Antioxidant and Antimicrobial Potentials of Endophytic Fungi Residing in Cynanchum Chinense

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ABSTRACT: 18 strains endophytic fungi were isolated from leaves and branches of Cynanchum chinense. The antioxidant and antibacterial activities of the broth of the 18 strains were studied by vitro methods. The antioxidant activities results showed that all tested strains have different degree of DPPH radical scavenging activities. At the concentration of 0.1mg/mL, 8 of 18 strains played a certain DPPH radical scavenging activities, with the scavenging rate more than 50%, of these, the extract of 1 strain played notable DPPH radical scavenging activity, with the scavenging rate of 84.5%. 15 strains showed a certain hydroxy radical scavenging abilities, but the scavenging activity was weak, with the max scavenging rates of 14.5% at the concentration of 0.8 mg/mL. The antibacterial activities results showed that 9 of 18 strains were active against the six test bacterium (Staphylococcus aureus, Bacillus licheniformis, Streptococcus uberis, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae.), but none played strong inhibitory effect, with (the inhibition zone was greater than 20mm).

1. INTRODUCTION

Endophytes are the micro-organisms that colonize the interior of the plant parts, without having any negative effect on the host (Breen, 1994), rather helping the plant by imparting resistance to host plant against biotic (Breen, 1994, Schulz, 1999, and Dingle, 2003) and abiotic stresses (West, 1994). Every plant species examined till date harbors endophytic fungi (Strobel, 2006). In addition to providing wide range of activities against plant pathogens and herbivores, endophytic fungi are known to produce bio-molecules of pharmaceutical and agricultural importance. Many of the bioactive metabolite from endophytic fungi act as plant defence activator and have been useful in novel drug discovery (Owen, 2004). Muscador albus, an endophytic fungus of rainforest plant, is known to produce volatile organic compound responsible for fumigant activity against stored grain pests (Strobel, 2006 and Strobel, 2001). Several antioxidant and antimicrobial metabolites, such as colletotric acid (Zou, 2000), griseofulvin (Park, 2005), are reported from endophytic fungi. Nematicidal activity is also reported from the culture filtrate of Fusarium oxysporum, an endophytic fungus of tomato.
Metabolites of endophytic fungi responsible for pesticidal activity have been reviewed by Kumar et al (Kumar, 2008).

Cynanchum chinense have immunomodulatory effect (Dajun, 2009) and antitumor property (Li, 2011). Present paper describes the diversity of endophytic fungi isolated from Cynanchum chinense and their antioxidant and antimicrobial potentials.

2. MATERIALS AND METHODS

2.1 Sample collection

Cynanchum chinense collected from Lanzhou, Gansu Province, China, May 2015. Then selected undamaged parts, washed with tap water and processed for isolation of endophytic fungi.

2.2 Media preparation

Malt extract agar medium [Malt extract (15 g/l); Agar (15 g/l), pH: 7.4–7.8] was used for isolation and purification of endophytic fungi. Wickerham medium [Malt extract (3 g/l); Yeast extract (3 g/l); Peptone (5 g/l); Glucose (Qualigens) -10 g/l; pH -7.2 to 7.4] was used for small scale multiplication of endophytic fungi, being used for extracting metabolite. Potato dextrose agar (PDA) was used for bioassay.

2.3 Isolation of endophytic fungi

Endophytic fungi were isolated from the healthy plants of Cynanchum chinense as per the procedure of described by Wang et al (2006) with minor modification in surface sterilization. The plant parts were surface sterilized with 75% ethanol for 2 min followed by 1% sodium hypochlorite for 3 min. Surface sterilized plant parts were dried on sterile blotting sheet, cut into small pieces, and transferred to malt agar plates, after taking imprint of dried sterile plant part. These plates were incubated at 28°C for 3-10 days. Hyphal tips of the developing fungal colonies were transferred to fresh malt agar plates to get pure culture.

2.4 Small-scale multiplication of endophytic fungi

Endophytic fungi showing antagonistic property against pathogenic bacteria were inoculated in potato liquid medium (300 ml in 500 ml conical flask) and incubated at 28°C for 3-4 weeks. Upon cooling of the media, each flask of potato liquid medium was inoculated with each endophytic fungus. One flask of medium was kept for control.
2.5 Antioxidant activity assay

2.5.1 DPPH radical scavenging activity

DPPH radical assay was performed as description in previous study (Khan, 2011) with minor modifications. Briefly, 2mL sample (0.05 mg/mL) and 2mL DPPH solutions (20mmol/L) were mixed, and subsequently incubated at room temperature for 30 min. Then, the supernatant was collected to measure the absorbance value at 540 nm. The percentage inhibition was calculated by the formula: Inhibition (%) = 100 × (Acontrol - Aextract)/Acontrol, (Acontrol and Aextract are the absorbance of control and sample at 540 nm, respectively).

2.5.2 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was evaluated according to the previous reported method with minor modification (Royer, 2011). Briefly, 1 mL FeSO4 solution (600 mmol/L) and 1 mL sample (0.5 mg/mL) were mixed in colorimeter tube, and 1mL hydrogen peroxide (1%) was added subsequently. Then, the mixture solution was incubated in a water bath at 37 °C for 30 min. Finally, the supernatant was collected to measure the absorbance value at 510 nm. The percentage inhibition was calculated by the formula: Inhibition (%) = 100 × (Acontrol - Aextract)/Acontrol, (Acontrol and Aextract are the absorbance of control and sample at 510 nm, respectively).

2.6 Bioassay of extracts of endophytic fungi against pathogenic bacteria

Bioassay of endophytic fungi fungal extracts against pathogenic bacteria was done by dual culture technique. Potato dextrose agar (PDA) medium was selected for dual culture as it favours growth of pathogenic bacteria, Staphylococcus Aureus, Bacillus licheniformis, Streptococcus uberii, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The dried extracts were dissolved in dimethyl sulfoxide to make 5.0000 mg/ml concentration. PDA media (30 ml) was poured in plates, and the filter paper (diameter 6mm) were soaked in extracts sample for half an hour, then dried and placed on inoculated indicator bacteria plates. After incubation at 37°C for 3-7 days, plates were observed and antagonism was expressed by the presence of inhibition zone at the point of interaction. To check if the growth inhibition is due to dimethyl sulfoxide, filter paper was soaked in filter paper and placed on the same plates. The diameters of the inhibition zones were measured.
3. RESULTS

3.1 Isolation and molecular identification of endophytic fungi

18 endophytic fungi were isolated from root and tuber of *Cynanchum chinense*.

3.2 Antioxidant of the endophytic fungi

All tested strains have different degree of DPPH radical scavenging activities. The extract of fermented liquid concentration at 0.1mg/mL, 8 of 18 strains played a certain DPPH radical scavenging activities, with the scavenging rate more than 50%. Of these, the extract of 1 strain played notable DPPH radical scavenging activity, with the scavenging rate of 84.5%.

15 strains showed a certain hydroxy radical scavenging abilities, but the scavenging activity was weak, with the max scavenging rates of 14.5% at the concentration of 0.8 mg/mL.

3.3 Bioactivity activity assay

Dual culture test revealed that 9 strain’s extracts of 18 endophytic fungi showed activity against the six test bacterial. Extracts of 1 strain showed high activity against the six test bacterial (the diameters of the inhibition zones are more than 10mm).

4. DISCUSSIONS

The present work is the first report of the antibacterial activity of endophytic fungi from *Cynanchum chinense*. 18 endophytic fungi were isolated and their antioxidant and antibacterial activity was studied. The results showed the endophytic fungi of *Cynanchum chinense* have a certain degree of antioxidant potentials and inhibition activity against pathogenic bacteria. But the activities are weak.

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REFERENCES


