Journal of Medicinal Plants Research Vol. 6(12), pp. 2295-2298, 30 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR011.272
ISSN 1996-0875 ©2012 Academic Journals

*Full Length Research paper*

# Antifungal activity of coptidis rhizoma against *Candida* species

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Accepted 12 March, 2012

**The extract of Coptidis Rhizoma was determined antifungal activity against five different *Candida* species. The extract showed antifungal activity against *Candida albicans* at 200 µg/ml, and *C. tropicalis* and *C. glabrata* at 50 µg/ml. A compound 1 was separated by thin layer chromatography (TLC) analysis and confirmed as berberine by high performance liquid chromatography (HPLC) analysis. The minimum inhibitory concentration (MIC) values of the compound 1 and berberine were 24 and 48 µg/ml against *C. tropicalis* and *C. glabrata*, and 30 and 50 µg/ml against *C. tropicalis* and *C. glabrata*. The $IC_{50}$ values of the compound 1 were 58 µM at 24 h and 40 µM at 48 h by MTT assay of cell viability using HaCaT cell line. The above results indicated that berberine was a good candidate to inhibit the propagation of *C. tropicalis* and *C. glabrata*, even in human skin.**

**Key words:** Anticandidial activity, coptidis rhizoma, MTT assay, HaCaT cell line.

## INTRODUCTION

Fungal infections have shown increased rate at the numerous surveys over the past decades. The most notable infections were due to *Candida* spp., which were found to be the fourth most common cause of nosocomical blood stream infection among hospitals during the 1980s (Pfaller, 1995; Abi-Said et al., 1997; Richardson and Kokki, 1998; Verduyn et al., 1993). This trend has continued into the 2000s. Notably, these infections were increased by species of *Candida* other than *C. albicans*.

The emergence of species other than *C. albicans* is clearly a concern. Urinary infection is the frequent fungal infection in patients with HIV infection and in people with long term antibiotics and steroid use. Fungal infections were generally treated by antibiotics such as amphotericin B (Amp B) and the zoles (Blumberg and Reboli, 1996; Girmenia and Martino, 1998). Data have reported that the resistance of *Candida* species to Amp B and the zoles has been increasing (Fonos and Cataldi, 2000; Gallis et al., 2000).

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## MATERIALS AND METHODS

The dried Coptidis Rhizoma was crushed with a pulverizer and extracted two times with a 70% ethanol at room temperature for 24 h. The extracts were evaporated to dryness using a rotary evaporator (Eyela, Japan). The extract powders were stored at -20°C until use. *Candida* species, *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilensis*, and *C. utils* were purchased from Korea Gene Bank (Daejeon, Korea). *Candida* species were culture on YM (Difco, USA) media with the extract for antifungal activity test. The isolated compounds separated by Thin Layer Chromatography on silica gel plates 60F254 (Merck, Darmstadt, Germany) were analyzed using HPLC (Varian, Walnut Creek, CA) equipped with PDA detector, and a Varian polar C18 reversed-phase column (4.6 × 250 mm, 0.45 µm). The mobile phase was composed of a phosphate buffer-acetonitrile solution containing 0.1% formic acid. The elution program for the mobile phase was as follows; 10% acetonitrile at 0 min, 30% acetonitrile at 10 min, 80% acetonitrile at 20 min, 80% acetonitrile at 25 min, 10% acetonitrile at 30 min. The flow rate was 1 ml/min and UV detection was dually performed at 290 nm and 340 nm. For cell viability assay, HaCaT cells line was obtained from Korean cell Line Bank (Seoul, Korea). Cells were cultured in DMEM containing FBS (10%), penicillin (100 U/ml), streptomycin (0.1 mg/ml) at 37°C in a humidified atmosphere of 5% $CO_2$. Cells were harvested after incubation for 24 h. Viability of cultured cells was determined by MTT method (Mosmann, 1983). Cells were added to each well in 96-well plates, and cultured for 24 h. After samples treatment, MTT (5 mg/ml in PBS) was added 100 µL to each well. Cells were incubated at 37°C for 30 min, and

**Table 1.** Antifungal activity of extract of Coptidis Rhizoma against *Candida* species.

| Species | Clear zone (mm) contents of extract (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 10 | 50 | 100 | 150 | 200 |
| *C. albicans* | - | - | - | - | 3 |
| *C. tropicalis* | - | 2 | 3 | 6 | 9 |
| *C. glabrata* | - | 3 | 4 | 4 | 5 |
| *C. parapsilensis* | - | - | - | - | - |
| *C. utils* | - | - | - | - | - |

-: Not detected.

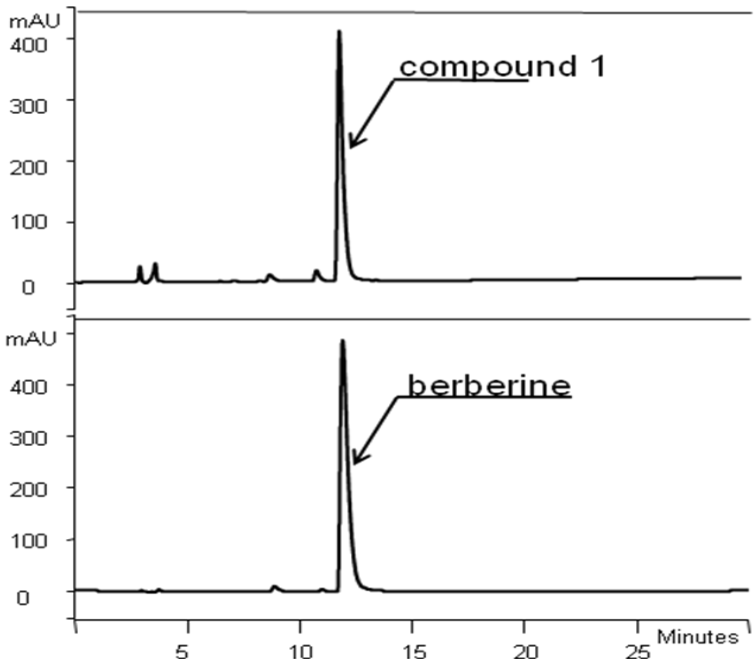


**Figure 1**. HPLC analysis of compound 1 isolated from coptidis rhizoma.

DMSO was added to dissolve the formazan crystals. The absorbance was measured at 560 nm with a spectrophotometer.

## RESULTS AND DISCUSSION

Anticandidial activity of Coptidis Rhizoma extract was determined against five different *Candida* sp. by agar diffusion assay. The extract of Coptidis Rhizoma showed antifungal activity against *Candida albicans* at 200 μg/ml, *C. tropicalis* and *C. glabrata* at 50 μg/ml. The extract showed best antifungal activity against *C. glabrata* at 50 μg/ml (Table 1). A compound 1 was separated by TLC analysis from Coptidis Rhizoma extract, and the above compound 1 was confirmed as berberine by HPLC analysis (Figure 1). The MIC (minimum inhibition concentration) of the compound 1 and berberine were determined and compared. The MIC values of the compound 1 and berberine were 24 and 48 μg/ml against *C. tropicalis* and *C. glabrata*, and 30 and 50 μg/ml against *C. tropicalis* and *C. glabrata* (Table 2). The difference of MIC values of both compounds was supposed an error of weighting materials. The $IC_{50}$ values of the compound 1 were 58 μM at 24 h and 40 μM at 48 h by MTT assay of cell viability using HaCaT cell line (Figure 2). The above results indicated that the compound 1, berberine, was a good candidate to inhibit the propagation of *C. tropicalis* and *C. glabrata*, even in human skin.

Candidiasis has been the most common fungal infection in HIV infected individuals and patients in hospital. Although *Candida* spp. resistant to antifungal agents have been a rarity until three decades ago, resistance of *Candida* spp. was spread in a serious infection disease of the hospital environment. Although

**Table 2.** MIC of a compound 1 from coptidis rhizoma.

| Species | Compound 1 (μg/ml) | Berberine (μg/ml) | Amphotericin B (μg/ml) |
|---|---|---|---|
| *C. albicans* | - | - | 0.2 |
| *C. tropicalis* | 24 | 30 | 0.4 |
| *C. glabrata* | 48 | 50 | 0.4 |
| *C. parapsilensis* | - | - | 0.4 |
| *C. utils* | - | - | 0.4 |

**-** : Not detected.

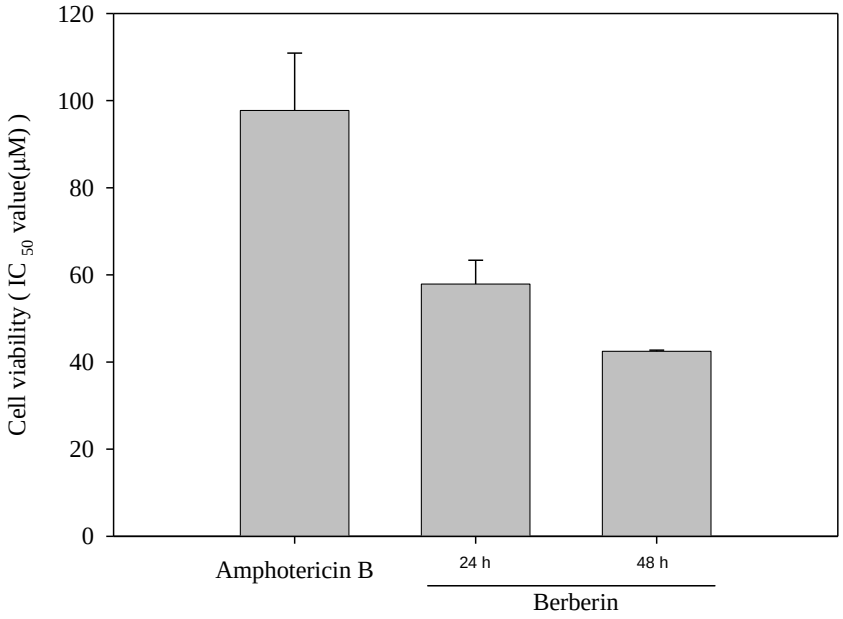


**Figure 2.** $IC_{50}$ values for viability on HaCaT cells. Growth inhibition of HaCaT cell after treatment with berberine and Amphotericin B. Cells were plated at $1x10^4$ cell/well per 96 well plate, and incubated for 24 h. The cells treated with variable concentrations of berberine for 24 and 48 h, and amphotericin B for 24 h and the viability were measured by MTT assay. The data represent means ±SD of five independent experiments.

uncommon, this outbreak should serve as a warning that multi-resistance of *Candida* strains may develop and spread within the hospital environment (Bodey, 1988; Schaberg et al., 1991; Masia and Gutierrez, 2002).

In this work, we did seek what a kind of the extract from Coptidis Rhizoma was responsible for the antifungal effect. It is suggested that the compound 1, berberine, can be applied as a phytomedicine for treatment of the Candidal infection (Williamson, 2001; Han and Lee, 2005).

## ACKNOWLEDGEMENT

This research was supported by the Academic Research Fund of Hoseo University in 2010 (2010-0131).

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