonopsis javanica* (Blume) Hook f. (*C. javanica*) is a precious herb in Oriental folk medicine with various functions. *C. javanica* root, which can be combined with bai zhu, huang qui, chenpi, black cohosh, and chaihu, is effective in alleviating many conditions such as poor appetite, insomnia, somber body, xerostomia, stomach pain, intestinal prolapse, uterus prolapse, and haemorrhoid. Furthermore, the root may be mixed with a five-flavour berry, balloon flower, and *Launaea sarmentosa* to improve blood circulation, frequent coughing, lung disease, asthma, and trouble breathing. Besides, the root is utilized to increase erythrocyte, low blood pressure, and low blood sugar, increase uretic, and cure kidney edema, especially in the case of the high presence of albumin in urea. A daily dosage is about 12–20 g by decoction, tablet, balm, and immersion with alcohol or mixture with other medicine [1-3].

In Vietnam, *C. javanica* grows sparsely in the Northern mountain regions (Lai Chau, Lao Cai, Ha Giang, and Son La) or the South plateau (Truong Son, Ngoc Linh Mountain), especially in

Central Higlands (Kon Tum, Gia Lai, Dak Lak, and Langbiang – Lam Dong province).

According to the Checklist of plant species in Vietnam, *Codonopsis javanica* (Blume) *Hook. f. & Thomson* (*C. javanica*) has the following taxonomical description:

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliosida*

Classification: *Asteriades*

Order: *Asterales*

Family: *Campanulaceae*

Genus: C*odonopsis*

Because of indiscriminate deforestation, uncontrolled exploitation, and uneven farming, the natural storage of *C. javanica* constantly declines. This species is currently listed in "Vietnam Red Data Book" (Part II- Plants, 2007), "The Red List of Medicinal Plants of Vietnam" (2006), Group IIA-Limited exploitation, Vulnerable species (VU) level and is necessary to be conserved according to the Decree No. 32/2006/ND-CP by the Government [4-5]. Therefore, it is crucial to study the farming process and to analyze the biochemical components and medicinal values of *C. javanica*. These practices significantly contribute to further maintaining the indigenous medicinal plants and developing the pharmaceutical industry in Vietnam.

2 Materials and methods

2.1 *C. javanica* root extraction

The Dangshen *C. javanica* samples were collected in Kon Tum province during the dry season (from January to April). Botanical identification was carried out at the University of Science, VNUHCM. The roots were washed and weighed. They were also subjected to slicing, drying, and grinding into fine powder prior to extraction.

The extraction of *C. javanica* root was carried out twice by soaking the powder in ethanol with a mass/volume ratio of 1:5 at 70 °C. The solvent was removed from the extract with a Heidolph rotary evaporator at 32 ± 1 °C at 74 rounds/min. This ethanol extract was then dried at 25–27 °C.

Dimethyl sulfoxide (DMSO, Sigma Aldrich) was used to dilute the extract, and the final concentration of DMSO did not exceed 0.05% [6-7]. No adverse effect due to the presence of DMSO was observed in this study.

2.2 Qualitative phytochemical analysis

The phytochemical groups of Dangshen *C. javanica* root extract were identified in the sample by using standard procedures improved by the Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City [8-10].

Total phenolic content: The root extract was mixed with distilled water and heated to 100 °C. A 0.1% $FeCl_3$ solution was added to the sample.

Flavonoids: The root extract was mixed with ethanol. Zinc powder was added to all samples, and the mixture was shaken. A few drops of HCl were added to the sample subsequently.

Saponins: The root extract was combined with boiling distilled water. The sample was then vigorously shaken until air bubbles appeared, and the sample was left to rest for 20 min for further observation and analysis.

Terpenoids: The root extract was mixed with chloroform. Concentrated HCl was then dropped gently into the sample.

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2.3 Antibacterial activity

Antibacterial screening of the *C. javanica* root extract was performed with the disc diffusion method and minimal inhibitory concentration (MIC) assay, as described previously [11].

Disc diffusion method: Bacterial strains (American Tissue Culture Collection, ATCC; Manassas, VA, USA) were inoculated in nutrient-enriched Brain Heart Infusion (BHI) culture medium so that the turbidity of the broth culture was adjusted to 0.5 MacFarland Standard (~1.5 × 10^8 CFU/mL). The bacterial suspension was streaked into the Mueller Hinton Agar (MHA) plate following the Kirby-Bauer method (disc diffusion method). The *C. javanica* root extract was diluted in a concentration-dependent manner on several 6-mm paper discs. Positive control was kanamycin (Sigma Aldrich), and negative control was DMSO. The experiments were carried out in triplicate.

The antimicrobial effect of the root extract was evaluated by measuring the inhibition zone diameter according to the following formula

I (mm) = $D - d$

where D is the diameter of the zone of inhibition, and d is the diameter of the paper disc.

Minimal inhibitory concentration: The bacteria were inoculated in the BHI culture at 37 °C, and the turbidity of the broth was adjusted to 0.5 MacFarland Standard (~1.5 × 10^8 CFU/mL). The bacterial suspension was then diluted to reach 1.5 × 10^6 CFU/mL. The *C. javanica* root extract with different concentrations was also added to the bacterial culture.

After 24 h of incubation, the MIC value was determined at the well containing the lowest concentration of the root extract in which there was no visible bacterial growth. The changing colour of resazurin (Sigma Aldrich) from blue to pink indicates that the bacteria are still alive. The experiments were performed in triplicate.

2.4 Antioxidant activity

The ability of the *C. javanica* root extract in scavenging free radical could be analyzed with the DPPH assay [12-13]. The extract was prepared in a concentration-dependent manner to develop the linear equation representing antioxidant capacity. Several samples at multiple concentrations were then added to previously prepared DPPH solutions. The blank included the root extract and methanol but no DPPH solution. The samples were kept in the dark at ambient temperature for 60 min. After incubation, the samples were subjected to absorbance measurements at 517 nm on a spectrophotometer (JenWay Genova Plus). The experiments were performed in triplicate.

DPPH free radical scavenging capability (I, %) was calculated from the formula

$$I\% = \frac{A1 - (A2 - A3)}{A1} \times 100\%$$

where $A1$ is the absorbance without the tested sample; $A2$ is the absorbance of the root extract, and $A3$ is the absorbance of the sample without the DPPH solution.

The IC_{50} value is the concentration of the root extract, capable of scavenging 50% of free radicals.

2.5 Cytotoxic activity

Cytotoxic assays were carried out in this study according to previous reports [6-7].

Cancer cell lines HepG2 (human hepatocyte carcinoma cancer, ATCC) and MCF-7 (human breast cancer, ATCC) were cultured with a density of 5 × 10^4 cells/mL. The cell culture medium consisted of high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco), 10%

Fetal Bovine Serum (FBS, Gibco), and penicillin/streptomycin. The cells were then incubated at 37 °C and 5% CO_2.

The *C. javanica* root extract and doxorubicin (DOX, as positive control) were diluted to various concentrations (0–100 μg/mL). The blank, negative and positive controls were also set up in a 96-well plate.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is based on the principle that MTT is converted in the oxidative-reductive reaction with the cell mitochondria to crystalized formazan. MTT was plausible to use with a few solutions to destroy the cell membrane and dissolve the formazan crystals. The absorbance of these solutions was then measured at 595 nm. This method evaluated cell viability through their respiratory activities. The percentage cell death (I, %) was then calculated from the formula

$$I\ (\%) = 100 - 100 \times (A_{595\text{sample}} - A_{595\text{blank}})/(A_{595\text{DMSO}} - A_{595\text{blank}})$$

where $A_{595\text{sample}}$ is the optical density (OD) measured at 595 nm for the cells treated with the root extract; $A_{595\text{blank}}$ is the OD measured at 595 nm for the blank wells, and $A_{595\text{DMSO}}$ is the OD measured at 595 nm for the cells treated with DMSO.

The IC_{50} value is the concentration of compounds capable of 50% anticancer viability. The result was represented as mean ± standard deviation (SD).

3 Results and discussion

3.1 Phytochemical screening

The chemical constituents of the Dangshen *C. javanica* root extract in this study (Table 1) are consistent with those of previous reports [1, 14, 15]. Phytochemical screening confirms the presence of secondary metabolites, such as saponins, terpenoids, alkaloids, and polyphenols, in the roots of *C. javanica*. The *C. javanica* roots collected in Kon Tum contain a large amount of saponins, one of the main medicinal components of Dangshen.

The saponins, terpenoids, alkaloids, and polyphenols display antioxidant, anti-inflammatory, anticancer, antihyperglycemic, and antidiabetic activities. Alkaloids can provide an underlying structure for developing different antibiotics with a diverse range of action [16]. Polyphenols possess scavenging or chelating antioxidant activity [17]. Based on the above findings, we conducted further experiments to determine the *C. javanica* root extract bioactivity.

Table 1. Phytochemical screening of *C. javanica* root extract

No.	Compound	Recognizing reaction	Positive reaction	Result
1	Polyphenols	$FeCl_3$ solution	Navy or green	+
2	Flavonoids	Zinc (Zn) powder and HCl solution	Pink and gas product	–
3	Terpenoids	Chloroform and HCl solution	Brown and two layers separation	++
4	Saponins	Bubbling	Stable white foam layer	+++
5	Alkaloids	Mayer/ Wager solution	Sepia precipitation	++

3.2 Antibacterial activity

The *C. javanica* root extract at a concentration of 50 μg/mL was analyzed for antibacterial effect against *Bacillus cereus* and *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* and *Escherichia coli* (gram-negative) with the disc diffusion method. The bactericidal activity was evaluated via the diameter of the inhibition zone.

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The data in Table 2 demonstrate that the 50 μg/mL *C. javanica* root extract was effective against all four bacterial strains, especially against *B. cereus*, with the largest zone of inhibition (7.7 mm).

Fig. 1 shows that the 200 μg/mL root extract sufficiently inhibits *E. coli* growth. The colour in the well remains blue, like the colour of the blank. However, the 20 μg/mL root extract does not repress *E. coli* growth. The colour of the well is pink, like the colour of the negative control (this well did not contain the root extract), and the 150 μg/mL root extract fully inhibits *E. coli* growth.

Nevertheless, the 130 μg/mL root extract did not completely limit bacterial growth. The colour in this well was pink-purple. These data confirm that the MIC value of the root extract against *E. coli* is 150 μg/mL.

Table 2. Antibacterial effect of *C. javanica* root extract

Bacterial strains	Diameter of zone of inhibition (mm)
P. aeruginosa	4.3 ± 0.6
S. aureus	4.0 ± 1.0
B. cereus	7.7 ± 0.6
E. coli	3.3 ± 0.6

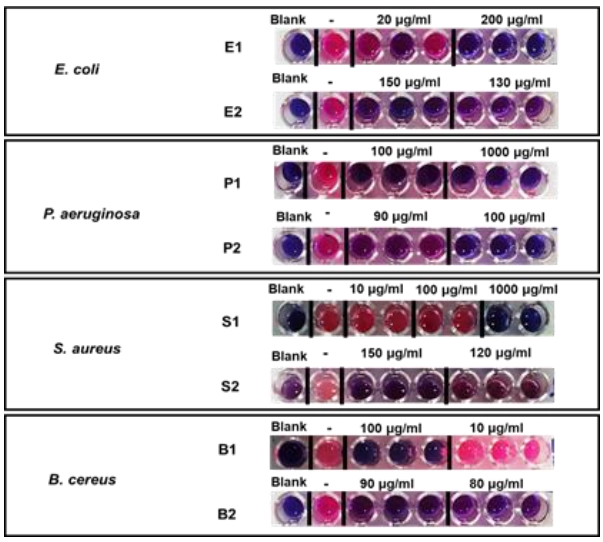

Fig. 1. MIC assays of *C. javanica* root extract for various bacterial strains

The remaining bacteria were also subjected to the extract, and the MIC values against *P. aeruginosa*, *S. aureus*, and *B. cereus* are 100, 150, and 90 μg/mL, respectively.

Thus, the *C. javanica* root extract is effective against all four tested bacterial strains, and the activity is strongest against *B. cereus*.

Previous studies revealed that various components of plant extracts, such as terpenoids, alkaloids, and phenolic compounds, could inhibit the growth of foodborne and spoilage bacteria, disrupting bacteria's enzymatic activity and damaging the proteins of the microbial cell membrane [18]. The components of *C. javanica* identified here and believed to be the most important in terms of biological activity are saponins, terpenoids, alkaloids, and polyphenols, as reported in Table 1. These compounds can serve as natural antimicrobial agents against infections or diseases caused by the tested microorganisms. Therefore, further studies should be performed to isolate and characterize the extract's bioactive compounds to develop of new antibacterial drugs.

3.3 Antioxidant activity

As shown in Fig. 2, the percentage of DPPH free radical scavenging increases with the concentration of the extract. This result reveals that the *C. javanica* root extract exhibits antioxidant capacity. However, this activity is lower than that of ascorbic acid (the scavenging ability was 92% for the root extract at 60 μg/mL and ascorbic acid at 20 μg/mL).

The antioxidant effect of the root extract is assessed via the IC_{50} value (Table 3). We can see that the root extract is about five times weaker than ascorbic acid.

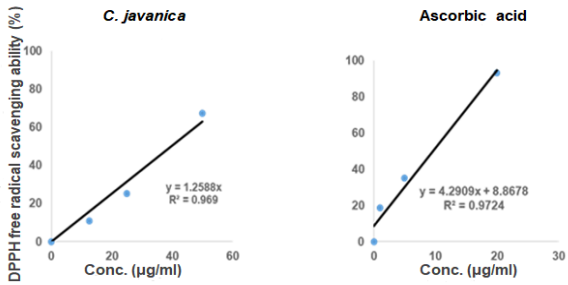

Fig. 2. Antioxidant capacity of *C. javanica* root extract

Table 3. DPPH radical scavenging activity (IC_{50} value) of *C. javanica* root extract

Sample	IC_{50} (µg/mL)
C. javanica extract	46.8 ± 6.8
Ascorbic acid	10.8 ± 0.1

3.4 Cytotoxic activity

To evaluate the potential anticancer activity, we first compared the *C. javanica* root extract's *in vitro* cytotoxicity with that of DOX for HepG2 and MCF-7 cell lines. As a standard therapeutic drug, DOX was used in this experiment and showed high toxicity for the tested cell lines. Fig. 3 shows that the root extract had cytotoxicity at 100 µg/mL, comparable with that of DOX at 6.25 µg/mL against HepG2 cells. Similarly, the root extract also suppressed MCF-7 cells at a concentration of 100 µg/mL, corresponding to that of DOX at 12.5 µg/mL. Meanwhile, DMSO at a concentration of 0.05%, corresponding to the concentration used to dissolve the root extract at 100 µg/mL, did not show any cytotoxic activities against HepG2 and MCF-7 cells.

Table 4 presents the IC_{50} value of the *C. javanica* root extract against HepG2 and MCF-7 cells. The extract exhibited cytotoxicity about 15 times weaker than DOX for HepG2 cells and 9.5 times for MCF-7 cells. Although the root extract did not show a cytotoxic activity as effective as DOX, it exhibited some cytotoxicity against HepG2 and MCF-7 cells. The findings are generally consistent with those of previous anticancer studies [19-21]. The cytotoxic or antiproliferative activity of *C. javanica* root extract might be mediated with its bioactive constituents.

Additionally, because the *C. javanica* root extract exhibited cytotoxic activity against both cell lines, further identifying the bioactive reagents responsible for this effect and analyzing how they work is required to understand the therapeutic benefits of this plant. In the future, researchers might look into cell signalling pathways and the activity of particular active compounds in the extract to determine its potential anticancer capabilities.

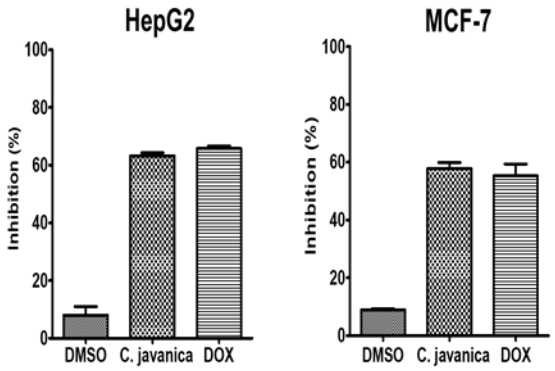

Fig. 3. Cytotoxic activity of *C. javanica* root extract on human hepatocyte carcinoma cells HepG2 and human breast cancer cells MCF-7

Table 4. IC50 values of different cancer cell lines-cytotoxicity assays of *C. javanica* root extract

Samples	IC_{50} (µg/mL)	
	HepG2	MCF-7
C. javanica extract	83.6 ± 2.7	95.3 ± 2.3
DOX	5.4 ± 0.2	10.9 ± 2.2

4 Conclusions

This study shows that the *C. javanica* root extract contains saponins, terpenoids, alkaloids, and polyphenols. It exhibits antibacterial potential against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*

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with an IC_{50} value of 150, 100, 150, and 90 μg/mL, respectively. This extract also displays a DPPH scavenging activity with an IC_{50} value of 46.8 ± 6.8 μg/mL, five times weaker than ascorbic acid. The MTT assay reveals that the root extract possesses a significant cytotoxic effect against HepG2 (IC_{50} = 83.6 ± 2.7 μg/mL) and MCF-7 cells (IC_{50} = 95.3 ± 2.3 μg/mL). Therefore, the root extract should be subjected to further research regarding its biological potential and possible use in the food industry and medicine.

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Conflict of interests

The authors declare that they have no conflicts of interest regarding the publication of this article.

References

1. Chung HM, Sinh PX, Tuyen NM. Initial study on chemical compounds of Vietnam Dang Sam *Codonopsis javanica* (Blume) Hook. Hanoi: National Institute of Medicinal Materials (NIMM); 2002.
2. Loi DT. The medicinal plants and herbs of Vietnam. 14th ed. Hanoi: Medical Publishing House; 2006.
3. VC Vo. Dictionary of Vietnamese Medicinal Plants. Hanoi: Medical Publishing House; 2012. p. 566-567.
4. Tap N. The Red List of Medicinal Plants in Vietnam. Journal of Medicinal Materials. 2006;11(3):97-105.
5. Ban NT. Vietnam Red Data Book (Part II - Plants). Hanoi: Publishing House of Natural Science and Technology; 2007.
6. Likhitwitayawuid K, Angerhofer CK, Cordell GA, Pezzuto JM, Ruangrungsi N. Cytotoxic and Antimalarial Bisbenzylisoquinolme Alkaloids from *Stephania erecta*. Journal of Natural Products. 1993;56(1):30-8.
7. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. JNCI: Journal of the National Cancer Institute. 1990;82(13):1107-12.
8. Ciulei I. Methodology for Analysis of Vegetable Drugs. Bucarest: Faculty of Pharmacy; 1982.
9. Saklani S, Sati B, Sati H, Mishra A. Pharmacognostic, phytochemical and antimicrobial screening of *Aphanamixis polystachya*, an endangered medicinal tree. Int J Pharm Pharm Sci. 2012;4(3):235-240.
10. Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City. Medical research methodology. Ho Chi Minh: Publishing House of Ho Chi Minh City University of Medicine and Pharmacy; 2015.
11. Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology. 2001;74(3):217-20.
12. Formagio ASN, Volobuff CRF, Santiago M, Cardoso CAL, Vieira MDC, Valdevina Pereira Z. Evaluation of Antioxidant Activity, Total Flavonoids, Tannins and Phenolic Compounds in *Psychotria* Leaf Extracts. 2014;3(4):745-57.
13. Yen GC, Duh PD. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. Journal of Agricultural and Food Chemistry. 1994;42(3):629-32.
14. Wang Z-T, Ma G-Y, Tu P-F, Xu G-J, Ng T-B. Chemotaxonomic study of Codonopsis (family Campanulaceae) and its related genera. Biochemical Systematics and Ecology. 1995;23(7): 809-12.
15. Duy TH, Quoc LPT, Cam TTH, Ngoc PTK, Dao DTA. Optimization of extraction and collection of saponins from *Codonopsis javanica* (Blume) Hook. f. by alpha-amylase enzyme. Journal of Science and Technology. 2015;4(99)1-3.
16. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Front Microbiol. 2019;10:1-28.
17. Baccarin T, Mitjans M, Lemos-Senna E, Vinardell MP. Protection against oxidative damage in human erythrocytes and preliminary photosafety assessment of Punica granatum seed oil nanoemulsions entrapping polyphenol-rich ethyl

acetate fraction. Toxicology in Vitro. 2015;30(1,Part B):421-8.

18. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-53.
19. Ueda J-y, Tezuka Y, Banskota AH, Tran QL, Tran QK, Harimaya Y, et al. Antiproliferative Activity of Vietnamese Medicinal Plants. Biological and Pharmaceutical Bulletin. 2002;25(6):753-60.
20. Mei P, Zhen-dong Z, Juan Y. Inhibitory Effects of 14 Polysaccharides on Sarcocarcinoma S (180) of Mice. Journal of Mountain Agriculture and Biology. 2010.
21. Yang D-S, Zi-Lei L, Xue W, Yang Y-P, Wei-Bing P, Ke-Chun LIU, et al. Chemical constituents from the roots of Campanumoea javanica and their antiangiogenesis activities. Chinese Traditional and Herbal Drugs. 2015;46:470-5.