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Bioactivity of some plant extracts against *Drechslera biseptata* and *Fusarium solani*

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Abstract

Phytochemical screening of *Alhagi maurorum*, *Calotropis procera* and *Datura innoxia* extracts revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, steroids and/or triterpenes. Moreover, anthraquinones were detected in *A. maurorum* and *C. procera* whereas alkaloids were detected in *D. innoxia* only. When ethyl alcohol extract prepared from *D. innoxia* was further fractionated by petroleum ether and chloroform, three fractions were detected. Four triterpenes subfractions (D1-D4) were isolated from petroleum ether fraction and identified as β-amyrin, daturadiol, daturadiol-3-acetate and daturaolone. Chloroform fraction contained alkaloids which were fractionated into 3 subfractions (D5-D7) and identified as atropine, hyoscine and hyoscyamine. Two coumarin subfactions were isolated from the remaining aqueous layer and identified as scopoletin and scopolin. All the selected plant extracts were effective in inhibiting growth, sporulation, conidial germination as well as cellulase and pectinase enzymes of *Drechslera biseptata* and *Fusarium solani*, except the remaining aquaeous layer fraction of ethanolic extract prepared from *D. innoxia* which exhibited stimulatory effect on spore production and nonsignificant effect on conidial germination as well as cellulase and pectinase enzymes of *D. biseptata*. Mostly *F. solani* was more susceptible to plant extracts than *D. biseptata*. It was evident that the antifungal activity of the plant extracts depended upon type of plant, solvent, extract concentration as well as fungal type and its phase of growth.

***Key words*: Antifungal activity, successive plant extracts.

Introduction

Root rot is one of the most serious plant diseases, causing large yield losses in a number of crops. To avoid the implication of yield losses due to plant disease, a variety of control methods are used. The use of chemical compounds toxic to fungi is the most commonly known method for controlling fungal diseases in field and greenhouse [19]. Although this method has been very effective in controlling plant fungal diseases, some major problems, including environmental pollution, phytotoxicity and the production of resistant pathogen populations, threaten to limit its use. In recent years, interests have been generated in the development of safer antifungal agents such as plant-based essential oils and extracts to control phytopathogens [18].

Plants are rich in a wide variety of secondary metabolites which have been found *in vitro* to have antimicrobial properties [15]. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties [23, 30]. Alam *et al.*[8] tested five plant extracts against conidial germination of *Fusarium oxysporum* and reported that the extract of *Calotropis procera* showed high inhibitory effect. Various plant extracts, e.g. *Azadirachta indica*, *Datura metel, Ocimum sanctum* and *Parthenium hysterophorus,* have been examined for their antifungal activity with the objective of exploring environmentally safe alternatives of plant disease control [31]. The methanol extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Ithania somnifera* and *Ziziphus mauritian* showed antimicrobial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, Xanthomonas axonopodis* pv. *malvacearum, Aspergillus flavus*, *Drechslera turcica* and *Fusarium verticillioides* [27].

Plant metabolites and plant-based pesticides appear to be better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides [41]. The first step towards this goal is the *in vitro* antimicrobial activity assay. This led the authors to screen *in vitro* the antifungal activity of *A. maurorum*, *C. procera* and *D. innoxia* against root rot pathogens *Drechslera biseptata* and *Fusarium solani*.

Materials and Methods

Plant materials: The plants used in this study were *Alhagi maurorum* medik., *Calotropis procera* (Ait). Ait. f. and *Datura innoxia* Mill. The tested plants were collected in February 2004 from Riyadh region, Kingdom of Saudi Arabia. The collected plants were identified and authenticated by Dr. Nagat Bokhary, Department of Botany and Microbiology, Faculty of Science, King Saud University.

Phytochemical screening:Phytochemical screening of plant extracts was carried out using the methods of Rizk [35] to test for the presence of carbohydrates and/or glycosides, anthraquinones, steroids and/or triterpenes, coumarin, saponins and alkaloids. The method of Geissman [20] was used to test the

presence of flavonoids and the method of Stahl and Schild [38] to test the presence of tannins.

Source and maintenance of fungi: Virulent root rot isolates of *Drechslera biseptata* (Sacc. & Roum) Richardson & Fraser and *Fusarium solani* (Mart.) App. and Wr. were obtained from King Saud and King Abdel Aziz Universities, respectively, Faculty of Science, Botany and Microbiology Department. Both isolates were maintained on potato dextrose agar (PDA) medium.

Extraction and fractionation of plant material: Thoroughly washed mature aerial parts of the tested plants were shade dried and ground by using a blender. A known weight of the dried material of each plant was crushed and successively extracted by maceration with 95% ethanol at room temperature until ethanol became colorless. The ethanolic extract was separated by suction, filtered through a Büchner funnel. Solvent in combined filtrates was evaporated under reduced pressure at 60°C until the extract was dried. Dry fractions were stored at 4ºC until use.

The ethanolic extract of the selected plants was divided into 3 portions: one was taken for phytochemical screening, second for successive extraction by petroleum ether followed by chloroform (for *D. innoxia* only) and the rest was suspended in Tween–80 for antifungal activity. Petroleum ether, chloroform and the remaining aqueous layer fractions obtained from ethanolic extract of *D. innoxia* were dried under reduced pressure at 60°C. The antifungal activity of each fraction was measured.

Column chromatography: The compounds in each fraction of *D. innoxia* were isolated and purified by using column chromatography and preparative TLC. The petroleum ether extract was fractionated on silica gel column. Elution was carried out with petroleum ether, benzene and chloroform, in increasing polarity. The fractions were collected and pooled according to their similarity. The compounds were identified by using the UV, FT-IR, ^{1}H-NMR, MS and FAB-MS spectra[7]. Chloroform extract was fractionated, isolated and identified as in petroleum ether extract, except elution was carried out by using benzene, chloroform and methanol, in increasing polarity. The active constituents in the remaining aqueous layer were isolated and purified by preparative TLC, and identified as carried out in petroleum ether extract.

Determination of antifungal activity: The selected extract was diluted in water by the aid of Tween 80, and sterilized by passing through 0.45 µm bacterial filter. Known volumes of the diluted extract was added to PDA medium to give the following concentrations: 0.1, 1, 10 and 20%. After pouring the medium into the Petri dishes the plates were inoculated with 10 mm disc cut from the margin of actively growing mycelium of the pathogen and incubated at 25°C. The colony diameter was measured and the number of spores produced was counted by a haemacytometer after 7 days [17]. Germination of conidia was studied employing microscope slides covered with droplets of conidial suspension and plant extract [17]. The plates containing the slides were incubated at 25°C until the germination percentage reached about 50% in the control and fix this time for the other treatment.

Production of cellulose and pectin degrading enzymes: Surface disinfected seeds of *Cucurbita maxima* Duchense (winter squash) were sown in pots containing autoclaved vermiculite. The pots were placed in the greenhouse and watered daily. Cell walls from fresh tissues of 10-day-old hypocotyls were used as a carbon source for growth and enzyme induction by *D. biseptata* and *F. solani* [32]. Conical flasks (100 mL) each containing 30 mL of the medium described by Bateman *et al.*[11] were amended with 0.5 g of the dried cell wall as a carbon source. Cell wall-amended medium was autoclaved, cooled, and supplemented with the selected plant extract to produce the following concentrations: 1, 10 and 20%. A 6 mm diameter agar disk bearing hyphae of either *D. biseptata* or *F. solani* cut from 7-days-old colonies was transferred to each flask and incubated at 25ºC for 9 days. At the end of incubation period growth culture was filtered and centrifuged at 15,000× g for 20 min at -5ºC. The supernatant was used as crude enzyme source. Cellulolytic activity of both pathogens was assayed by incubating 0.5 mL of crude enzyme with 4.5 mL of 0.1% (w/v) carboxy methyl cellulose in 0.1 M potassium phosphate buffer (pH 7) at 30ºC for one hour. Reaction was stopped by boiling for 10 min. The increase in liberated reducing groups was measured photometrically by the method described by Miller [29]. Enzyme unit = amount of enzyme which produce one mg of glucose in one hour under condition of the experiment.

Pectate lyase was assayed by incubating reaction mixtures containing 0.5 mL of 2% (w/v) citrus pectin, 1.5 mL of 0.05 M Tris-HCl buffer (pH 8), 0.1 mL of 0.02 M $CaCl_2$ and 0.5 mL of cell wall degrading enzyme source at 30ºC for one hour. The reaction was stopped by boiling the mixture for 5 min, after cooling enzyme activity was measured photometrically as the increase in absorbance at 235 nm. One unit of pectate lyase was defined as an increase of one absorbance unit in one hour at 30ºC [26].

Statistical analysis: All values given in this work are means for 3-5 replicates; the experimental data was subjected to analysis of variance and the significance determined using LSD at level 1 and 5% [39].

Results

The preliminary phytochemical screenings of the ethanolic extracts of *A. maurorum*, *C. procera* and *D. innoxia* revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, steroids and/or triterpenes. Anthraquinones were detected in *A. maurorum* and *C. procera* whereas alkaloids were detected in *D. innoxia* only (Table 1). The crude ethanolic extract of *D. innoxia* partitioned with petroleum ether and chloroform, resulted in the fractions: petroleum ether, chloroform and the remaining aqueous layer. Four triterpenes subfractions (D1-D4) were isolated from petroleum ether fraction:

Compound D_1: melting point 191-194°C; IR (KBr) ν_{max} 3292 and 1035 cm^{-1} (OH group) and 2945, 2850, 1460 and 1385 cm^{-1} (aliphatic methylene and methyl groups). ^{1}H-NMR methyl signals at 0.98, 0.79, 0.92, 0.96, 1.12, 0.82 and 0.86 and olefinic proton resonating at δ 5.17 (^{1}H, t, J = 4 Hz, H-12); MS m/z (rel. int %) 218 (100), 203, 207 and 189 (pentacyclic triterpene amyrin). According to Ahmed and Rahman[7], this compound was identified as β-amyrin.

Compound D_2: melting point 260-264°C; IR (KBr) ν_{max} 3500 (OH) and 818 cm^{-1} ^{1}H-NMR(CDCl3, 100MHz) δ 5.2 (^{1}H, t, J=2.8 Hz, H-12), 4.50 (^{1}H, br s), 3.10 (^{1}H, t, J =7.0 Hz, H-3), 1.3, 1.22, 1.2, 1.1 and 1.04 (each 3H, s, H-25, 26, 24, 27 and 23), 0.84 (6H, s, H-29, 30) and 0.81 (3H, s, H-28); MS m/z (rel int %) 442, 427, 409, 218(100) 203.

Table 1. Phytochemical screening of ethanol extract prepared from the selected plants and fractions prepared from *Datura innoxia.*

	A. maurorum	*C. procera*	*D. innoxia*	Petroleum ether	Chloroform	Aqueous layer
	Ethyl alcohol					
Crystalline sublimate	-	-	-	-	-	-
Volatile substances	-	-	-	-	-	-
CHO &/or glycosides	+	+	+	-	-	+
Tannins	+	+	+	-	-	+
Flavonoids	+	+	+	-	-	+
Saponins	-	-	-	-	-	-
Alkaloids	-	-	+		+	+
Steroids &/or triterpens	+	+	+	+	-	-
Anthraquinones	+	+	-	-	-	-

+ Present; - Absent.

According to Ahmed and Rahman[7], this compound was identified as daturadiol.

Compound D_3: m.p. 230-235°C; IR (KBr) ν_{max} 3420, 1720 and 1260 cm^{-1}. ^{1}H-NMR (CDCl3, 100 MHz) δ 5.2 (^{1}H, t, J = 3Hz, H-12), 4.40 (^{1}H, br s, H-6) , 2.0 (3H, s,COCH3). MS m/z (rel int%) 484 , 218 (100), 203 and 189. According to Ahmed and Rahman[7], this compound was identified as daturadiol-3-acetate.

Compound D_4: m.p. 277-279 °C; IR(KBr) ν_{max} 3480 and 1690 cm^{-1}.^{1}H-NMR (CDCl3 100 MHz) δ 5.19 (^{1}H, s, H-12) , 4.40 (^{1}H, br s,H-6) , 0.88 (6H, s, H-29, 30) MS m/z (rel int %): 440 and 218 (100). According to Ahmed and Rahman[7], this compound was identified as daturaolone.

Chloroform fraction contains alkaloids which were fractionated into 3 subfractions (D5-D7):

Compound D_5 was identified by determination of melting point (115-118°C), mixed melting point and cochromatography with authentic sample. According to Stahl and Schild [38], this compound was identified as atropine.

Compound D_6 was identified by determination of melting point (57-59°C), mixed melting point and cochromatography with authentic sample. According to Stahl and Schild [38], this compound was identified as hyoscine.

Compound D_7 was identified by determination of melting point (106-108°C), mixed melting point and cochromatography with authentic sample. According to Stahl and Schild [38], this compound was identified as hyoscyamine.

Two coumarin subfactions were isolated from the remaining aqueous layer (D8-D9):

Compound D_8: m.p.213-214ºC; in the ^{1}H-NMR it gave a duplicate at 6.26 and 7.95, J= 16 Hz for position 3 and 4, respectively [41].^{13}C-NMR was in accordance with Al-Jaber[9]. This compound was identified as scopolitin

Compound D_9: m.p.213-214°C; ^{1}H-NMR (400MHz), DMSO-d6 δ 7.97 (^{1}H, d, J = 9.5Hz , H-4), 7.29 (^{1}H, S, H-5) , 7.15 (1H, S, H-8) , 6.32(^{1}H , d, J = 9.5Hz , H-3), 5.35 (^{1}H , d, J = 4.2Hz, H-1') , 3.82 (3H, S, OCH3, 3.1 S-3.73(sugar H)13C-NMR (100 MHz , DMSO-d6) δ 160.6 (C-2), 149.7 (C-7) ,148.8 (C-9) , 145.9 (C-6) , 144.2 , (C-4) , 113.2 (C-3) , 112.3 (C10), 109.7 (C-5), 103.0 (C-8) , 99.7(C-1') , 77(C-5') , 76.5 (C-3'), 73.1 (C-2') , 69.5 (C-4') , 60.7 (C-6') and 56.1 (OCH3). According to Ahmed and Rahman[7], this compound was identified as scopolin.

Antifungal activity: Crude ethanolic extract of *A. maurorum*, *C. procera* and *D. innoxia* inhibited significantly growth and sporulation of *D. biseptata* and *F. solani* (Table 2). The inhibition was directly correlated to the extract concentration. However, the lower concentration of *A. maurorum* (0.1%) stimulated significantly sporulation of *F. solani*.

D. innoxia ethanolic extract was successively extracted by petroleum ether and chloroform. The fractions of *D. innoxia* ethanolic extract also were effective in controlling fungal growth and sporulation of both pathogens, particularly *F. solani,* which failed completely to sporulate at 20% of petroleum ether and the remaining aqueous layer fractions (Table 3). However, the remaining aqueous layer fraction stimulated significantly spore production by *D. biseptata.*

Crude ethanolic extract, petroleum ether and chloroform fractions of *D. innoxia* inhibited significantly spore germination (Table 4) as well as the two cell wall degrading enzymes cellulase and pectinase (Table 5) of both pathogens. The tables also showed that the remaining aqueous layer fraction exhibited nonsignificant effect on conidial germination and enzyme activity of *D. biseptata* whereas*, F. solani* was susceptible to this fraction.

Petroleum ether fraction was the most effective among ethyl alcohol fractions in retarding growth and sporulation (Table 3) whereas chloroform fraction was the most effective in inhibiting germination and enzyme activity (Tables 4 and 5) of both pathogens. The results of this work revealed that crude ethanol extract prepared from *D. innoxia* was more potent in controlling *D. biseptata* and *F. solani* than its fractions of petroleum ether, chloroform or the remaining aqueous layer.

Table 2. Growth (mm) and sporulation (number of spores×10^4) of *Drechslera biseptata* and *Fusarium solani* after incubation for 7 days at 25°C on PDA medium supplemented with various concentrations of ethanol extract prepared from the selected plants.

	D. biseptata						*F. solani*					
	Growth			Sporulation			Growth			Sporulation		
	A. maurorum	***C.** procera*	*D. innoxia*	*A. maurorum*	***C.** procera*	*D. innoxia*	*A. maurorum*	***C.** procera*	*D. innoxia*	*A. maurorum*	***C.** procera*	*D. innoxia*
0.0	85.0	85.0	85.0	18.3	18.3	18.3	85.0	85.0	85.0	45.3	45.3	45.3
0.1	81.3	83.5	82.5	18.0	15.0	10.7	84.5	81.3	82.0	59.7	13.3	21.0
1.0	82.0	80.3	80.0	16.3	13.0	9.30	84.2	83.3	80.0	40.7	4.70	2.70
10.0	79.0	80.0	63.0	5.00	13.3	9.00	83.0	81.0	65.8	13.3	2.70	1.00
20.0	77.3	77.5	43.0	4.30	10.3	1.30	77.0	67.0	35.0	1.30	0.00	0.00
LSD												
1%	2.71	4.71	2.46	4.09	3.22	6.21	3.16	5.89	2.98	17.15	1.75	3.19
5%	1.98	3.43	1.79	2.98	2.35	4.53	2.31	4.30	2.17	12.52	1.27	2.33

Table 3. Growth (mm) and sporulation (number of spores×10^4) of *Drechslera biseptata* and *Fusarium solani* after incubation for 7 days at 25°C on PDA medium supplemented with various concentrations of petroleum ether, chloroform or aqueous layer fraction prepared from ethanolic extract of *Datura innoxia*.

	D. biseptata						*F. solani*					
	Growth			Sporulation			Growth			Sporulation		
	Pet ether	Chl	Aq layer	Pet ether	Chl	Aq layer	Pet ether	Chl	Aq layer	Pet ether	Chl	Aq layer
0.0	85.0	85.0	85.0	18.3	18.3	18.3	85.0	85.0	85.0	45.3	45.3	45.3
0.1	81.0	84.5	85.0	15.7	16.3	36.9	85.0	85.0	85.0	14.9	43.4	38.3
1.0	77.0	84.5	80.0	15.0	16.0	32.9	82.0	84.3	85.0	12.9	23.9	34.5
10.0	51.5	72.0	76.0	11.0	11.3	23.2	74.0	79.3	70.5	1.90	23.9	10.0
20.0	46.5	64.0	75.0	10.0	10.3	20.0	69.3	71.3	63.3	0.00	19.4	0.00
LSD												
1%	6.52	7.29	5.65	2.56	1.82	3.71	1.79	4.33	4.22	3.57	3.31	3.45
5%	4.82	5.40	4.18	1.87	1.32	2.71	1.33	3.20	3.12	2.60	2.42	2.51

Pet ether: Petroleum ether; Chl: Chlorofrm; Aq layer: Aqueous layer.

Table 4. Percentage germination (%) of conidia of *Drechslera biseptata* and *Fusarium solani* in presence of various concentrations of ethanol extract prepared from *Datura innoxia* and its fractions petroleum ether, chloroform or aqueous remaining layer.

	D. biseptata				*F. solani*			
	EtOH	Pet ether	Chl	Aq layer	EtOH	Pet ether	Chl	Aq layer
0.0	50.2	50.2	50.2	50.2	50.0	50.0	50.0	50.0
0.1	36.6	50.0	40.6	50.0	41.5	47.7	50.0	50.0
1.0	35.0	44.4	39.0	49.3	42.0	45.5	40.1	49.8
10.0	33.4	43.2	37.4	49.2	18.2	40.7	34.5	49.7
20.0	22.8	41.8	34.0	49.2	10.3	40.2	31.5	35.7
LSD								
1%	1.99	5.30	1.45	1.48	5.33	5.99	5.38	3.22
5%	1.45	4.01	1.06	1.08	3.89	4.37	3.92	2.35

EtOH: Ethanol; Pet ether: Petroleum ether; Chl: Chlorofrm; Aq layer: Aqueous layer.

Table 5. Effect of different concentrations of ethanol extract prepared from *Datura innoxia* and its fractions petroleum ether, chloroform or aqueous remaining layer on cellulase and pectinase enzymes secreted by *Drechslera biseptata* and *Fusarium solani*.

	D. biseptata								*F. solani*							
	Cellulase				Pectinase				Cellulase				Pectinase			
	EtOH	Pet ether	Chl	Aq layer	EtOH	Pet ether	Chl	Aq layer	EtOH	Pet ether	Chl	Aq layer	EtOH	Pet ether	Chl	Aq layer
0.0	0.16	0.16	0.16	0.16	35.6	35.6	35.6	35.6	0.13	0.13	0.13	0.13	28.1	28.1	28.1	28.1
1.0	0.09	0.14	0.11	0.16	25.0	31.2	30.6	35.5	0.06	0.08	0.07	0.08	12.7	19.7	15.5	19.3
10.0	0.07	0.10	0.09	0.16	18.9	27.6	25.0	35.3	0.05	0.06	0.05	0.07	11.9	15.1	14.7	14.9
20.0	0.03	0.05	0.05	0.16	12.5	20.9	18.1	34.9	0.02	0.04	0.02	0.03	9.80	11.8	11.4	13.6
LSD																
1%	0.08	0.04	0.03	0.03	0.87	1.76	2.04	1.34	0.02	0.01	0.012	0.01	0.88	0.70	1.26	3.03
5%	0.06	0.03	0.02	0.02	0.63	1.27	1.47	0.96	0.01	0.007	0.009	0.007	0.63	0.50	0.91	2.18

EtOH: Ethanol; Pet ether: Petroleum ether; Chl: Chlorofrm; Aq layer: Aqueous layer.

Discussion

The presence of carbohydrates and/or glycosides, tannins, flavonoids, steroids and/or triterpenes in the selected plants, in addition to antraquinones in *A. maurorum* and *C. procera* and alkaloids in *D. innoxia,* may explain the antifungal activity of the plant extracts against *D. biseptata* and *F. solani*. Alkaloids[12] flavonoids [28], sterols, coumarins, phenols and tannins [4, 34], steroids[40] and anthraquinones [6] are known to possess potent antifungal activity.

In our investigation ethanolic extracts of the selected plants, in addition to petroleum ether, chloroform and the remaining aqueous layer fractions of *D. innoxia,* retarded significantly growth parameters as well as cellulase and pectinase activity of *F. solani*. Sharma and Trivedi [36] concluded that *Datura stramonium* and *Calotropis procera* had antifungal activity against *Fusarium oxysporum* f. sp. *cumini.* Chitra *et al.* [13] found that extracts prepared from *D. innoxia* inhibited pectinase and cellulase enzymes produced by the phytopathogenic fungus *Colletotrichum capsici*. The results of this work indicated that the pattern of inhibition depended largely upon type of the plant [10], solvent used for extraction and extract concentration [2], tested organism and phase of growth [25]. Crude ethanolic extract prepared from *D. innoxia* was shown to have better efficacy against the tested pathogens than other extracts prepared from *A. maurorum* or *C. procera*. The variation in antifungal activity of the selected plants may be attributed to the difference in chemical nature and concentration of the active constituents of each plant [5]. At the same time, the different types of *D. innoxia* extracts exhibited a variation in activity and degrees of inhibition.This may be attributed to the variation in the active constituents of each extract of *D. innoxia*. It is well known that each solvent has the capacity to extract certain type of chemicals that result in variable activity of each extract of the same plant [22]. Fractions of petroleum ether, chloroform and the remaining aqueous layer in this investigation contain triterpenes (β-amyrin, daturadiol, daturadiol-3-acetate and daturaolone), tropane alkaloids (atropine, hyoscine and hyoscyamine) and coumarins (scopolitin and scopolin), respectively. Cristani *et al.*[14] reported that the antimicrobial activity of terpenes is related to their ability to affect not only permeability but also other functions of cell membranes. These compounds might cross the cell membrane, thus penetrating into the interior of the cell and interact with critical intracellular sites. Kocòr *et al*.[24] isolated two pentacyclic triterpenes (daturadiol and daturaolone) from *D. innoxia* Mill. which is a known source of tropane alkaloids. Tropane alkaloids, isolated from *Datura stramonium*[21], and hyoscyamine and scopolamine[1] have high inhibitory effect aginst fungi. These results are in harmony with our findings.

Mostly, *D. biseptata* was resistant to all plant extracts more than *F. solani*. In previous study, *D. biseptata* was tolerant to antifungal agents more than *Fusarium moniliforme* [3]. Moreover, the remaining aqueous layer fraction in the current study exhibited inhibitory effect on growth, stimulatory effect on sporulation and nonsignificant effect on conidial germination and cell wall degrading enzymes of *D. biseptata*, whereas its effect was inhibitory in case of *F. solani.* These findings are in line with those of Ng *et al*.[33] who concluded that coumarins have no antimicrobial effect at 25 mg/L, meanwhile Shukla *et al.*[37] reported that scopolitin was effective against *A. alternate* and inhibited conidial germination of some fungi. These findings support our results which concluded that the remaining aqueous layer fraction which contains two types of coumarins had different activities according to type of organism and phase of growth. In addition, Dixit and Tripathi [16]observed stimulatory effect on *Fusarium nivale* and *Cephalosporium* sp. caused by *Brassica juncea* and *B. pekinensis*.

Although crude ethanolic extract prepared from *D. innoxia* and its fractions showed different levels of activity against growth, sporulation, germination and cell wall degrading enzymes of the tested pathogens in this study, the former extract exhibited stronger antifungal activities. Synergism due to the presence of larger number of biologically active compounds in the crude ethanolic extract than in its fractions may increase its antifungal activity and explain our results. Abdel-Motaal *et al*.[1] concluded that the combination of hyoscyamine and scopolamine showed higher antifungal activity than hyoscyamine alone.

Conclusions

This study demonstrates that extracts of the selected plants exhibited an effective antifungal potential against both root rot pathogens, *D. biseptata* and *F. solani*, which offers the possibility of developing strategies for controlling plant pathogens with natural extracts or bioactive metabolites of medicinal plants.

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