RESEARCH ARTICLE

STUDY ON THE ANTIOXIDANT, ANTIBACTERIAL, AND CYTOTOXIC ACTIVITIES IN VITRO OF ROOT EXTRACTS FROM *CODONOPSIS JAVANICA* IN KON TUM PROVINCE, VIETNAM

Hai Ha Pham Thi^a, Kien Cuong Tran^a, Thu Nha Nguyen Thi^a, Lan Phan-Hoang Nguyen^{a,c}, Thanh Luan Nguyen^{b*}

^a NTT High-Tech Institute, Nguyen Tat Thanh University, 298-300A Nguyen Tat Thanh Street, Ward 13, District 4, Ho Chi Minh City 700000, Vietnam.

^b Department of Veterinary Medicine, Institute of Applied Science, Ho Chi Minh City University of Technology (HUTECH), 475A Dien Bien Phu Street, Ward 25, Binh Thanh District, Ho Chi Minh City 700000, Vietnam.

^c Biochemistry and Molecular Biology Department, Gettysburg College, 300 North Washington Street, Gettysburg, PA 17325.

*Corresponding author Email: nt.luan@hutech.edu.vn

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ABSTRACT

Dangshen *Codonopsis javanica* performed high medicinal properties in herbal remedies; however, there is currently not much specific analysis for phytochemicals and bioactivities of this plant. In this study, the ethanol extract of *C. javanica* roots obtained from Kon Tum Province, Vietnam contained several substances as saponin, phenolic acid, terpenoid and alkaloid. The antibacterial effect of the extract showed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus* with the IC₅₀ value of 150, 100, 150 and 90 µg/ml, respectively. The antioxidant capacity of the extract also displayed IC₅₀ value of 46.8 ± 6.8 µg/ml. Moreover, the anticancer ability of the extract exhibited on two human cancer HepG2 cells (IC₅₀ = 83.6 ± 2.7 µg/ml), and MCF-7 cells (IC₅₀ = 95.3 ± 2.3 µg/ml). Hence, this study for the first time provide the basic data for further research of bioactivities of natural compounds Dangshen *C. javanica*.

KEYWORDS

Antibacterial, Anticancer, Antioxidant, Bioactive Compounds, Dangshen *Codonopsis javanica*.

1. INTRODUCTION

Dangshen *Codonopsis javanica* (Blume) Hook f. (*C. javanica*, belongs to *Campanulaceae*) is a precious herb in Oriental folk medicine with various function in tonic and complementing the spleen, mediating fatigue and poor appetite, improving the immune system and fitness, reducing stress, etc. (Hoang et al., 2002; Do, 2006; Vo, 2012). In Vietnam, Dangshen *C. javanica* is scattered from the Northern mountainous region to the South-Central Coast, especially in Kon Tum Province, Vietnam. This specie recently has been listed in "Vietnam Red Data Book, Part II- Plants, 2007" for prime preservation (Nguyen, 2006; Nguyen et al., 2007). Therefore, it is crucial to study the farming process and to analyze the biochemical components as well as medicinal values biochemical components of *C. javanica*. These make significant contribution to further maintaining the indigenous medicinal plants, and developing the pharmaceutical industry in Vietnam.

2. MATERIALS AND METHODS

2.1 *C. javanica* root extract preparation

The *C. javanica* roots were collected in Kon Tum Province, Vietnam during dry season. The ethanol extract of the roots was carried out by twice soaking the fine powder in ethanol (EtOH) with the mass/volume ratio of 1:5 at 70°C in order to collect the EtOH root extract. The solvent was removed from the ethanol extract using Heidolph rotary evaporator at 32°C at the speed of 74 rounds/min. This ethanol extract was then dried at 25°C.

2.2 Analyzing the chemical constituents

Total phenolic content. Dang Shen *C. javanica* root extract was mixed with distilled water then heated to 100°C. FeCl₃ 0.1% was added.

Flavonoid. The root extract was mixed with ethanol. Zinc powder was added to all the samples and the mixture was shaken. HCl were added subsequently.

Saponin. The root extract was combined with 100°C distilled water. This sample was then strongly shaken, air bubbles appeared, and the sample was left to rest for 20 min for further observation and analysis.

Terpenoid. The root extract was mixed with Chloroform (CHCl₃). HCl were then dropped into the samples.

2.3 Antibacterial activities of root extract

2.3.1 Disc diffusion method

The bacterial suspension was streaked into MHA agar plate following Kirby-Bauer method (Disc diffusion method). *C. javanica* extract was diluted in DMSO into various concentrations. Several 6mm paper discs were loaded with differing extract concentrations and the same amount of DMSO as the amount used for dilution. The antimicrobial effect of the extract was evaluated by measuring zone of inhibition diameter, according to the following formula:

$$I \text{ (mm)} = D - d$$

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D represents diameter of the inhibition zone while d represents the diameter of paper discs

2.3.2 Minimal inhibitory concentration (MIC)

The bacterial suspension was then diluted to reach 1.5×10^6 CFU/ml. *C. javanica* extract with different concentrations were also added to the bacterial culture. After 24h of incubation, MIC concentration was determined at the well containing the lowest concentration of the *C. javanica* extract in which there was no visible bacterial growth. The changing color of resazurine (Sigma Aldrich) from blue to pink indicated that the bacteria were still alive.

2.4 Antioxidant activities

The *C. javanica* extract was prepared at different concentrations to develop the linear equation representing the antioxidant capacity of the extract. Several tested samples at multiple concentrations were then added into previously prepared DPPH solution. Blank samples were also set without DPPH solution. All the samples were kept in the dark at room temperature for about 60 min. After the incubation period, the absorbance was measured at 517 nm using an ELISA reader (JenWay Genova Plus). The experiment was done in triplicate.

2.5 Cytotoxic activities against cancer cell lines

Cancer cell lines HepG2 (human hepatocyte carcinoma cancer, ATCC) and MCF-7 (human breast cancer, ATCC) were cultured with high glucose Delbecco's Modified Eagle's Medium (DMEM, Gibco), 10% Fetal Bovine Serum (FBS, Gibco), and antibiotics at 37°C and 5% CO₂. Dangshen *C. javanica* extract and Doxorubicin, which is positive control, were both subjected to dilution in culture medium to achieve in a concentration-dependent manner. Blank wells also added with no cell. MTT assay based on the principle that MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) involves in the oxidative-reductive reaction with the cell mitochondria and generate crystalized formazan. It is plausible to use a few different solutions to both destroy the cell membrane and dissolve the formazan crystals. Then the absorbance of these solutions is then measured at 595 nm. This method evaluates cell viability through their respiratory activity.

3. RESULTS AND DISCUSSION

3.1 *C. javanica* root chemical constituents

The qualitative analysis of the chemical constituents of Dangshen *C. javanica* root were presented in Table 1, similar to other extraction reports (Wang et al., 1995; Hoang et al., 2002; Truong et al., 2015). The data confirmed the presence of multiple secondary metabolites in the root such as saponin, terpenoid, alkaloid and polyphenol. Saponin, one of the main medicinal components of Dangshen, highly exposed in *C. javanica* root collected in Kon Tum Province.

Table 1: Phytochemical analysis result of *C. javanica* extract

No.	Compounds	Recognizing reaction	Positive reaction	Result
1	Polyphenol	FeCl ₃ solution	Navy or green	+
2	Flavonoid	Zinc (Zn) powder and HCl solution	Pink and gas product	-
3	Terpenoid	Chloroform and HCl solution	Brown and two layers separation	++
4	Saponin	Bubbling	A stable white foam layer	+++
5	Alkaloid	Mayer/ Wager solution	Sepia precipitate	++

Note: -: negative; +: positive; ++: many; +++: high amount

3.2 Antibacterial activities

3.2.1 Disc diffusion method

Root extract at the concentration 50 mg/ml was subjected to antibacterial analysis against four bacterial strains, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, using disc diffusion method. Data in Table 2 demonstrated that 50 mg/ml of *C. javanica* extract was effective against all four bacterial strains, especially against *B. cereus* with the highest 7.7 mm of zone of inhibition.

3.2.2 MIC-dilution method

MIC result was demonstrated in Figure 1. At strip E1, 200 µg/ml of the extract was able to fully inhibit *E. coli* growth, as the color in the well was blue which was similar to the color of the blank well. However, 20 µg/ml of the extract did not restrain *E. coli* growth, as the color in the well was pink which was similar to the color of negative control well (this well did not contain the extract). In strip E2, the extract was diluted to achieve lower concentration 150 µg/ml and 100 µg/ml. The extract at concentration 150 µg/ml was capable of fully inhibiting *E. coli* growth. Nevertheless, at concentration 130 µg/ml, the extract did not completely restrain the bacterial growth. The color in this well was pink- purple, as a result of the combination between pink and blue. These data confirmed that the MIC value of the extract against *E. coli* was 150 µg/ml.

MIC data of the extract against three remaining bacteria was also analyzed similarly to that of *E. coli*. The MIC value of the *C. javanica* extract against *P. aeruginosa*, *S. aureus*, and *B. cereus* was 100 µg/ml, 150 µg/ml, and 90 µg/ml, respectively. Thus, *C. javanica* extract was effective against all four tested bacterial strains. It especially showed the strongest activity against *B. cereus* because the MIC value was 90 µg/ml, which was the lowest.

Figure 1: MIC assay (resazurin was used as reagent) result of *C. javanica* extract showing its antibacterial ability against four bacterial strains *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*.

Table 2: The antibacterial effect of *C. javanica* extract against four bacterial strains was shown as the zone of inhibition diameter. The data were presented as mean $\pm$ SD

Bacterial strains	Zone of inhibition diameter (mm)
<i>P. aeruginosa</i>	4.3 $\pm$ 0.6 mm
<i>S. aureus</i>	4.0 $\pm$ 1.0 mm
<i>B. cereus</i>	7.7 $\pm$ 0.6 mm
<i>E. coli</i>	3.3 $\pm$ 0.6 mm

3.3 Antioxidant activities

Based on Figure 2, as the concentration of the extract increased, the DPPH scavenging percentage also increased. This result affirmed that *C. javanica* extract presented its antioxidant capacity in a concentration-dependent manner, in which the percentage of free radical scavenging was directly proportional to the extract's concentration. The scavenging ability of the extract was 92% at 60 µg/ml, while that of ascorbic acid was also 92% but only at 20 µg/ml.

Figure 2: The antioxidant capacity of *C. javanica* extract (A) and ascorbic acid (B) were demonstrated by their DPPH free radical scavenging ability.

The antioxidant effect of the extract was also elaborated by IC_{50} value in Table 3. As shown in Table 3, IC_{50} value of *C. javanica* extract and ascorbic acid (positive control) were 46.8 ± 6.8 mg/ml and 10.8 ± 0.1 mg/ml, respectively. This result also revealed the passable antioxidant capacity of the extract, which was only 5 times weaker than ascorbic acid.

Table 3: IC_{50} value showing the antioxidant capacity of *C. javanica* extract and ascorbic acid

Samples	IC_{50} (mg/ml)
<i>C. javanica</i> extract	46.8 ± 6.8
Ascorbic acid	10.8 ± 0.1

3.4 Cytotoxic activities

Dangshen *C. javanica* extract exhibited cytotoxicity at 100 μ g/ml, corresponding to the ability of DOX at the concentration 6.25 μ g/ml against HepG2 cells in Figure 3. Similarly, the extract also against MCF-7 cells at 100 μ g/ml, corresponding to the ability of DOX at the concentration 12.5 μ g/ml. Meanwhile, DMSO at 1.0% corresponding to the concentration used to dissolve the extract at 100 μ g/ml did not show any cytotoxic activity against both HepG2 and MCF-7 cells.

Figure 3: *C. javanica* cytotoxic activity against human hepatocyte carcinoma HepG2 cells and human breast cancer MCF-7 cells at 100 μ g/ml. Negative control was DMSO at 100 μ g/ml. Positive control was Doxorubicin at 6.25 μ g/ml (HepG2) and at 12.5 μ g/ml (MCF-7).

Table 4 presented the IC_{50} value demonstrating the cytotoxic activity of Dangshen *C. javanica* against HepG2 and MCF-7 cells to be low. *C. javanica* extract specifically exhibited cytotoxicity about 15 times weaker than DOX for HepG2 cells and 9.5 times for MCF-7 cells. This experiment for the first time showed the cytotoxicity of *C. javanica* root extract against HepG2 and MCF-7 cells. The findings were generally consistent with previous anticancer studies (Ueda et al., 2002; Peng et al., 2011; Yang et al., 2015).

Table 4: IC_{50} value presenting the cytotoxic activity of *C. javanica* extract and DOX against HepG2 and MCF-7 cell-line. The data were presented as mean $\pm$ SD.

Samples	IC_{50} (μ g/ml)	
	HepG2	MCF-7
<i>C. javanica</i> extract	83.6 ± 2.7	95.3 ± 2.3
DOX	5.4 ± 0.2	10.9 ± 2.2

4. CONCLUSION

In this study, the roots of *C. javanica* collected in Kon Tum Province, Vietnam were subjected to ethanol extraction in order to achieve the crude

extract. Phytochemical analysis showed multiple chemical constituents, including saponin, terpenoid, alkaloid, and polyphenol in the *C. javanica* root extract. The bioactivity experiments also affirmed the antibacterial, antioxidant, and anticancer activities of the *C. javanica* root. Two methods used in this study to examine the antibacterial effect of the extract from *C. javanica* against four bacterial strains were agar plate diffusion and MIC value. *C. javanica* extract had antibacterial potential against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus* with the IC_{50} value of 150, 100, 150 and 90 μ g/ml, respectively.

DPPH assay was performed the antioxidant capacity of *C. javanica* extract. *C. javanica* extract displayed antioxidant effect with IC_{50} value of 46.8 ± 6.8 μ g/ml, which was only 5 times weaker than ascorbic acid. Moreover, MTT assay was carried out on human hepatocyte carcinoma HepG2 cells and human breast cancer MCF-7 cells to study the cytotoxic ability of *C. javanica* extract. *C. javanica* extract inhibited HepG2 cells ($IC_{50} = 83.6 \pm 2.7$), and MCF-7 cells ($IC_{50} = 95.3 \pm 2.3$). Taken together, this study for the first time shows the diverse bioactivity of Dangshen *C. javanica* from Kon Tum Province, Vietnam such as antibacterial, antioxidant, and anticancer activities. Therefore, the extract is recommended to be used in the food industry and medicine. Additionally, the extracts can be used in further research of bioactivities of natural compounds.

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