

Full Length Research Paper

Antimicrobial and antitumor activities of crude secondary metabolites from a marine fungus *Penicillium Oxalicum* 0312F₁

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The extract of fermentation product of the *P. oxalicum* strain 0312F₁ was isolated through gel (Sephadex LH-20) column chromatography to give fractions A, B, C, D, E and F. Then, antimicrobial activity was evaluated by dual culture method and micro-dilution method. As a result, the water soluble extract of marine fungus *Penicillium oxalicum* 0312F₁ had inhibitory activity against *Alternaria solani*. We also tested antitumor activity using methyl thiazolyl tetrazolium (MTT) method. The results showed that the fractions F gave the maximum inhibition rate (higher than 70%) against both human gastric cancer cell line (SGC-7901) (SGC-7901 cell line) and human hepatic cancer cell line (BEL-7404) (BEL-7404 cell line), at the concentration 0.5 mg/mL. While at the concentration of 1 mg/mL, the inhibition rates of fractions D, E, F against SGC-7901 cell line was higher than 70%, the inhibition rates of fractions C and F against BEL-7404 cell line was higher than 70%. There were antimicrobial and antitumor active fractions in secondary metabolites from marine fungus *P. oxalicum* 0312F₁.

Key words: Antimicrobial, antitumor, crude secondary metabolites, *Penicillium oxalicum*.

INTRODUCTION

Recently, the interest in studying extreme environments has increased. These habitats may accommodate new species, which may be used as unique sources of secondary metabolites of biotechnological or pharmaceutical potential. The species found in extreme environments may also produce novel chemical

compounds (Stierle et al., 2006; Hujšlová et al., 2010). Fungal plant pathogens, which infect all major crops, are a threat to global food security; they cause serious losses both in the field and post-harvest, and some may produce mycotoxins (Strange and Scott, 2005). Since the discovery of penicillin, the micromycetes have been

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famous as producers of secondary metabolites with biological activity, including antibacterial, antifungal, antitumor, immunosuppressant, cholesterol-lowering agents and mycotoxins (Shen et al., 2009; Lopes et al., 2012).

Penicillium sp. has been reported as bioactive for antitumor activity (Han et al., 2007) and antimicrobial activity (Li et al., 2001; Yang, 2002; Chen and Zhang, 2006; Tan et al., 2006; Wang et al., 2013). Compounds isolated from the EtOAc extract of *Penicillium* sp. exhibited both cytotoxic and antimicrobial activity (Subramani et al., 2013; Wang et al., 2013). Also, compounds isolated from crude extract of *Penicillium* sp. only showed cytotoxic activity (Rand et al., 2005). It is reported that compounds showed moderate cytotoxic activity against the human hepatocellular liver carcinoma cell line (Gao et al., 2011). There are also new compounds isolated from *Penicillium* sp. with cytotoxic activity, which should be unique resource for developing new pharmaceuticals (Lin et al., 2012; Li et al., 2013).

As part of our previous investigation on the anti-phytoviral, antimicrobial and antitumor activities of marine fungi, fungi attracted our attention because the fungi strains found exhibited relatively high bioactivity (Shen et al., 2009, 2010; Tan et al., 2012).

The aim of this study was to investigate the antimicrobial and antitumor activities of the crude extract of a marine fungus, *Penicillium oxalicum*. The water soluble extract had moderate antimicrobial activity against plant pathogenic fungi. And then, the water soluble extract was concentrated to give a fraction subjected to Sephadex LH-20 to afford six fractions, which had moderate to high potential antitumor activity. The results will guide us to isolate active compounds from active crude fractions by the bio-guided method.

MATERIALS AND METHODS

Tested strains

Marine-derived *P. oxalicum* strain 0312F₁ (Genbank accession no. EU926977), and the pathogenic fungi (*Colletotrichum orbiculare*, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Colletotrichum graminicola*, *Rhizoctonia cerealis*, *Alternaria solani* and *Fusarium graminearum* Schwabe) were all maintained on potato dextrose agar (PDA) medium. And they were all incubated at 28°C for 48 h and then stored at -20°C in tubes with PDA medium in fridge.

Cancer cell lines

Human gastric cancer cell line (SGC-7901) and hepatic cancer cell line (BEL-7404) were cultured under 5% CO₂ at 37°C in RPMI 1640 medium supplemented with foetal bovine serum and penicillin-streptomycin (100 IU/mL).

Extraction procedure

The marine-derived *P. oxalicum* strain 0312F₁ was cultured at 28°C

for 7 days in Erlenmeyer flasks (1 L), each containing 100 g rice and 200 mL glucose-peptone-yeast medium. The mycelium and medium were twice extracted with MeOH (ca 30 L). The extract was concentrated *in vacuo* to yield a residue, which was dissolved in H₂O and filtered. The water-soluble fraction was then desalted by Dianion-HP20 column eluting by H₂O. The MeOH extract was concentrated to give a fraction of 7.8 g, which was subjected to Sephadex LH-20, eluted with MeOH-H₂O (0, 1:20, 1:10, 1:5, 2:5, 1) to afford six fractions (A: 1.2 g, B: 1.7 g, C: 1.8 g, D: 1.9 g, E: 0.8 g, F: 0.4 g).

Antimicrobial activity assay

The inhibitory activity of marine fungus 0312F₁ against pathogenic fungi was evaluated by duel culture method (Shen et al., 2010). And the inhibitory activity of crude extract of marine fungus 0312F₁ against pathogenic fungi was evaluated by micro-dilution method (Ríos Dueñas et al., 2011). The concentration of crude extract of marine fungus 0312F₁ against pathogenic fungi was 2 mg/mL. And the concentrations of crude extract of marine fungus 0312F₁ in EC₅₀ evaluation were 2, 1, 0.5, 0.25 and 0.125 mg/mL, respectively.

Cytotoxic activity assay

Human gastric cancer cell line SGC-7901 and hepatic cancer cells BEL-7404 were plated into 96-well tissue culture dishes at a density of 1×10³ per well in 180 mL medium and allowed to attach at 37°C for 24 h, followed by the addition of 20 mL solutions of different fractions of crude extract samples (dimethyl sulfoxide (DMSO) was added as the vehicle, ≤1%), incubated at 37°C for another 72 h. Then, 20 mL methyl thiazol tetrazolium (MTT) (2 mg/mL) was added to each well and incubated at 37°C for 4 h. Finally, the supernatants were removed and the formazan crystals were dissolved by adding 200 mL DMSO. The absorbance at 570 nm was determined by a microplate reader. 5-Fluorouracil and cis-diaminedichloroplatinum (CDDP) were used as positive control (Shen et al., 2009).

Statistical analysis

The IC₅₀ values were determined from concentration-effect curves by linear regression analysis. Statistical analysis was performed using SPSS version 13.0, and data were presented as the arithmetic mean ± standard deviation.

RESULTS AND DISCUSSION

Inhibitory activity of marine fungus 0312F₁ against pathogenic fungi

The inhibitory activity of marine fungus 0312F₁ against pathogenic fungi was evaluated by duel culture method. Table 1 shows that inhibitory activity of strain 0312F₁ against pathogenic fungi was weak.

Inhibitory activity of crude extract of marine fungus 0312F₁ against pathogenic fungi

In Table 2, the crude extract of marine fungus 0312F₁

Table 1. Inhibitory effect of marine fungus 0312F₁ against pathogenic fungi.

Pathogenic fungi	Inhibition rate (%)
<i>C. orbiculare</i>	37.21±1.82
<i>F. oxysporum</i>	35.90±1.27
<i>P. aphanidermatum</i>	39.58±2.03
<i>C. graminicola</i>	21.74±0.93
<i>R. cerealis</i>	26.67±1.27
<i>A. solani</i>	12.50±1.22
<i>F. graminearum Schwabe</i>	20.70±0.92

Values are mean of three replicates.

Table 2. Inhibitory activity of crude extract of marine fungus 0312F₁ against pathogenic fungi.

Pathogenic fungi	Inhibition rate (%)
<i>C. orbiculare</i>	-
<i>F. oxysporum</i>	15.91±1.81 ^b
<i>P. aphanidermatum</i>	6.67±2.02 ^c
<i>C. graminicola</i>	-
<i>R. cerealis</i>	-
<i>A. solani</i>	58.62±0.92 ^a
<i>F. graminearum Schwabe</i>	56.00±0.76 ^a

Values are mean of three replicates. The concentration of water soluble extract was 2 mg/mL; -: no inhibitory activity. Means in the same column with different letters are significantly different (n=3, P<0.05).

was determined for inhibitory activity against pathogenic fungi. The metabolites displayed weak to high antimicrobial activity against all pathogenic fungi. The metabolites showed much higher antimicrobial activity against *A. solani* and *F. graminearum Schwabe*, with nearly 60% inhibition. The results indicated that the active fraction of metabolites of strain 0312F₁ should be in water soluble extract.

A large number of marine-derived fungal extracts have antimicrobial activity mainly related to *Penicillium* species (Shen et al., 2010; Lopes et al., 2012). New antifungal compounds usually come from natural sources, which involves screening of microorganisms and plant extracts (Augustine et al., 2005; Bevan et al., 1995).

Growth of 7 plant pathogens in controlled culture and culture of water soluble extract from strain 0312F₁ was evaluated by micro-dilution method. *R. cerealis* was the fastest growing of all the controlled plant pathogens (Figure 1E). After inoculation for two days, its radius was 4.5 cm, while *C. graminicola* was the most slowly growing. Growth of 7 plant pathogens increased in a time dependent manner (Figure 1). In Figure 1F, after inocula-

tion with water soluble extract, *A. solani* was growing slowly. The phenomenon indicated that the crude extract of strain 0312F₁ had definite inhibitory activity against *A. solani*.

The EC₅₀ value of water soluble extract of strain 0312F₁ against *A. solani* was then determined. The inhibition rates were 58.60, 40.00, 32.00, 21.33 and 9.02%, under the concentrations of 2, 1, 0.5, 0.25 and 0.125 mg/mL, respectively. The EC₅₀ value was 1.43 mg/mL by calculation (Figure 2). The results indicated that the inhibition rates increased in a concentration dependent manner.

Cytotoxic activity of fractions of crude extract of marine fungus 0312F₁

The culture extracts of *Penicillium* strains possessing some extent of antifungal ability were evaluated as a possible source of anti-tumor products on human tumor cell lines (Nicoletti et al., 2008). Our MTT assay indicated the inhibitory activity of fractions D, E and F against SGC-7901 cells, which were afforded after water soluble extract was subjected to Sephadex LH-20, and was higher than 70% (with inhibition 70.04, 71.35, and 79.30%, respectively) under the concentration of 1 mg/mL. Furthermore, the inhibition rate of fractions F was high (up to 73.24%) under the concentration of 0.5 mg/mL (Figure 3).

Similarly, the inhibitory activity of fractions C and F against BEL-7404 cells was higher than 70% (with inhibition of 72.38 and 78.13%, respectively). Under the concentration of 0.5 mg/mL, the inhibition rate of fractions F was also high (up to 74.74%) (Figure 4).

Secondary metabolites produced by microorganisms are the main source of the skeletons; some have been used directly in field (Yang et al., 2008). Cytotoxic compounds should be isolated from the active fractions C-F. And fraction F was the most active fraction to obtain cytotoxic compounds against SGC-7901 and BEL-7404 cells.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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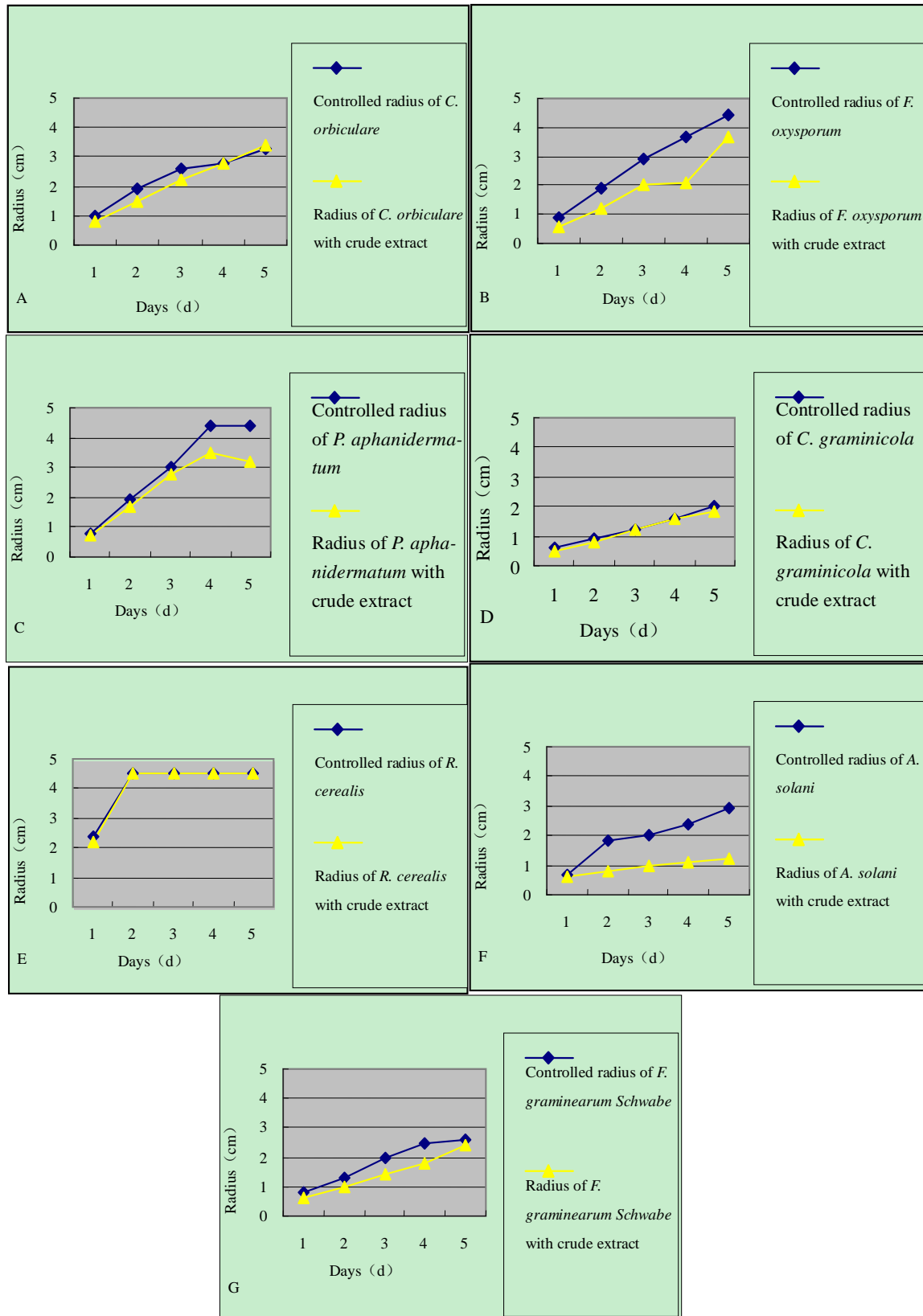


Figure 1. Inhibitory effects of water soluble extract from strain 0312F₁ on the growth of plant pathogens in controlled culture and culture of water soluble extract from strain 0312F₁. A-G: Growth of 7 plant pathogens in controlled culture and culture of water soluble extract from strain 0312F₁.

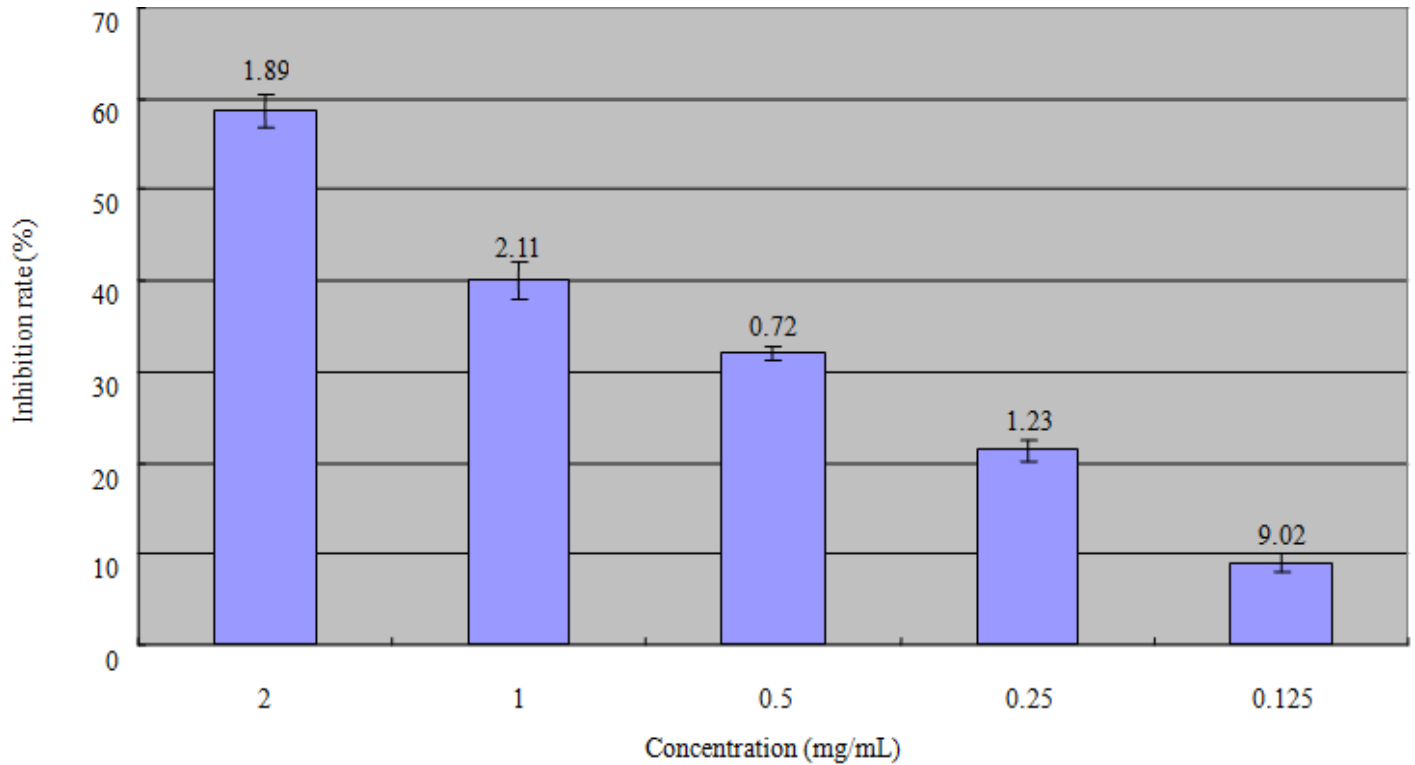


Figure 2. Inhibitory activity of crude extract of marine fungus 0312F₁ against *A. solani*. Values are mean of three replicates.

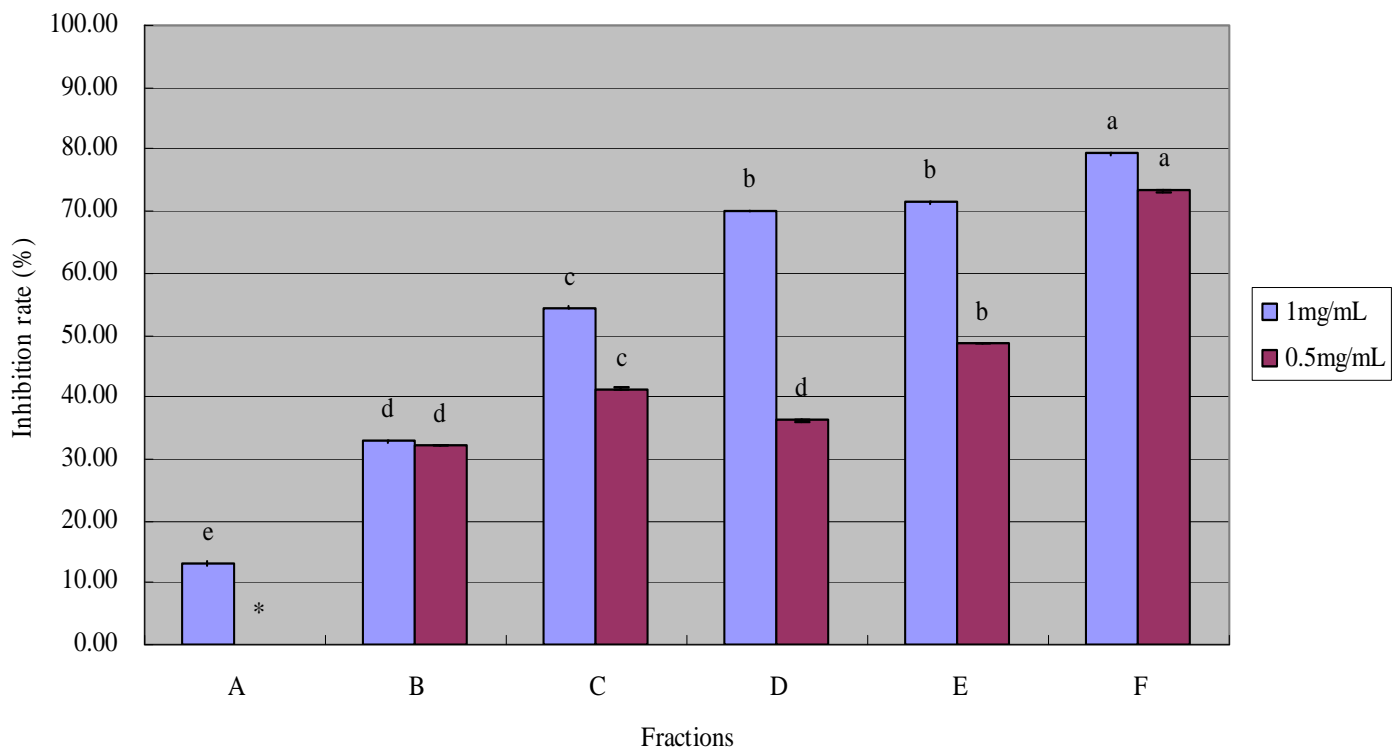


Figure 3. Inhibitory activity of fractions of crude extracts of strain 0312F₁ against SGC-7901 cells. Values are mean of three replicates. Different letters in the column with the same color (at the same concentration) are significantly different ($P < 0.05$); *: no inhibitory activity.

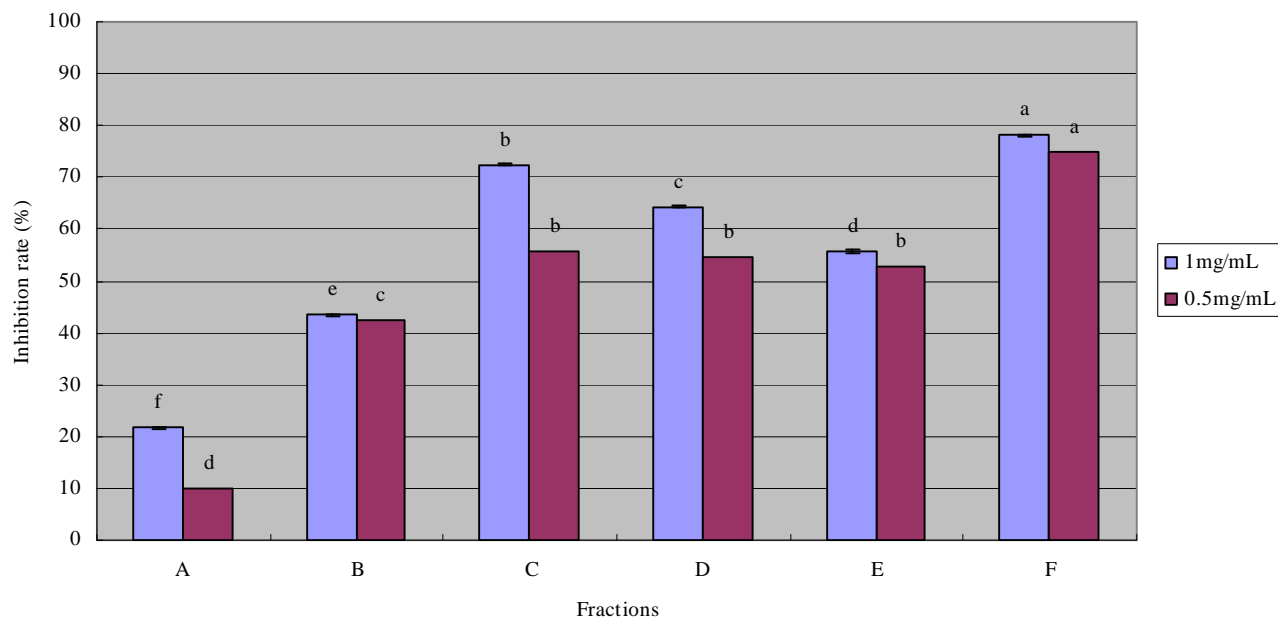


Figure 4. Inhibitory activity of fractions of crude extracts of strain 0312F₁ against BEL-7404 cells. Values are mean of three replicates. Different letters in the column with the same color (at the same concentration) are significantly different ($P < 0.05$).

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