Herbicidal and insecticidal activity of secondary metabolites from endophytic and soil fungi

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Running title: Bioactivity of fungal secondary metabolites

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ABSTRACT

Fungi have been shown to be a good source for lead molecules in drug discovery and development. However, many compounds derived from fungi were not evaluated for their bioactivity against economic agricultural and public health pests. Twelve fungal secondary metabolites (1-12) were evaluated for herbicidal activity against *Silybum marianum* and insecticidal activity against *Culex pipiens* larvae. Among the tested metabolites, brefeldin A (6) and 6-eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one (11) showed potent herbicidal activity against *S. marianum* with complete inhibition of seed germination at 500 mg L^{-1}. Compound 6 revealed an exceptional herbicidal activity as it caused complete inhibition of root growth and strong reduction in shoot growth (I = 74.5%) and germination (10.0%) at 25 mg L^{-1}. In addition, dehydroaustin (9), phomaxanthone A (4) and deacetylphomaxanthone A (5) displayed a potent toxicity against fourth larval instar of *C. pipiens* with LC_{50} values of 3.27, 57.03 and 63.50 mg L^{-1}, respectively. Based on the results of this study, compounds 4-6, 9 and 11 should be developed as natural pesticides.

Keywords: Natural products, Fungal metabolites, Bioactivity, *Silybum marianum*, *Culex pipiens*.

INTRODUCTION

Fungi are a rich source of diverse natural compounds that have the potential for the discovery of new drugs and agrochemicals (Porras-Alfaro *et al.* 2011). It has been estimated that only 5% (around 70,000) of fungi total populations (1.5 million) have been explored (Sharma *et al.*, 2016). This confirms fungi as possible alternatives to plants for producing valuable natural products.

Although the soil fungi are well-known as a source for important antibiotics and drugs (Wasser, 2002; Sethi *et al.*, 2013) much effort has been devoted in last two decades to study the secondary metabolites form marine and endophytic fungi as new sources for drugs and agrochemicals (Butler *et al.*, 2014; Segaran *et al.*, 2019).

Interest in microbial metabolites as pesticides arises to overcome the problems associated with the continuous use of synthetic pesticides, such as adverse effects of pesticide residues on human, non-target organisms and environment, and emergence of pest resistance (Aktar *et al.*, 2009). In fact **the** microbial metabolites have several advantages to be good candidates in pest control, such as structure diversity, wide-spectrum of bioactivity, new modes of action and biodegradability (Gerwick and Sparks, 2014). Several pesticides of microbial origin have been introduced into field of pest management include: avermectin, bialaphos, milbemycin, kasugamycin, blasticidin S, polyoxin, mildiomycin, validamycin and tetranactin (Tanaka and Omura, 1993). Therefore, it is highly important to investigate new fungal metabolites and examine their bioactivities against economic pests.

Silybum marianum (L.) Gaertn. (Asteraceae) is a dangerous weed in many parts of Middle East, Africa, Australia, and North and South America (Holm *et al.*, 1997). In Egypt, it is considered as an invasive weed that spread through wheat fields in both new reclaimed lands and desert areas. *Culex pipiens* L. (Diptera: Culicidae) is the most widespread mosquito species in Egypt (Zahran *et al.*, 2017; Abdelgaleil *et al.*, 2017). This insect is able to transmit several pathogens, such as West Nile virus, *Bancroftian flariasis* and Rift Valley Fever virus (Meegan *et al.*, 1980).

In our continuous efforts to search for new natural products with possible application in pest management, twelve fungal secondary metabolites (**1**-**12**) were examined for their effect on the germination and growth of roots and shoots of *S. marianum*. In addition, the toxicity of these compounds was tested against the fourth larval stage of *C. pipiens* to explore their potential for the control of these economic pests.

MATERIALS AND METHODS

Test organisms

Field biotype seeds of Holy thistle, *Silybum marianum* (L.) Gaertn. (Asteraceae), were collected from Faculty of Agriculture Farm, Alexandria (31° 12' 56.30" N, 29° 57' 18.97" E). The plant seeds were identified by Prof. FathAllah Zaitoon of the Plant Pathology Department, Faculty of Agriculture, Alexandria University. Voucher specimen (SM-1909) was deposited in Department

of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University. Uniform and undamaged seeds were used for the germination and seedling growth tests. Seeds were examined for their germination before experiments. The seed germination was 80% after 12 days of sowing. *Culex pipiens* L. (Diptera: Culicidae) was reared in an insectary at 27±2 °C and 75±5% **relative humidity (R.H.)** at the Department of Applied Entomology and Zoology, Alexandria University, Egypt as described by Zahran *et al.* (2017).

Fungal strains and isolation of secondary metabolites

Strains of different endophytic and soil fungi were isolated from different sources (Table 1), identified by BEX Co. Ltd., Japan, using a DNA analysis of the 18S rDNA regions and had been deposited in the laboratory of natural products at the Faculty of Agriculture of Yamagata University. After fermentation on unpolished rice media (unpolished rice 1000 g + 35 ml of 3.5% NaCl at 25ºC for four weeks), the fungal growth media were extracted with MeOH. The MeOH extracts were concentrated and partitioned into *n*-hexane and EtOAc. The EtOAc extracts **were subjected to silica gel column chromatography with mixtures** of *n*-hexane–EtOAc (100:0 – 0:100, v/v), and mixtures of EtOAc-MeOH (50:50 – 0:100, v/v). The rustling fractions were further chromatographed on ODS by eluting with H_2O and an increasing ratio of MeOH (100:0 to 0:100) or silica gel by eluting with a mixture of $CHCl_3$-MeOH (90:10, v/v). For HPLC analysis, a reversed phase HPLC (Semipreparative HPLC with Shimadzu pump and UV LC-10A detector (set at 210 nm) on a Mitysil ODS column (150 × 6.0 mm i.d.) at a flow rate of 2.0 mL/min, solvent systems, MeOH:H_2O, 60:40 – 80:20, v/v) and PTLC (EtOAc – *n*-hexane, 70:30 – 30:70, v/v) were used. The purity of isolated compounds was higher than 95%.

Germination and seedling growth inhibition bioassay

The effect of fungal secondary metabolites (**1**-**12**) on seed germination, and root and shoot growth of *S. marianum* was evaluated using a bioassay method explained by Abdelgaleil *et al.* (2009). The tested compounds were dissolved first in DMSO and diluted with distilled water containing 0.02% of Triton-X 100 to give final concentration of 500 mg L^{-1}. Three replicates with 10 seeds in each one were prepared for each compound. The compound solutions (6 ml) were transferred to each Petri dish (9 cm) lined with filter paper (Whatman No. 2). Petri dishes were then placed in polyethylene bags which were expanded and closed to prevent moisture loss. In the control treatment, a solution of 0.5% of DMSO and 0.02% of Triton-X 100 in distilled water was added.

All treatments were kept at 20±2 ºC and a 12-h photoperiod for 12 days. Then, the number of germinated seeds and length of root and shoot were taken. Growth inhibition (I %) of root and shoot lengths was calculated from this equation:

$$I\ (\%) = [1 - T/C] \times 100$$

Where, T and C are the root or shoot lengths (cm) in treatment and control. Moreover, two compounds (**6** and **11**) were further evaluated on germination and seeding growth at a series of concentrations of 25, 50, 100, 250 and 500 mg L^{-1}.

Larvicidal bioassay

The toxicity of fungal secondary metabolites (**1-12**) was carried out against *C. pipiens* larvae following a recommended method of the World Health Organization (WHO, 1996) with slight modifications. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water containing 0.1% of Tween-20 to give three final concentrations (100, 250 and 500 mg L^{-1}). Water in control treatments was mixed with DMSO (0.5%) and Tween-20 (0.1%). Twenty *C. pipiens* larvae were separately put into 200-ml plastic cups containing 100 ml of the compound solutions. Each concentration was replicated three times. All treatments were kept under the same insect rearing conditions for 24 h. Then, the number of dead larvae was counted and mortality (%) was calculated. Three compounds (**4**, **5** and **9**) were further tested at concentrations of 10, 25, 50, 70 and 100 mg L^{-1}. Mortality data of these compounds were subjected to probit analysis to calculate LC_{50} values for these compounds (Finney, 1971).

Statistical analysis

Significant differences among mean values of germination percentages, root and shoot lengths were determined (P = 0.05) by using a one-way analysis of variance (ANOVA) followed by Tukey's HSD test. The LC_{50} values of compounds were estimated by probit analysis using the SPSS 21.0 software (Statistical Package of Social Sciences Inc., USA).

RESULTS

Isolation, structure elucidation of isolated secondary metabolites

Twelve secondary metabolites (**1-12**) have been isolated from different fungal strains (Table 1) using different chromatographic techniques, including silica gel and ODS columns, PTLC and HPLC. Among the isolated metabolites, nine compounds (**1, 2, 4**-**7** and **10**-**12**) were isolated from

endophytic fungi and three compounds (**3**, **8**, and **9**) were isolated from soil fungi. The chemical structure (Fig. 1) of the isolated compounds was elucidated on the basis of their spectroscopic data of ultraviolet (UV), infrared (IR), high resolution mass spectroscopy (HRMS) and nuclear magnetic resonance (NMR). The isolated compounds belong to ten different chemical groups of natural products (Table 1).

Effect of fungal metabolites on germination and seedling growth of *S. marianum*

Effect of fungal secondary metabolites (**1-4** and **6-12**) on germination, root and shoot growth of *S. marianum* at concentration of 500 mg L^{-1} is shown in Table 2. Among the **11** tested metabolites, compounds **3**, **4**, **6**, **8**, **9**, **10** and **11** caused reduction in seed germination after 12 days of treatment. Compounds **6** and **11** induced complete inhibition of seed germination at this concentration. In addition, seven compounds (**2**, **3**, **4**, **6**, **8**, **11** and **12**) revealed significant inhibition in root growth compared with control treatment. **Similarly,** compounds **6** and **11** which showed complete inhibition of germination, compounds **3**, **4** and **12** strongly inhibited root growth with 87.5, 80.4 and 74.8% inhibition, respectively. Conversely, compounds **1** and **10** significantly increased the root growth. Similarly, compounds **3**, **4**, **8** and **12**, **significantly reduced** in shoot growth. The results revealed that the tested compounds caused higher inhibitory effects on **plant establishment by reducing** root and shoot growth than on seed germination. Also, some of the tested compounds had greater inhibitory effects on root growth than on shoot growth.

The previously presented results showed that compounds **6** and **11** caused complete inhibition of seed germination at 500 mg L^{-1}. Therefore, these two compounds were further evaluated at lower concentrations (25, 50, 100, 250 and 500 mg L^{-1}). Compound **6** caused complete inhibition of root growth at all tested concentrations (Table 3). Likewise, it induced complete inhibition of germination and shoot growth at all tested concentrations except 25 mg L^{-1}. This compound showed strong inhibition of germination (10.0%) and shoot growth **(74.5%)** at 25 mg L^{-1}. In addition, compound **11** induced significant reduction in seed germination at all of the tested concentrations. Also, it caused strong inhibition of root and shoot growth at 100 and 250 mg L^{-1}.

Larvicidal activity of fungal metabolites against *C. pipiens*

Toxicity of twelve fungal metabolites against the fourth larval instar of *C. pipiens* at concentrations of 100, 250 and 500 mg L^{-1} is presented in Table 4. Four compounds (**4**, **5**, **8** and **9)** caused complete mortality (100%) of larvae at 500 mg L^{-1}, while three compounds (**4**, **5** and **9)**

caused complete mortality of larvae at 250 mg L^{-1}. At 100 mg L^{-1}, compound **9** was the only compound that caused complete mortality of larvae. Also, compounds **4** and **5** induced strong larval toxicity at this concentration with 95.0 and 90.0 % mortality, respectively. Comparative toxicity results (Table 5) indicated that compound **9** revealed the highest larval toxicity (LC_{50} = 3.27 mg L^{-1}), followed by compound **4** (LC_{50} = 57.03 mg L^{-1}) and compound **5** (LC_{50} = **63.50** mg L^{-1}).

DISCUSSION

New pest management strategies are based on decrease the use of synthetic pesticides and increase the use of natural alternatives. Therefore, introducing new natural products with low risk on human and environment, and new modes of action is highly needed in the field pest management nowadays (Schrader *et al.*, 2010). In this regard, microorganisms are a rich source for natural compounds with successful use in pest management (Singh, and Yadav, 2020; Saad *et al.*, 2021). **Several hundred species of fungi are known to infect and kill pests. The most important groups are the Hyphomycetes within genera like *Beauveria, Metarhizium, Verticillium, Paecilomyces* and *Hirsutella* (Keller, 1998). Moreover, fungi are also known for producing toxic compounds against pests mainly form soil fungi of genera *Aspergillus* and *Penicillium*. Secondary metabolites with insecticidal and antifeedant properties were also found in endophytic and phytopathogenic fungi (Berestetskiy and Hu, 2021).** In this study, the herbicidal and insecticidal activities of twelve secondary metabolites isolated from fungi were investigated to explore their possible use in pest control programs.

The results showed that most of the tested compounds reduced seed germination and seedling growth of *S. marianum* at concentration of 500 mg L^{-1}. Among the eleven tested metabolites, compounds **6** and **11** revealed excellent herbicidal activity with complete inhibition of germination and seedling growth at this concentration. Moreover, compound **6** showed strong herbicidal activity at 25 mg L^{-1}. Based on the obtained results, this compound is among the most effective natural herbicides reported so far. In our previous study, compounds **6** and **11** were also the most potent inhibitors of seed germination, and growth of root and shoot of *Echinochloa crus-galli* at concentration of 2mM (Saad *et al.*, 2021). Also, compound **6** was found to be more effective than compound **11** against *E. crus-galli*. The herbicidal activity of compound **11** was further supported by the studies of Shiono and Murayama (2005) who stated that this compound inhibited the root

194 growth of lettuce by 46% at concentration of 50 mg L^{-1}. In addition, Compound **6** has been
195 described to inhibit wheat germination and the root growth of *Allium cepa* (Betina, 1992)
196 Comparing the inhibitory effects of tested compounds on the seed germination with seedling
197 growth of *S. marianum* indicated that the tested compounds were more effective against seedling
198 growth than germination. These findings are in agreement with previous studies on many natural
199 compounds in which the seedling growth were more sensitive than germination (Leather and
200 Einhellig, 1984; Abdelgaleil and Hashinaga, 2007; Saad *et al.*, 2012). Also, the results of current
201 study revealed that the tested compounds were more potent inhibitors to root than to shoot with
202 few exceptions. The higher sensitivity of roots could be due to the roots are the first to expose and
203 absorb tested compounds from growing media (Turk *et al.*, 2002). Similar observations were
204 reported on the inhibitory effects of other natural compounds on seedling growth (Wang *et al.*,
205 2007; Gouda *et al.*, 2016).
206 The results of larvicidal bioassays against *C. pipiens* demonstrated that among the tested
207 metabolites, compounds **9**, **4** and **5** **showed** a promising larvicidal activity. In particular, compound
208 **9** which displayed the highest toxicity with LC_{50} = 3.27 mg L^{-1}. Based on our results, this
209 compound is among the most toxic natural compounds reported so far against *C. pipiens* or other
210 mosquito species. This finding is supported by previous study of Geris *et al.* (2008) who found that
211 compound **9** was highly toxic against third instar larvae of *Aedes aegypti* with LC_{50} value of 2.9
212 mg L^{-1}. Compound **9** was more toxic to *C. pipiens* larvae than thymol (LC_{50} = 37.95 mg L^{-1}),
213 carvacrol (LC_{50} = 44.38 mg L^{-1}), cinnamaldehyde (LC_{50} = 58.97 mg L^{-1}), eugenol (LC_{50} = 86.22
214 mg L^{-1}) and cuminaldehyde (LC_{50} = 38.94 mg L^{-1}) (Radwan *et al.*, 2008; Zahran and Abdelgaleil,
215 2011). Similarly, compound **9** showed higher toxicity than curcumin (LC_{50} = 19.07 mg L^{-1})
216 isolated from *Curcuma longa* (Sagnou *et al.*, 2012). [44] Moreover, this compound showed similar
217 toxicity to caulerpin (LC_{50} = 1.99 mg L^{-1}) and caulerpinic acid (LC_{50} = 4.89 mg L^{-1}) isolated from
218 *Caulerpa racemosa* (Alarif *et al.*, 2010).
219 The results of the current study indicate that the tested compounds showed high selectivity against
220 the examined pests. For example, compounds **6** and **11** showed promising herbicidal activity
221 against *S. marianum* while had no insecticidal activity. Likewise, compounds **5** and **9** possessed
222 potent insecticidal activity against *C. pipiens* but were not active against the tested weed. This
223 selectively is crucial for the development of these compounds as new bio-pesticides.

In conclusion, this study described, for the first time, the herbicidal and insecticidal activities of twelve fungal metabolites against the economic agricultural pests, *S. marianum* and *C. pipiens*. Among the tested metabolites, brefeldin A (**6**) and 6-eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one (**11**) revealed promising herbicidal activity against *S. marianum*, in particular, brefeldin A (**6**) which could be developed as a new natural herbicide. In addition, dehydroaustin (**9**) exhibited remarkable larvicidal activity against *C. pipiens* indicating its possible use in integrated management programs of mosquitoes.

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Table 1. Names, chemical class and source of fungal secondary metabolites.

Name	Chemical group	Fungus (fungal origin)	Reference
Nodulisporone B (**1**)	Phenylisobenzofuranones	*Nodulisporium* sp. SH-1 (*Xylaria polymorpha*)	Hayasaka *et al.*, 2011
19-(α-D-glucopyranosyloxy)isopimara-7,15-dien-3β-ol (**2**)	Isopimarane diterpenoids	*Paraconiothyrium* sp. MY-42 (unidentified plant)	Shiono *et al.*, 2011
Pencolide (**3**)	Maleimides	*Penicillium sclerotiorum* (Soil)	Lucas *et al.*, 2007
Phomaxanthone A (**4**)	Dimeric xanthones	*Phomopsis* sp. (*Rhizophora mucronata*)	Elsaesser *et al.*, 2005
Deacetylphomaxanthone A (**5**)	Dimeric xanthones	*Phomopsis longicolla* (*Sonneratia caseolaris*)	Ronsberg *et al.*, 2013
Brefeldin A (**6**)	Macrolides	*Penicillium brefeldianum (Pinellia ternata)*	Hutchinson *et al.*, 1983
Anthracobic acid A (**7**)	Polyketides	*Anthracobia* sp. (unidentified plant)	Shiono. 2006
Fasciculol C (**8**)	Lanostane triterpenoids	*Neamatoloma fasciculare* (Mushroom)	Ikeda *et al.*, 1977; Kim *et al.*, 2013
Dehydroaustin (**9**)	Meroterpenoids	*Penicillium brasilianum* (Soil)	Schürmann *et al.*, 2010
Pyrrocidine A (**10**)	Alkaloids	*Acremonium zeae* (*Zea mays*)	Wicklow and Poling, 2009
6-Eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one (**11**)	Cyclohexenones	Xylariaceous endophytic fungus (YUA 026) (unidentified plant)	Shiono *et al.*, 2005
Secalonic acid A (**12**)	Dimeric xanthones	*Claviceps purpurea (Secale cereale*)	Masters and Bräse, 2002

Table. 2 Effect secondary metabolites isolated from endophytic fungi on germination and seedling growth of *Silybum marianum* after 12 days of sowing at 500 mg L^{-1}[a].

Compound	Germination[b] (% ± SE)	Root		Shoot	
		Length (cm) (Mean ± SE)	I[c] (%)	Length (cm) (Mean ± SE)	I (%)
Control	86.7±3.34ab	11.39±0.22bc	0.0	2.16±0.06abc	0.0
1	86.7±3.34ab	14.32±0.14a	-28.2	2.33±0.13ab	-7.87
2	86.7±3.34ab	5.47±0.23d	52.0	1.86±0.24bc	13.9
3	76.7±3.34b	1.42±0.06g	87.5	1.33±0.17d	38.4
4	80.0±5.78b	2.23±0.25f	80.4	1.30±0.10d	39.8
6	0.0±0.0c	0.0±0.0h	100.0	0.0±0.0e	100.0
7	96.71±3.34a	11.93±0.11b	-4.74	2.33±0.25ab	-7.87
8	83.3±3.34ab	5.05±0.42d	55.7	1.35±0.09d	37.5
9	80.0±5.78b	11.12±0.17c	2.37	2.53±0.06a	-17.1
10	83.3±6.67ab	13.82±0.17a	-21.3	1.80±0.26c	16.7
11	0.0±0.0c	0.0±0.0h	100.0	0.0±0.0e	100.0
12	90.0±5.78ab	2.87±0.11e	74.8	1.31±0.16d	39.4
df[d]	11	11		11	
F[e]	63.74	801.70		29.30	
P[f]	<0.00	<0.00		<0.00	

[a] Values are means ± SE of three replicates with 10 seeds in each one.
[b] Values followed by different letters are significantly different at 0.05 probability level.
[c] I = Inhibition.
[d] df = Degrees of freedom.
[e] F = *F*-statistic. [f] P = *P*-value.

Table. 3 Effect of brefeldin A and 6-eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one on germination and seedling growth of *Silybum marianum* after 12 days of sowing at different concentrations[a].

Compound	Conc. (mg L^{-1})	Germination (% ± SE)[b]	Root		Shoot	
			Length (cm) (Mean ± SE)	I[c] (%)	Length (cm) (Mean ± SE)	I[c] (%)
Control	0.0	75.0±2.89a	6.33±0.49a	0.0	1.96±0.21a	0.0
Brefeldin A (**6**)	25	10.0±5.78d	0.0±0.0d	100.0	0.50±0.28c	74.5
	50	0.0±0.0d	0.0±0.0d	100.0	0.0±0.0d	100.0
	100	0.0±0.0d	0.0±0.0d	100.0	0.0±0.0d	100.0
	250	0.0±0.0d	0.0±0.0d	100.0	0.0±0.0d	100.0
	500	0.0±0.0d	0.0±0.0d	100.0	0.0±0.0d	100.0
6-Eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one (**11**)	25	60.0±5.78b	6.27±0.40a	0.95	1.63±0.03a	16.8
	50	56.7±3.33b	4.50±0.17b	28.9	1.87±0.20a	4.59
	100	43.3±3.33c	0.83±0.27c	86.9	1.20±0.17b	38.8
	250	6.7±3.33d	0.02±0.02d	99.7	0.17±0.17cd	91.3
	500	0.0±0.0d	0.0±0.0d	100.0	0.0±0.0d	100.0
df[d]		10	10		10	
F[e]		88.34	139.35		32.99	
P[f]		<0.00	<0.00		<0.00	

[a] Values are means ± SE of three replicates with 10 seeds in each one.
[b] Values followed by different letters are significantly different at 0.05 probability level.
[c] I = Inhibition.
[d] df = Degrees of freedom.
[e] F = *F*-statistic.
[f] P = *P*-value.

Table. 4 Mortality percentages of *Culex pipiens* fourth instar larvae after 24 h of treatment with secondary metabolites isolated from endophytic fungi at different concentrations.

Compound	Mortality (%) after 24h		
	100 mg L^{-1}	250 mg L^{-1}	500 mg L^{-1}
Control	0.0	0.0	0.0
Nodulisporone B (**1**)	0.0	0.0	0.0
19-(α-D-glucopyranosyloxy) isopimara-7,15-dien-3β-ol (**2**)	0.0	-[a]	-
Pencolide (**3**)	0.0	0.0	0.0
Phomaxanthone A (**4**)	95.0	100.0	100.0
Deacetylphomaxanthone A (**5**)	90.0	100.0	100.0
Brefeldin A (**6**)	0.0	0.0	46.7
Anthracobic acid A (**7**)	0.0	0.0	10.0
Fasciculol C (**8**)	0.0	6.25	100.0
Dehydroaustin (**9**)	100	100.0	100.0
Pyrrocidine A (**10**)	0.0	0.0	15.0
6-Eopxy-4-hydroxy-3-methoxy-5-methyl-cyclohex-2-en-1-one (**11**)	0.0	0.0	38.9
Secalonic acid A (**12**)	0.0	0.0	0.0

a= Not tested.

Table 5. Comparative toxicity of fungal metabolites against *Culex pipiens* fourth instar larvae after 24h of exposure.

Compound	LC_{50}[a] (mg L^{-1})	95% confidence limits (mg L^{-1})		Slope ± SE	Intercept ± SE	(χ^2)[b]	*P*[c]
		Lower	Upper				
Phomaxanthone A (**4**)	57.03	52.62	61.39	6.75±0.81	-11.85±1.47	0.001	0.978
Deacetylphomaxanthone A (**5**)	63.50	58.75	68.41	6.50±0.72	-11.73±1.31	0.006	0.941
Dehydroaustin (**9**)	3.27	0.38	6.53	1.11±0.30	-0.57±0.40	0.181	0.671

[a] The concentration causing 50% mortality.
[b] Chi square value.
[c] Probability value.

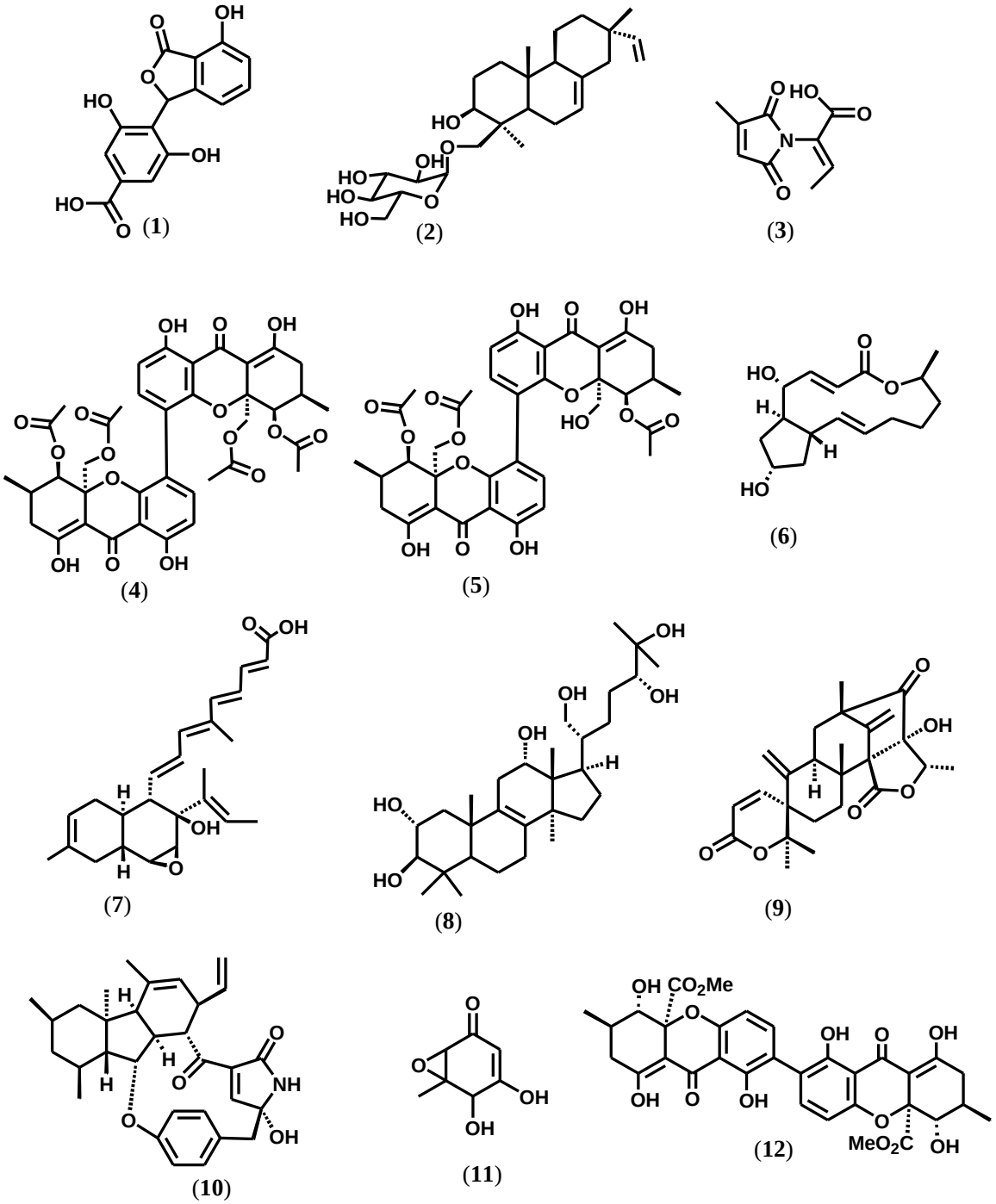

Figure 1. Chemical structure of fungal metabolites (**1-12**).