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Phenolics and antifungal activities analysis in industrial crop Jerusalem artichoke (*Helianthus tuberosus* L.) leaves

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ABSTRACT

Spoilage of industrial fruits and vegetables in storage and transportation due to fungal infection results in significant losses, and new natural antifungal treatments would have a large economic value. The extracts of antifungal compounds and phenolic acids from Jerusalem artichoke (*Helianthus tuberosus* L.) leaves were investigated for potential use in enhancing preservation of fruits and vegetables in storage. Either crude leaf extract or *n*-butanol fraction was active against *Botrytis cinerea, Colletotrichum gloeosporioides, Phytophthora capsici* Leonian and *Rhizoctonia cerealis*, with the values of IC_{50} (half of the maximum inhibitory concentration) ranging from 2.166 to 2.534 g L^{-1} for the crude leaf extract and 0.232–1.911 g L^{-1} for *n*-butanol fraction. The severity of grey mould caused by *B. cinerea* was significantly reduced by *n*-butanol fraction applied at 1 and 2 g L^{-1} (the control efficiency of 71.3% and 77.8%, respectively, compared with commercial preparation *Carbendazim*. Following *in vitro* activity-guided fractionation by bioautography, six phenolic acids were separated from *n*-butanol fraction. Among them, caffeic acid, 3,4-dicaffeoylquinic acid and 1,5-dicaffeoylquinic acid played a dominant role and were active in bioassays against *Gibberella zeae*, with respective minimum inhibitory concentrations (MIC) being 108, 60 and 4.2 μg mL^{-1}. These results imply that Jerusalem artichoke leaves might be a potential source of natural fungicides.

1. Introduction

Plants contain a large variety of phytonutrients, of which many have antioxidant properties. Antioxidant compounds include vitamins, phenols carotenoids and flavonoids (Vadakkemuriyil Divya Nair et al., 2013). Phenolics are secondary plant metabolites found in the majority of herbs, vegetables, and tea (Shao et al., 2008). In recent years many studies have demonstrated that free radicals are the leading cause of degenerative disease (Shao et al., 2008). Plant antioxidants work as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers and enzyme inhibitors. Many of these protective biological effects are attributed to polyphenol contents of plants. The demand for natural antioxidants (plant phenolics) has increased tremendously over the past five years. New phenolic products are continuously been developed and consumed (Shao et al., 2008, 2009; Bakowska-Barczak and Kolodziejczyk, 2011; Giri et al., 2012; Vadakkemuriyil Divya Nair et al., 2013). Our results have good significance, as this study provided economic and efficient ways to enhance phenolic content for commercial use. *Helianthus tuberosus* L. (Asteraceae), also known as Jerusalem artichoke and an important industrial crop, is a sunflower species originating from the eastern North America. It has been naturalized and cultivated widely across the tropical, temperate, boreal and arid to semi-arid zones. Jerusalem artichoke has both economic and ecological values. Given its wide ecological adaptation (it is resistant to drought, salt, cold and wind stress), Jerusalem artichoke has been used in food, pharmaceutical, feed, sugar, paper, cosmetics, and bioethanol industry, and in desert and tideland control (Denoroy, 1996; Shao et al., 2009; Mohamed Hussein Hamdy Roby et al., 2013).

Jerusalem artichoke has been reported to have various pharmacological activities, such as aperient, cholagogue, diuretic,

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stomachic and tonic, and has been used as a folk medicine for the treatment of bone fracture, diabetes, rheumatism, skin wounds, swelling and pain (Talipova, 2001; Baba et al., 2005; Pan et al., 2009; Jin et al., 2013). In addition, previous phytochemical studies have disclosed that effective compounds such as coumarins, unsaturated fatty acids, polyacetylenic derivatives, phenols and sesquiterpenes have been isolated from Jerusalem artichoke (Pan et al., 2009). These Jerusalem artichoke compounds were found to possess antioxidant, antimicrobial, antifungal and anticancer activities (Ahmed et al., 2005; Pan et al., 2009; Yuan et al., 2012; Meng et al., 2012).

There are myriads of plant species that might be used as fungicides, with many organic compounds in plants found to possess antifungal or antimicrobial activities (Wilkins et al., 1989). Antibacterial activities in genus *Helianthus* have been reported in the early 1980s (Spring et al., 1981). The sesquiterpene compounds isolated from the aerial parts of *Helianthus annuus* L. may have antibacterial (annuithrin) or antifungal activities (niveusin B) (Spring et al., 1982). Other compounds such as alkaloids, flavonoids and phenolics may also have antifungal properties (Vokou et al., 2006). Phenolic compounds are widely distributed in plants and have been ascribed putative roles in protection against infection by plant pathogens (Wen et al., 2003) and/or insects (*e.g.* chlorogenic acid; Sinden et al., 1988; Friedman, 1997; Percival et al., 1999). Twelve phenolic compounds (including chlorogenic acid) were effective in inhibiting *Xylella fastidiosa* growth (Maddox et al., 2010), whereas a new phenolic compound had antifungal activity against *Sclerotinia sclerotiorum* (Prats et al., 2007).

Previous studies demonstrated that crude extract of Jerusalem artichoke leaves possessed antifungal or antimicrobial activities (Liu et al., 2007; Han et al., 2010). The main constituents in the leaves of Jerusalem artichoke are chlorogenic and isochlorogenic acids that have good antioxidant properties. In general, the activity of phenolics is related to their chemical structures (Yuan et al., 2012); based on structural features of Jerusalem artichoke phenolics, its leaf extracts could exhibit antifungal capacities. However, to the best of our knowledge, antifungal or other anti-phytopathogenic activities of Jerusalem artichoke main phenolics have not previously been reported.

Hence, the antifungal and anti-phytopathogen activities of different fractions/extracts of Jerusalem artichoke were investigated by the activity-guided method. Furthermore, we tested the fractions/extracts for their capacity to enhance preservation of fruits and vegetables in storage, which is a novel way to exploit new natural fungicides.

2. Materials and methods

2.1. Chemicals and plants

3-*o*-caffeoylquinic acid was obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Captan, Cyprodinil and Azoxystrobin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other standard samples were obtained from Yuanye Biological Technology Co., Ltd. (Shanghai, China). All other analytical-grade chemicals were obtained from Shoude Experiment Equipment Co., Ltd. (Nanjing, China). AB-8 macroporous resin and polyamide resin were obtained from Cangzhou Bon Adsorber Technology Co., Ltd. (Cangzhou, China). The leaves of Jerusalem artichoke (identified as cultivar NanYu (Long et al., 2009) by Prof. Zhaopu Liu, College of Resource and Environmental Science, Nanjing Agricultural University) were collected from Dafeng District (Jiangsu, China) in October 2010. The leaves were air-dried at room temperature.

2.2. Extraction and isolation of bioactive compounds

The dried and milled leaves (2 kg) were refluxed under vacuum overnight using 95% (v/v) ethanol (EtOH) at room temperature for three times. After evaporation under reduced pressure, the 255.91 g of dried EtOH crude extract (CE) was suspended in 1.0 L water and partitioned sequentially with petroleum ether (PE, 3 × 1 L), chloroform (Chl, 3 × 1 L), ethyl acetate (EA, 3 × 1 L) and *n*-butanol saturated with water (NB, 3 × 1 L). After removing the solvents, four fractions were obtained. The yields of PE, Chl, EA, NB and water fractions were 10.55, 37.25, 40.82, 50.59 and 115.60 g, respectively. Our bioassays found that the NB fraction showed the strongest antifungal activity.

The NB fraction was purified (N01) on a AB-8 macroporous resin column (5.5 cm × 50 cm). After rinsing with distilled water, the residue was chromatographed through a 80–100 mesh polyamide resin column, eluting with a series of ethanol/water from 10:90 (v/v) to 100:0 (v/v) to generate seven sub-fractions (N02–N08). Sub-fractions N03 and N08 were the most active in our study.

Sub-fraction N03 was separated by preparative HPLC (methyl alcohol:H_2O = 40:60) to yield compounds 1 and 2. In addition, sub-fraction N08 was further purified by preparative HPLC (methyl alcohol:H_2O = 40:60) to give compounds **3**, **4**, **5** and **6**. The structures of compounds **1**–**6** were determined by Mass Spectrometer (MS) and Nuclear magnetic resonance spectroscopy (NMR). The spectral data of the compounds were in agreement with those previously published (Lee et al., 2010; Peng et al., 2000; Tolonen et al., 2002).

2.3. Fungal strains and culture conditions

Nine plant pathogens *i.e. Botrytis cinerea* (*B. cinerea*), *Colletotrichum gloeosporioides*, (*C. gloeosporioides*), *Phytophthora capsici* Leonian, *Rhizoctonia cerealis* (*R. cerealis*), *Exserohilum turcicum* (*E. turcicum*), *Gaeumannomyces graminis*, *Gibberella zeae* (*G. zeae*), *Pyricularia grisea* and *Sclerotinia sclerotiorum*, donated by Jiangsu Academy of Agricultural Sciences, were incubated on potato dextrose agar (PDA) at 23–25 °C in the dark.

2.4. Bioassay and statistical analysis

The antifungal activities of CE and different fractions against the plant pathogens were evaluated by the growth inhibition bioassay (Dan et al., 2010). The tested reagents were dissolved in acetone and added to the sterile culture medium (PDA) at the specified concentrations. Following thorough mixing, the media were poured into Petri dishes (9 cm i.d.). The small amount of acetone added with each reagent had no effect on the growth of pathogens. The 4 mm × 4 mm agar plugs infected with fungi were incubated on agar plates, one plug per plate, at 23–25 °C in the dark. Colony growth diameters were measured for 7–10 days. All treatments were tested in quadruplicate, and plates without any additives were used as controls. The IC50 (half maximal inhibitory concentration) values and *p*-test analyses were carried out by SPSS 13.0 software, so did mean values and standard deviations.

Growth inhibition was calculated as follows:

$$\text{inhibition}\,\% = \left(\frac{C - T}{C - 4}\right) \times 100$$

C and *T* were the averages from four replicates of hyphal extension (mm) in the control and the treatment, respectively. The diameters of agar plugs were 4 mm.

2.5. Bioautography

The sub-fractions N01–N08 were screened for activity against plant pathogenic fungi by TLC bioautography (Danelutte et al.,

2003; Alcerito et al., 2002; Niu et al., 2006). Fractions (50 g L^{-1}) were developed on TLC plates with chloroform/ethyl acetate/formic acid 5:4:1. The chromatograms were carefully dried for complete removal of solvents, and overlaid by agar seeded with inoculum of *R. cerealis* (approximately 3.0×10^5 cells mL^{-1}). *B. cinerea* and *C. gloeosporioides* (approximately 3.0×10^5 conidia mL^{-1}) were in liquid potato-dextrose broth. All the inoculums were incubated in darkness in a moistened chamber at 23–25 °C for 72 h. Inhibition zones were observed as a clear halo against a dark background. The fungicide technical standards *Azaxystrobin, Captan and Cyprodinil* were used as controls at 2 μg per dot (Jayasinghe et al., 2003; Meepagala et al., 2003).

2.6. Microdilution assay

Spores were washed from the surface of 7- to 10-day-old agar plates with sterile 0.85% (w/v) saline solution containing 0.1% (v/v) Tween 80. The spore suspension was adjusted with sterile saline solution to a concentration of approximately 1.0×10^5 in a final volume of 100 μL per well. Microdilution tests (Hänel and Raether, 1988; Daouk et al., 1995; Wanger et al., 1995; Espinel-Ingroff et al., 2002; Favre et al., 2003; Prats et al., 2007) were performed in 96-well plates in order to determine the antifungal activity of the samples against phytopathogenic fungi *E. turcicum* and *G. zeae*. The samples were diluted in distilled water to give serial twofold dilutions that were added to each medium, resulting in concentrations ranging from 0.49 to 1000 μg mL^{-1}. The plates were incubated at 23–25 °C for 7–10 days.

The MIC (minimal inhibitory concentration) was defined as the lowest concentration resulting in no visible fungal growth after the incubation time.

2.7. Application in fruit storage

The application in storage was evaluated by assessment of disease severity (Kim et al., 2000). Mature and healthy red tomato (*Lycopersicon esculentum* cv. Suhong 2003) fruit (120–130 g per fruit, 5.5 cm across) from Jiangsu province (China). The fruits were free from visible wounds and diseases. Before each trial, fruits were washed with water followed by 75% (v/v) ethanol for 60 s, rinsed in sterile water and air-dried. All experiments were arranged in a completely randomized split-plot design with three replicates each containing 10 fruits per treatment. The concentrations of different extracts at 1 and 2 g L^{-1} were prepared by dissolving the required amounts in sterile Tween 20 (0.1%, v/v) solution. The fruits were then injected with 100 μL of a 5×10^4 mL^{-1} spores suspension or had 4 mm mycelial disks placed onto them, one disc per fruit. (A hole was bored into each fruits with a 5 mm cork borer. The disc was put in the hole and the core of tissue was replaced and sealed with Vaseline; Oladiran and Iwu, 1993). The inoculated fruits were covered with plastic tape to maintain a moist condition. The commercial antifungal preparation *Carbendazim* was used as a reference.

The disease severity index of grey mould on the tomato fruits was rated on a scale of 0–5 (0 = no disease symptom, 1 = decay up to 0.5 cm in diameter without sporulation, 2 = decay between 0.5 and 1.0 cm in diameter with sporulation beginning,

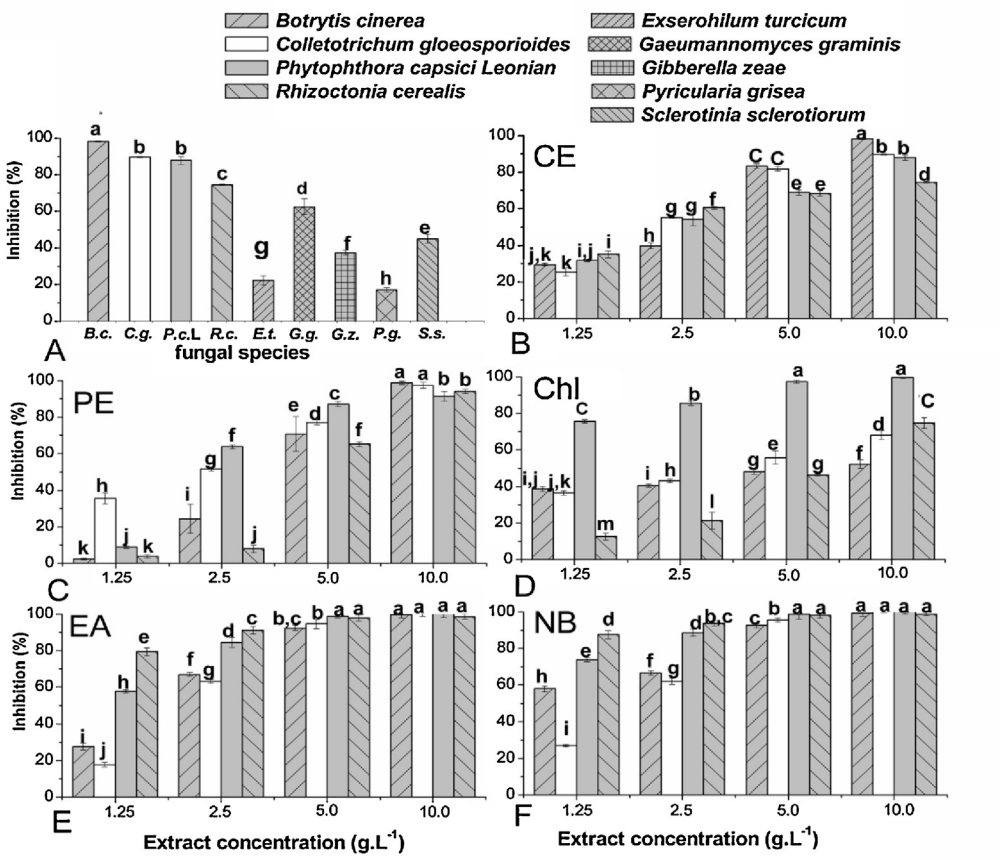

Fig. 1. Antifungal activities of extracts from Jerusalem artichoke leaves. (A) Antifungal activities of ethanolic crude extracts (10 g L^{-1}) from Jerusalem artichoke leaves against nine fungal species. (B–F) Antifungal activities of ethanolic crude extracts and different fractions from Jerusalem artichoke leaves against four fungal species. CE: crude extract; PE: petroleum ether fraction; Chl: chloroform fraction; EA: ethyl acetate fraction; NB: *n*-butanol fraction.

Table 1
IC_{50} Values of different Jerusalem artichoke leaf fractions against phytopathogens.

Fractions[a]	IC_{50} ($g\,L^{-1}$)			
	B. c.	*C. g.*	*P. c.* L.	*R. c.*
CE	2.241	2.234	2.534	2.166
PE	3.446	2.095	2.853	4.574
Chl	7.504	3.340	0.875	5.140
EA	1.859	2.071	1.154	0.300
NB	1.263	1.911	0.839	0.232

[a] CE: crude extract; PE: petroleum ether fraction; Chl: chloroform fraction; EA: ethyl acetate fraction; NB: *n*-butanol fraction.
B. c.: Botrytis cinerea; C. g.: Colletotrichum gloeosporioides; P. c. L.: Phytophthora capsici Leonian; R. c.: Rhizoctonia cerealis.

3 = decay between 1.0 and 2.5 cm in diameter with sporulation, 4 = decay between 2.5 and 4.0 cm in diameter with sporulation and mycelium, and 5 = fruit completely rotten and heavily covered with mycelium) as the percentage of diseased fruit area (ARO, 1993; Oladiran and Iwu, 1993; Soylu et al., 2010). Disease severity (DS) was calculated according to the formula given by Tariq et al. (1998):

DS% = ∑(number of fruits in that rating × severity rating)/(total number of fruits assessed × highest scale) × 100;

$$\text{Effectiveness}\,\% = \left(\frac{C-T}{C-T}\right) \times 100$$

where C = mean disease severity in the control, and T = mean disease severity in the relevant treatment.

3. Results

3.1. Antifungal activity

The crude extract of Jerusalem artichoke leaves caused 16.9 to 98.2% growth inhibition of nine phytopathogenic fungi when applied at 10 $g\,L^{-1}$ (Figs.1A and 2A). The stronger activity was noted against *B. cinerea, C. gloeosporioides*, *Phytophthora capsici* Leonian and *R. cerealis* than other species.

When various fractions were compared across a range of concentrations, there was little difference in antifungal activity at 10 $g\,L^{-1}$ for the Chl, EA and NB fractions, whereas the PE fraction showed significantly lower inhibition of all fungi except *Phytophthora capsici* Leonian (Fig. 1C). Interestingly, the PE fraction was significantly more effective against *Phytophthora capsici* Leonian than against other fungi across the whole range of concentrations tested.

The large variation in antifungal activity of various fractions against four fungi was found at 1.25 $g\,L^{-1}$ (Fig. 1C–F). Of the four extracts, the NB fraction showed the strongest antifungal activity against *B. cinerea, C. gloeosporioides* and *R. cerealis*, whereas the Chl and NB fractions were the strongest of the four against *Phytophthora capsici* Leonian (Fig. 1D and F and Fig. 2B–E).

The NB fraction had the lowest and the Chl fraction the highest IC_{50} values against *B. cinerea, C. gloeosporioides* and *R. cerealis* (Table 1). In contrast, the Chl and NB fractions had equally low IC_{50} values against *Phytophthora capsici* Leonian (Table 1).

Table 3
Bioautographically determined mean inhibitory zones (diameter, in mm) of *B. cinerea, C. gloeosporioides* and *R. cerealis* upon exposure to *n*-butanol sub-fractions or commercial antifungal agents as control.

Sub-fractions	B. cinerea	C. gloeosporioides	R. cerealis
N01	NA	NA	NA
N02	5.5 ± 0.5	NA	NA
N03	10.0 ± 1.0	8.7 ± 1.6	11.0 ± 1.7
N04	6.1 ± 0.1	3.6 ± 0.4	3.0 ± 0.2
N05	2.0 ± 0.3	4.1 ± 0.1	3.5 ± 0.2
N06	4.1 ± 0.5	1.0 ± 0.3	NA
N07	3.0 ± 0.1	3.8 ± 0.1	2.6 ± 0.3
N08	8.3 ± 0.3	12.1 ± 0.8	11.3 ± 0.9
Azoxystobin	21.0 ± 0.9	30.2 ± 0.3	25.0 ± 0.1
Captan	12.9 ± 0.8	10.3 ± 0.6	22.0 ± 0.2
Cyprodinil	18.4 ± 0.2	23.2 ± 0.2	15.0 ± 0.1

The sub-fractions were applied as 50 $g\,L^{-1}$ in 2 μL of acetone onto a silica TLC plate.
NA: not active.

3.2. Effects of the crude leaf extract and different fractions on disease development in vivo

Disease development on infected tomato fruits was controlled *in vivo* using fractions from Jerusalem artichoke leaves found to be effective in *in vitro* studies. Crude extract and the EA and NB fractions significantly decreased disease severity on tomato in comparison to the *Carbendazim* control (Table 2 and Fig. 2F). The higher dose (2 $g\,L^{-1}$) was more effective than the lower dose (1 $g\,L^{-1}$) in suppressing *B. cinerea* infection (Table 2). The NB fraction offered stronger protection against *B. cinerea* infection than crude extract and the EA fraction (Table 2).

3.3. Bioautography

The most active NB fraction was divided into 8 sub-fractions (N01–N08) by polyamide resin column eluted with gradient ethanol/water from 10:90 (v/v) to 100:0 (v/v). Eight subfractions at the concentration of 50 $g\,L^{-1}$ were screened for their antifungal activities by bioautography *via* thin-layer chromatography (TLC) on silica gel in comparison with commercial fungicides Azoxystobin, Captan and Cyprodinil. Good antifungal activities (clear zones with fungal mycelical or reproductive stroma absent) against *B. cinerea, C. gloeosporioides* and *R. cerealis* were noted for N03 and N08 sub-fractions (Table 3).

3.4. Separation and identification of phenolic compounds

Using HPLC, we isolated six compounds from sub-fractions N03 and N08. These were identified as 3-*o*-caffeoylquinic acid (3-CQA), caffeic acid, 3,4-dicaffeoylquinic acid (3,4-DiCQA), 3,5-dicaffeoylquinic acid (3,5-DiCQA), 1,5-dicaffeoylquinic acid (1,5-DiCQA) and 4,5-dicaffeoylquinic acid (4,5-DiCQA) by comparing their UV, MS and ^{1}H and ^{13}C NMR spectral data with the data reported in previous studies (Peng et al., 2000; Tolonen et al., 2002; Lee et al., 2010).

Table 2
Effects of different fractions from leaves of Jerusalem artichoke on the infection caused by *B. cinerea* on tomato fruits.

Concentrations ($g\,L^{-1}$)	Control	*Carbendazim*		CE		EA		NB	
	%DS[a]	%DS	% Protection	%DS	% Protection	%DS	% Protection	%DS	% Protection
1	56.8a	29.1b	48.8	21.7d	61.8	17.4e	69.4	16.3f	71.3
2		24.6c	56.7	15.8f	72.2	13.5g	76.2	12.6h	77.8

[a] DS: Disease severity. The means in a column followed by the same letters represent values that are not significantly different according to Duncan's test ($P \leq 0.05$)
CE, crude extract; EA, ethyl acetate fraction; NB, *n*-butanol fraction.

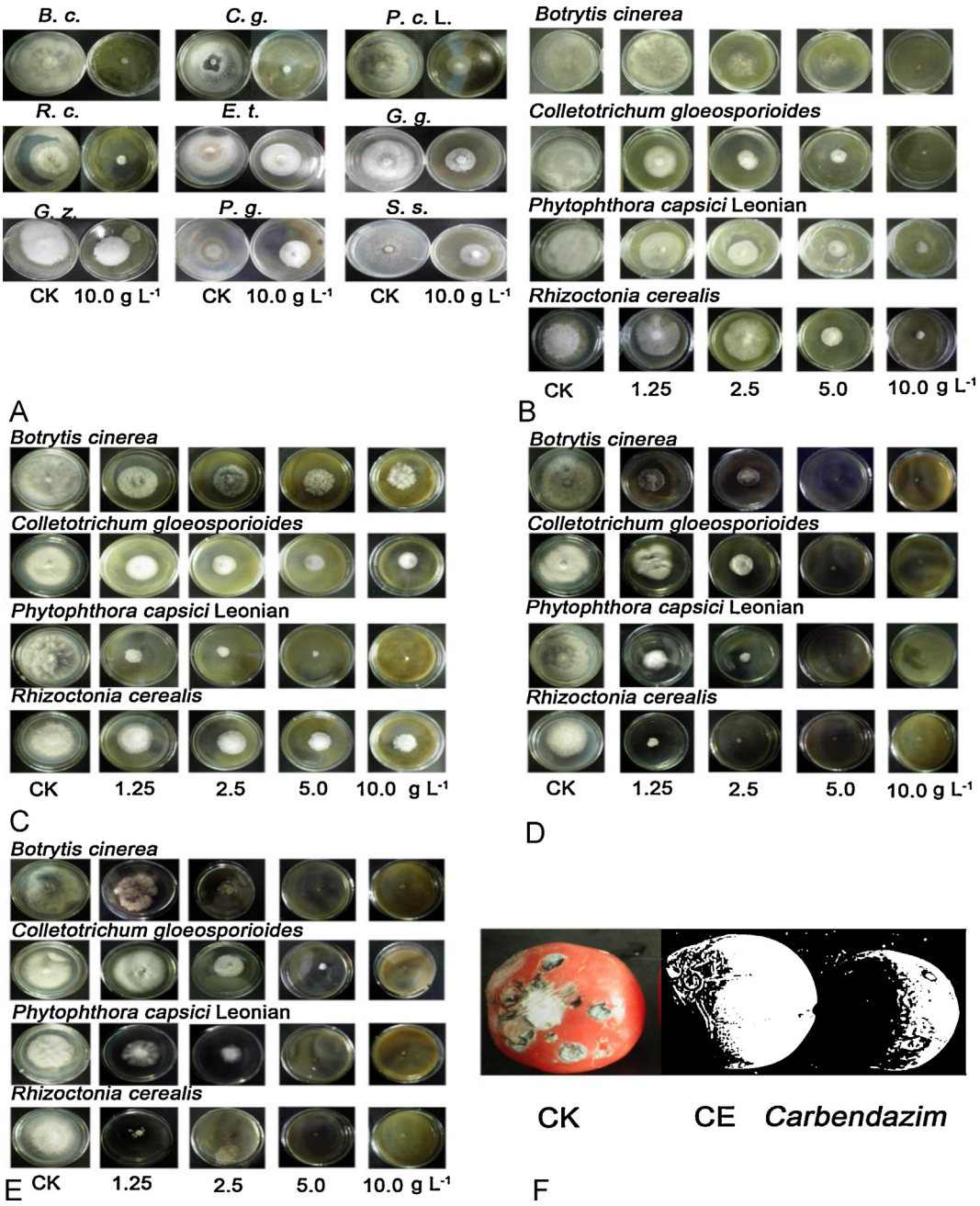

Fig. 2. Visual comparisons of toxicites of crude extract, PE, Chl, EA and NB fractions from Jerusalem artichoke leaves against four fungal species. (A) Effect of crude extract (CE) of Jerusalem artichoke leaves on radial growth of nine fungal species (10 g L^{-1}). B c., *Botrytis cinerea*; C g., *Colletotrichum gloeosporioides*; P. c. L., *Phytophthora capsici* Leonian; R. c., *Rhizoctonia cerealis*; E. t., *Exserohilum turcicum*; G. g., *Gaeumannomyces graminis*; G. z., *Gibberella zeae*; P. g., *Pyricularia grisea*; S. s, *Sclerotinia sclerotiorum*. (B–E) Visual comparisons of toxicites of PE, Chl, EA and NB fractions from Jerusalem artichoke leaves (1.25–10 g L^{-1}) against four fungal species. (F) Control effects of crude extract (CE) (1 g L^{-1}) on the infection caused by *Botrytis cinerea* on tomato fruits.

3.5. Antifungal activities of pure phenolic compounds

The antifungal activities of six phenolic compounds were evaluated by a microdilution assay. Purified caffeic acid, 3,4-DiCQA and 1,5-DiCQA were active in bioassays against *G. zeae*, with MIC values of 108, 60 and 4.2 μg mL^{-1} (Table 4).

4. Discussion

In vitro antifungal bioassay, across the crude extract and the four fractions, it appeared the highest IC_{50} were required for *B. cinerea* and the lowest for *Phytophthora capsici* Leonian, suggesting the former to be more difficult to control with the antifungal components from Jerusalem artichoke leaves than the latter. The results were consistent with the previous studies in which crude extracts of Jerusalem artichoke leaves were used as antifungal or antimicrobial agents. Nevertheless, it was notable that the NB fraction was more active than the extracts in previous studies (Liu et al., 2007; Han et al., 2010). The radial growths of *B. cinerea*, *C. gloeosporioides*, *Phytophthora capsici* Leonian and *R. cerealis* were completely inhibited by the EA and NB fractions at 10 g L^{-1} (Fig. 1E, F and Fig. 2D, E). Hence, the constituents of these two fractions were worth studying further. According to the previous research (Yuan et al., 2012), the main constituents in the

Table 4
Antifungal activity [expressed as minimum inhibitory concentration (MIC)] of the phenolic compounds isolated from the leaves of Jerusalem artichoke.

MIC ($\mu g\,mL^{-1}$)	Compounds					
	3-CQA	Caffeic acid	3,4-DiCQA	3,5-DiCQA	1,5-DiCQA	4,5-DiCQA
E. turcicum	>290	>215	>120	>80	>210	>180
G. zeae	>290	108	60	>80	4.2	>180

leaves of Jerusalem artichoke were chlorogenic and isochlorogenic acids, with chlorogenic acid being effective in inhibiting mycelium growth (Maddox et al., 2010). It remains to be established whether the main antifungal activity in the EA and NB fractions comes from chlorogenic acid or other compounds.

In vivo, crude extract and the NB fraction could be applied onto the preservations of Fruits and vegetables in storage for antifungal agent in comparison to *Carbendazim* (Table 2 and Fig. 2F). To the best of our knowledge, fractionation of Jerusalem artichoke leaf extracts and demonstration of antifungal activities of various fractions have not previously been reported neither *in vivo* nor *in vitro* conditions.

Based upon the estimated MIC values in microdilution assay of pure phenolic compounds, we found that caffeic acid, 3,4-DiCQA and 1,5-DiCQA played a dominant role against *G. zeae* in the Jerusalem artichoke leaf extracts, which is consistent with previous research (Kumaraswamy et al., 2011). According to another report, 3,5-DiCQA (isochlorogenic acid) was found to be active against *Rhizopus stolonifer* (Stange et al., 2001). We explored the structure-activity relationships of phenolic acids and found that the 3,4-DiCQA and 1,5-DiCQA were two isomers of isochlorogenic acids, with the isomers significantly varying in bioactivity (Yuan et al., 2012). Isomers 3,4-DiCQA and 1,5-DiCQA exhibited higher activities than the other two isomers (3,5-DiCQA and 4,5-DiCQA) (Table 4). Therefore, the antifungal activity of isomers appears to be affected by the position of the caffeoyl group on quinic acid.

Phenolic acids, besides their known antioxidant and radical-scavenging activity, were reported to reduce inflammation, and act as an antispasmodic and therapeutic agent and an inhibitor of reproduction of the human immunodeficiency virus (Stange et al., 2001). The antimicrobial activity of phenolic acids was reported for caffeic, chlorogenic, ferulic and *p*-coumaric acids (Wen et al., 2003). Čižmárik and Matel (1970) reported the antimicrobial activity of caffeic acid against *S. aureus, Corynebacterium diphtheriae, Proteus vulgaris, Mycobacterium tuberculosis, Helminthosporum carbonum* and *Streptomyces scabies*. Caffeic acid and chlorogenic acid were also used to inhibit *Listeria monocytogenes* and *Legionella pneumophila* (Furuhata et al., 2002). Caffeic, chlorogenic, isochlorogenic and *p*-coumaric acids were also found to be inhibitory to root-rotting fungi *in vitro*, while ferulic acid directly inhibited *Sclerotinia sclerotiorum* (Duke et al., 2003; Martínez, 2012).

The six phenolic acid tested in the present study had only a slight inhibitory effect on *E. turcicum*, but caffeic acid, 3,4-DiCQA and 1,5-DiCQA were significantly more active against *G. zeae.* This result indicated that the antimicrobial activity of phenolic acids may often be contradictory (Wen et al., 2003). Differences in experimental methodologies, medium composition and limitations due to the poor solubility of many phenolic compounds were likely responsible for contradictory results. However, the key was a narrow selective spectrum of inhibition (Boonyakiat, 1983). For example, chlorogenic acid inhibited *Penicillium expansum, Fusarium oxysporum* and *Mucor piriformis*, but slightly stimulated the growth of *B. cinerea* (Duke et al., 2003). Hence, the antifungal activities of the *n*-butanol sub-fractions of Jerusalem artichoke leaf extract may be due to interplay between the structure and function, which deserves further study.

In conclusion, this study was the first report on the antifungal activities of phenolic acids/fractions extracted from Jerusalem artichoke leaves being used for preservation of fruits. The *n*-butanol fraction was the most active. Activity-guided isolation led to the separation of six phenolic compounds. Caffeic acid, 3,4-dicaffeoylquinic acid and 1,5-dicaffeoylquinic acid were the major compounds responsible for the antifungal activities against *G. zeae*. The leaves of *H. tuberosus* L. are a promising source of natural fungicides.

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