Journal of
Microbial & Biochemical Technology

Lee and Choi, J Microb Biochem Technol 2015, 7:5
DOI: 10.4172/1948-5948.1000226

Short Communication Open Access

Antiviral and Antimicrobial Activity of Medicinal Plant Extracts

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Abstract

Antimicrobial activity of methanolic extracts of *Chamaecyparis obtuse* (CO), *Chrysanthemum boreale* (CB) and *Cryptomeria japonica* (CJ) against four bacteria (*Staphyloscoccus aureus, Bacillus cereus, Escherichia coli* and *Yesinia enterocolitica*) was evaluated by a disk diffusion method. Anti-human rhinovirus (HRV) 3 activity and cytotoxicity of them were evaluated by a cytopathic effect reduction method. The methanolic extracts of CB showed antimicrobial activity against tested four microorganisms with diameter of inhibition zones from 0.5 to 2.0 mm. The other extracts showed weak antimicrobial activity against *Bacillus cereus* and didn't exhibit antimicrobial activity against *Escherichia coli* and *Yesinia enterocolitica*. The antiviral assays demonstrated that methanolic extracts of CO and CJ possessed strong antiviral activity against HRV3 with 77.8% and 93.7%, respectively, at a concentration of 100 μg/mL with no cytotoxicity. However, methanolic extracts of CB did not show anti-HRV3 activity. Therefore, the antimicrobial of CB and antiviral activity of CO and CJ will be further investigate in preventing bacteria pathogens or HRV3-mediated injuries in pathological situations.

Keywords: Antimicrobial; Antiviral; Cytotoxicity; HRV3; Phytochemicals

Introduction

The increasing resistance of the microorganisms towards antibiotics has been led to serious health problems in the recent years [1]. Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the life-span of antifungal agents such as the azoles, antiviral agents such as the non-nucleoside reverse transcriptase inhibitors, and various antibacterial agents including β-lactams and quinolones [2-4]. This problem encourages the researchers to study the new agents which can effectively inhibit microbial growth.

An alternative approach to overcome these issues might be using natural antimicrobial or antiviral products and phytochemicals. The Middle East has unique niches for medicinal plants, which have been used for treating diseases and infections for thousands of years in traditional medicine. It has been shown previously that plants and their aromatic products have potential antimicrobial activities [5]. A previous paper was reported *Raoulia australis* showed anti-human rhinovirus activity [6].

In this study, we aimed to evaluate the antibacterial potential of the *Chamaecyparis obtuse* (CO), *Chrysanthemum boreale* (CB) and *Cryptomeria japonica* (CJ) extracts against four bacteria (*Staphyloscoccus aureus, Bacillus cereus, Escherichia coli* and *Yesinia enterocolitica*). We also assessed the antiviral activity of these extracts against human rhinovirus (HRV) 3.

Material and Methods

Fresh leaves of CO, CB and CJ were purchased from UNIQ F and F Co., Ltd. (Seoul, Korea). Ten grams of air-dried and powdered materials was extracted from each plant using with 95% methanol (100 mL). The obtained organic solutions from each plant were collected separately and filtered using Whatman no 42. The solution was evaporated by using a vacuum evaporator under 40°C to get the crude dried extract.

Staphyloscoccus aureus, Bacillus cereus, Escherichia coli and *Yesinia enterocolitica* was obtained from the Chungcheongnam-Do Health and Environment Research Institute in Korea. Nutrient Agar, Brain Heart Infusion broth and Brain Heart Infusion Agar bought from Difco USA.

The antimicrobial activity of the CO, CB and CJ extracts was carried out by the disk diffusion method [7] using 100 μL of suspension containing 10^8 CFU/mL of bacteria and 106 CFU/mL of yeast spread on nutrient agar or brain heart infusion agar and potato dextrose agar medium, respectively. The disks (Whatman, 8 mm in diameter) which impregnated with 0.05, 0.1, 0.5 and 1 μg/mL of three extracts were placed on the inoculated agar. The diameters of the inhibition zones were measured in millimeters

Human rhinovirus (HRV) 3 was provided by ATCC (American Type Culture Collection, Manassas, VA, USA) and was propagated in human epitheloid carcinoma cervix (HeLa) cells at 32°C. HeLa cells were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 0.01% antibiotic-antimycotic. Ribavirin and Sulforhodamine B (SRB) was purchased Sigma-Aldrich (St. Louis, MO, USA). Assays of antiviral activity and cytotoxicity were evaluated by the previous reported SRB method using cytopathic effect (CPE) reduction [8]. Infectivity of virus stock was determined by the SRB method and was determined as infectivity of the virus by SRB ID_{50} (50% infective dose). The results were transformed to percentage of controls and were drawn the dose-response curves. Ribavirin was used as positive, and DMSO was used as negative control.

The effect of 3 extracts on HRV3-induced CPE was observed. Briefly, Vero cells were seeded onto a 96-well culture plate at a concentration of 2×10^4 cells per well. Next day, medium was removed and washed with PBS. Then, 0.09 mL of diluted virus suspension and 0.01 mL of medium supplemented with each ginsenoside of 100 μg/mL were added. After incubation at 37°C in 5% CO_2 for 2 days, the morphology of cells was

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Received August 25, 2015; **Accepted** September 01, 2015; **Published** September 08, 2015

Citation: Lee JH, Choi HJ (2015) Antiviral and Antimicrobial Activity of Medicinal Plant Extracts. J Microb Biochem Technol 7: 286-288. doi:10.4172/1948-5948.1000226

observed under microscope of 320 X magnifications (AXIOVERT10, ZEISS, Germany), and images were recorded.

Results and Discussion

The antimicrobial activity of the CO, CB and CJ extracts against four bacteria is shown in Figure 1 and 2. The data obtained, indicated that *Streptococcus aureus* was the most sensitive microorganism against three extracts with dose dependent inhibition zone. CB extracts showed inhibitory effect with diameter of inhibition zones ranging from 0.5 to 2.0 mm at concentration of 1 μg/mL against four bacteria. CO extracts among the three extracts only showed inhibitory effect with diameter of inhibition zone of 0.7 mm at concentration of 1 μg/mL. CJ exhibited inhibitory effect against *Staphyloscoccus aureus* and *Bacillus cereus* with diameter of inhibition zone ranging from 0 to 1.0 mm.

As shown in Figure 3, CO and CJ extracts showed anti-HRV3 activity at concentration of 100 μg/mL. CO and CJ extracts did not exhibit cytotoxicity at concentrations of 100 μg/mL. However, CO extracts did not show anti-HRV3 activity at tested concentration and exhibited cytotoxicity against HeLa cells at concentrations of 100 μg/mL. Also, After 2 day infections of HeLa cells with HRV3, Mock cells (Figure 4A) or cells treated with 100 μg/mL CO and CJ extracts (Figure 4C and 4E) or ribavirin (Figure 4I) showed typical spread-out shapes and normal morphology. Infection with HRV3 in the absence of the oils resulted in a severe CPE (Figure 4B). Addition of the CO and CJ extracts and ribavirin on infected HeLa cells inhibited the formation of a visible CPE (Figure 4D, 4F and 4J). However, the addition of CB extracts in HRV3-infected HeLa cells didn't prevented CPE with showing cytotoxicity (Figure 4G and 4H).

The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have substantially threatened the current antibacterial therapy [9]. MDR bacterial infections often lead to increased mortality, longer length of stays in hospitals, and higher cost of treatment and care [9,10].

The inclusion of traditionally used medicines including phytomedicine, if they prove safe and effective, into national health

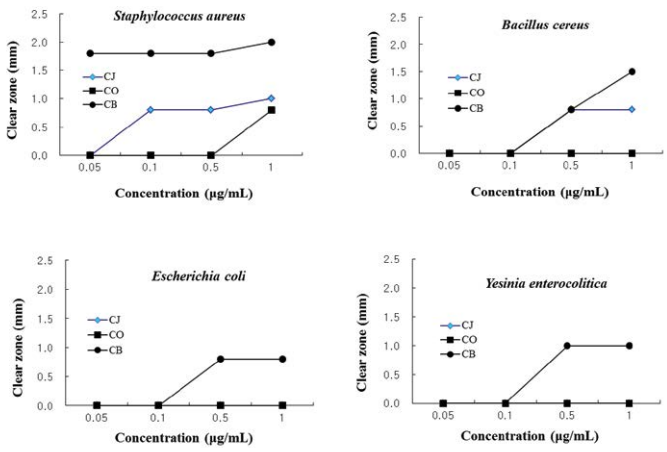

Figure 1: Antimicrobial activity of the *Chamaecyparis obtuse*, *Chrysanthemum boreale* and *Cryptomeria japonica* extracts against *Staphyloscoccus aureus, Bacillus cereus, Escherichia coli* and *Yesinia enterocolitica* (A). The paper disks treated with 0.05, 0.1, 0.5 and 1 μg/mL of three extracts and were incubated at 37°C after placed on the inoculated agar. Values represent the means of 3 independent experiments. CO, *Chamaecyparis obtuse*; CB, *Chrysanthemum boreale*; CJ, *Cryptomeria japonica*.

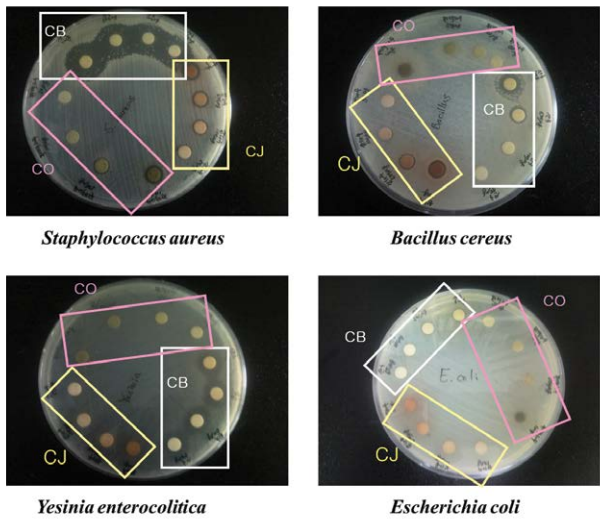

Figure 2: Morphology of clear zone by paper disk diffusion. The disks which impregnated with 0.05, 0.1, 0.5 and 1 μg/mL of *Chamaecyparis obtuse*, *Chrysanthemum boreale* and *Cryptomeria japonica* extracts were placed on the inoculated agar. The inoculated plates were incubated at 37°C. The morphology was investigated under camera and a photograph taken. CO, *Chamaecyparis obtuse*; CB, *Chrysanthemum boreale*; CJ, *Cryptomeria japonica*.

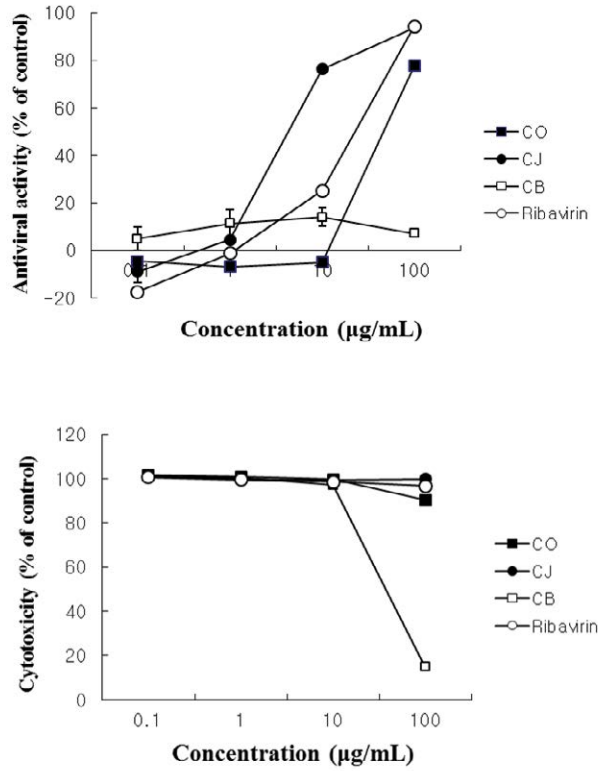

Figure 3: Antiviral activity of the *Chamaecyparis obtuse*, *Chrysanthemum boreale* and *Cryptomeria japonica* extracts against HRV3 in HeLa cells. Antiviral activity of three extracts against HRV3 in HeLa cells; A, Antiviral activity of three extracts against HRV3 in HeLa cells. The antiviral activity was investigated by CPE reduction assay. Results are presented as the mean percentage values obtained from three independent experiments carried out in triplicate ± S.D. CO, *Chamaecyparis obtuse*; CB, *Chrysanthemum boreale*; CJ, *Cryptomeria japonica*.

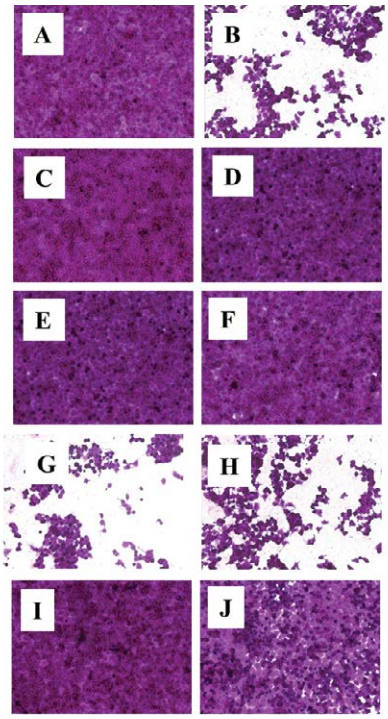

Figure 4: The effect of the *Chamaecyparis obtuse*, *Chrysanthemum boreale* and *Cryptomeria japonica* extracts on HRV3-induced CPE. The effects of three extracts on HRV3-induced CPE. Culture medium 96-well tissue culture plates were removed and the cells were washed with PBS. Then, 0.09 mL of diluted virus suspension and 0.01 mL of medium three extracts or ribavirin of 100 μg/mL were added. After incubation at 32°C in 5% CO_2 for 2 days, the morphology of cells was investigated under microscope and a photograph taken. (A) Non-infected cells; (B) virus-infected cells without extracts; (C) non-infected cells with CO extracts; (D) virus-infected cells with CO extracts; (E) non-infected cells with CJ extracts; (F) virus-infected cells with CJ; (G) non-infected cells with ribavirin; (H) virus-infected cells with ribavirin; (I) non-infected cells with CB extracts; (J) virus-infected cells with CB extracts. CO, *Chamaecyparis obtuse*; CB, *Chrysanthemum boreale*; CJ, *Cryptomeria japonica*.

care system is suggested by World Health Organization [11]. Although a large number of medicinal plants (>500 plants) have been reported to be used by people since a long time for primary health care, there has been a paucity in data regarding their in vitro or in vivo efficacy [12].

In this study, we aimed to determine the in vitro antimicrobial or antiviral activity of extracts from some selected medicinal against the 4 bacterial pathogens and HRV3. CB among the tested three medicinal plant extracts showed strong antimicrobial activity against *Staphyloscoccus aureus, Bacillus cereus, Escherichia coli* and *Yesinia enterocolitica*. However, CB didn't exhibit anti-HRV3 activity. It showed cytotoxicity at HeLa cells with showing abnormal morphology. Taken together, these results indicate that some cytotoxic materials containing within CB will be influenced cytotoxicity of HeLa cells. Therefore, antiviral activity of CB didn't measure at HeLa cells because cytotoxicity of the extracts.

In conclusion, the present study described that CB extracts possesses strong antimicrobial activity against *Staphyloscoccus aureus, Bacillus cereus, Escherichia coli* and *Yesinia enterocolitica*. CO and CJ possess anti-HRV3 activity. It will be interesting to further investigate the antimicrobial or antiviral activity of CO, CB and CJ in preventing bacteria pathogens or HRV3-mediated injuries in in vivo pathological situations.

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