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Acroindoles A–C, chlorinated indole derivatives from an actinomycete of the genus Acrocarpospora

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Abstract

Acroindoles A–C (**1** – **3**), three new chlorinated indoles, were isolated from the culture extract of a rare actinomycete *Acrocarpospora*. Through the NMR and MS analyses, these compounds were identified to possess a 6-chloroindole nucleus with an *N*-acylated aminoacetyl or a hydroxyacetyl substituent at 3-position. Compounds **1** – **3** inhibited the radicle elongation of lettuce seeds down to 30 ~ 86% of the nontreated seeds at 10 μg ml^{-1}. In addition, **1** was antimicrobial against *Staphylococcus aureus* and *Kocuria rhizophila* with MICs ranging from 25 to 50 μg ml^{-1}.

Introduction

Actinomycetes possess an exceptionally high ability to produce structurally diverse bioactive compounds [1]. As a producer of medicinal and agrochemical products, they have made a substantial contribution to human welfare and economical development [2]. However, extensive studies on actinomycetes over decades led to the frequent reisolation of compounds of known structural classes from major genera such as *Streptomyces*. Approximately 70% of actinomycete metabolites are derived from *Streptomyces*, whereas a number of actinomycete genera are uninvestigated in natural product research [3]. In the last few years, we actually discovered several new classes of bioactive compounds from unexploited genera such as *Allokutzneria*, *Catellatospora*, *Krasilnikova*, and *Pseudosporangium* [4–8].

The genus *Acrocarpospora* was first characterized by Tamura and coworkers in 2000 [9]. This genus belongs to the family *Streptosporangiaceae* and currently contains six species [10]. Cheng and coworkers reported the isolation of azaphilone-type polyketides and abietane-related diterpenoids from an *Acrocarpospora punica* strain [11, 12]. However, secondary metabolites of these classes have been exclusively found from fungi or plants, posing a question about the true origin of these compounds. During our ongoing investigation of new bioactive compounds from underexploited actinomycete genera, we recently found a new tetracenomycin from *Acrocarpospora* sp. RD005083 [13]. In this study, we further explored the secondary metabolites from another *Acrocarpospora* strain coded RD011456 and isolated three chloroindole derivatives, designated as acroindoles A–C (**1** – **3**). We herein describe the isolation, structure determination, and bioactivity of **1** – **3** (Fig. 1).

Results and Discussion

The producing strain *Acrocarpospora* sp. RD011456 was obtained from NBRC (Biological Resource Center, National Institute of Technology and Evaluation) [14]. This strain was cultured in three production medium (A3M, A11M, A16) which are routinely used in our laboratory. In the HPLC analysis of the culture extracts, notable production of secondary metabolites was recognized in the extract from A16 culture. Large-scale culture was then carried out in A16 medium and the whole culture broth was extracted with 1-butanol. After evaporation, the extract was consecutively fractionated using silica gel and ODS column chromatographies, followed by reverse-phase HPLC purification to yield acroindoles A (**1**), B (**2**), and C (**3**).

Compound **1** was obtained as a white amorphous solid. The molecular formula $C_{12}H_{11}ClN_2O_2$ was determined by HR-ESITOFMS analysis that showed a deprotonated molecular ion $[M - H]^-$ at *m/z* 249.0432 (Δ + 0.1 mmu, calculated for $C_{12}H_{10}{}^{35}ClN_2O_2$). The peak pattern of the mass spectrum corroborated the presence of one chlorine atom in **1** (Figure S1). The IR spectrum suggested the presence of NH and/or OH bond(s) (3303 and 3169 cm^{-1}) and carbonyl (1630 cm^{-1}) group(s). Comprehensive analysis of ^1H and ^{13}C NMR and HSQC spectra identified 12 resonances assignable to one each of ketone and acyl carbonyl carbons (δ_C 190.5 and 169.6), four nonprotonated sp^2 carbons (δ_C 136.9; 127.4; 124.2; 114.0), four sp^2 methines (δ_H/δ_C 7.53/111.9; 7.22/122.1; 8.13/122.4; 8.45/134.5), one methylene (δ_H/δ_C 4.44/45.7), and one methyl group (δ_H/δ_C 1.91/22.5) (Table 1).

Mutually coupled sp^2 methines H4 and H5 (*J* = 8.4 Hz) displayed intense three-bond HMBC correlations to C6 and C7a, and to C7 and C3a, respectively, along with two-bond correlations from H4 to C3a and H5 to C6 (Fig. 2). In addition, an sp^2 methine H7 was weakly coupled with H5 (*J* = 1.6 Hz) and was correlated with C3a, C6, and C7a in the HMBC spectrum. These correlation data established a 1,2,4-trisubstituted benzene ring. This ring structure was extended to include two sp^2 carbons C2 and C3 by HMBC correlations from H4 to C3 and H2 to C3, C3a, and C7a, providing an indole skeleton. HMBC correlations from H2 and H9 to a carbonyl carbon C8 (δ_C 190.5) defined the linkage of C8-C9 fragment to C3. Methylene proton H9 was coupled with an exchangeable proton at δ_H 8.18 (t, 5.6 Hz) which was deduced to be an amide proton by HMBC correlations from H9 to a nitrogen atom (N10, δ_N 109.2) and a carbonyl carbon C11 (δ_C169.6). HMBC correlations from a singlet methyl H12 to C11 and N10 evidenced an acetoamide terminus of the chain part connecting at C3. Finally, a chlorine atom was positioned at C6 to complete the structure of **1**.

The molecular formula of **2** was determined to be $C_{13}H_{13}ClN_2O_2$ by HR-ESITOFMS that gave a deprotonated molecular ion $[M - H]^-$ at *m/z* 263.0589 (Δ + 0.2 mmu, calculated for $C_{13}H_{12}{}^{35}ClN_2O_2$), corresponding to one methylene increment from **1**. The 1D and 2D NMR data of **2** were mostly similar to those of **1** (Table 2). The presence of an ethyl group consisting of H13 methyl and H12 methylene was readily identified in a COSY spectrum. HMBC correlations from H12 and H13 to C11 connected this ethyl group at C11 carbonyl carbon in place of a methyl group H12 in **1**.

The HR-ESITOFMS of **3** showed a deprotonated molecular ion $[M - H]^-$ at *m/z* 208.0168, establishing the molecular formula $C_{10}H_8ClNO_2$ (Δ + 0.3 mmu, calculated for $C_{10}H_7{}^{35}ClNO_2$). The presence of a 6-chloroindole skeleton and the substitution of a carbonyl carbon C8 at C3 was apparent from the NMR data (Table 3, Fig. 2). A singlet proton resonance (δ_H/δ_C 4.57/65.4) was assigned to an oxygenated methylene H9 connecting at C8 by its ^1H and ^{13}C chemical shifts and an HMBC correlation from H9 to C8. Compound **3** was thus determined as a 6-chloroindole bearing a hydroxyacetyl group at C3 (Fig. 2).

In an antimicrobial assay, only **1** exhibited weak activity against Gram-positive bacteria (Table 4). All compounds were not cytotoxic against P388 murine leukemia cells at 100 μM. In contrast, **1**–**3** showed

plant growth inhibitory effect in seed germination assay. Radicle elongation of germinated seeds of lettuce (*Lactuca sativa*) was inhibited by these compounds in a concentration-dependent manner at concentrations higher than 0.1 μg ml^{-1} (Fig. 3). Compound **2** showed the strongest inhibitory activity, suppressing the radicle elongation down to 86% at 10 μg ml^{-1}.

In summary, during the evaluation of the biosynthetic potential of the genus *Acrocarpospora*, three new 6-chloroindole derivatives were found from *Acrocarpospora* sp. RD011456 through HPLC/UV-based metabolite analysis. 3-(Aminoacetyl)indole has been obtained from the extract of a sponge [15] and its *N*-acetyl derivative has been isolated from *Streptomyces* and *Cladosporium* [16, 17]. Meanwhile, chlorinated derivatives of these compounds have not been reported previously. Although an *N*-propionyl derivative of 3-(aminoacetyl)indole is known in the field of synthetic chemistry, there have been no reports of it as a natural product. 3-(Hydroxyacetyl)indole has been isolated from various organisms such as *Pseudomonas* and an algae, but this is the first finding of its chlorinated congener [18, 19]. The results obtained in this study support the view that underexplored actinomycete genera, such as the genus *Acrocarpospora*, are promising sources for the discovery of new bioactive compounds.

Table 1
NMR data for **1** in DMSO-d_6

No.	δ_C[a]/δ_N	type	δ_H, mult (*J* in Hz)[b]	HMBC[c]
1	145.3[d]	NH	nd[e]	
2	134.5	CH	8.45, s	1, 3, 4, 9, 10
3	114.0	C		
3a	124.2	C		
4	122.4	CH	8.13, d (8.4)	3, 3a, 6, 7a
5	122.1	CH	7.22, dd (8.4, 1.6)	3a, 6, 7
6	127.4	C		
7	111.9	CH	7.53, d (1.6)	3a, 6, 7a
7a	136.9	C		
8	190.5	C		
9	45.7	CH_2	4.44, d (5.6)	3, 8, 10, 11
10	109.2[d]	NH	8.18, t (5.6)	9, 11
11	169.6	C		
12	22.5	CH_3	1.91, s	10, 11

[a] Recorded at 125 MHz.

[b] Recorded at 500 MHz.

[c] HMBC correlations are from proton(s) stated to the indicated carbon/nitrogen

[d] Determined from the projection data of ^{1}H-^{15}N HMBC spectrum.

[e] Not detected.

Table 2
NMR data for **2** in DMSO-d_6

No.	δ_C[a]/δ_N	type	δ_H, mult (*J* in Hz)[b]	HMBC[c]
1	146.0[d]	NH	nd[e]	
2	134.5	CH	8.45, s	1, 3, 3a, 7a, 8
3	114.0	C		
3a	124.2	C		
4	122.9	CH	8.13, d (8.5)	3, 3a, 6, 7a
5	122.4	CH	7.22, dd (8.5, 1.7)	3a, 7
6	127.9	C		
7	112.0	CH	7.53, d (1.7)	3a, 6, 7a
7a	137.5	C		
8	190.6	C		
9	45.6	CH_2	4.43, d (5.6)	8, 10, 11
10	110.0[d]	NH	8.08, t (5.6)	9, 11
11	173.3	C		
12	28.3	CH_2	2.19, q (7.5)	11, 13
13	9.9	CH_3	1.03, t (7.5)	11, 12

[a] Recorded at 125 MHz.

[b] Recorded at 500 MHz.

[c] HMBC correlations are from proton(s) stated to the indicated carbon/nitrogen

[d] Determined from the projection data of ^{1}H-^{15}N HMBC spectrum.

[e] Not detected.

Table 3
NMR data for **3** in DMSO-d_6

No.	δ_C[a]	type	δ_H, mult (*J* in Hz)[b]	HMBC[c]
2	134.3	CH	8.39, s	3, 3a, 7a, 8
3	113.2	C		
3a	124.2	C		
4	122.5	CH	8.15, d (8.5)	3, 3a, 6, 7a
5	122.1	CH	7.22, dd (8.5, 1.4)	3a, 6, 7
6	127.4	C		
7	111.9	CH	7.54, d (1.4)	3a, 6, 7a
7a	136.8	C		
8	194.6	C		
9	65.4	CH_2	4.57, s	8
9-OH			5.02, brs	

[a] Recorded at 125 MHz.

[b] Recorded at 500 MHz.

[c] HMBC correlations are from proton(s) stated to the indicated carbon.

Table 4
Antimicrobial activity of **1**–**3**

microorganisms	MIC (μg ml^{-1})			
	1	**2**	**3**	**control**
Staphylococcus aureus FDA209P JC-1	25	>100	>100	0.78[a]
Kocuria rhizophila ATCC9341	50	>100	>100	0.78[a]
Escherichia coli NIHJ JC-2	> 100	>100	>100	1.3[a]
Candida albicans NBRC0197	>100	>100	>100	0.78[b]

[a]kanamycin sulfate. [b]amphotericin B

Experimental section

General experimental procedure

UV spectra were recorded on a Shimadzu UV-1800 spectrophotometer. IR spectra were measured by a PerkinElmer Spectrum 100 spectrometer. NMR spectra were obtained on a Bruker AVANCE NEO 500 spectrometer in DMSO-d_6, referenced to the residual solvent signals for proton at δ_H 2.50 and carbon at δ_C 39.5 as internal standards. HR-ESITOFMS spectra were recorded on a Waters UPLC (column: Waters UPLC BEH C_{18} 2.1 mm × 50 mm, 1.7 μm) – Waters Synapt G2 mass spectrometer integrated LC-MS system. An Agilent HP1200 system equipped with a diode array detector was used for HPLC analysis and purification.

Microorganism

Acrocarpospora sp. RD011456 was obtained from NBRC (Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan). This strain showed 99.4% similarity in the 16S rRNA gene sequence (1431 nucleotides, DDBJ accession number LC688273) to *Acrocarpospora corrugata* NBRC 13972^T (accession number NR_041245).

Fermentation

Strain RD011456 was maintained on ISP-2 agar medium [yeast extract (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) 0.4%, malt extract (Difco Laboratories, Detroit, Michigan, USA) 1.0%, glucose 0.1%, and agar 2.0% in distilled water (pH 7.3)]. Well-grown colonies were inoculated into 500-ml K-1 flasks each containing 100 ml of the ISP-2 seed culture medium [yeast extract (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) 0.4%, malt extract (Difco Laboratories, Detroit, Michigan, USA) 1.0%, and glucose 0.1% in distilled water (pH 7.3)]. Flasks were placed on a rotary shaker operated at 200 rpm at 30°C for 7 days. The seed culture (5 ml) thus prepared was transferred into 500-ml K-1 flasks each containing 100 ml of A16 production medium consisting of glucose 2.0%, Pharmamedia (Traders Protein, Memphis, TN, USA) 1.0%, $CaCO_3$ 0.5%, and Diaion HP-20 (Mitsubishi Chemical, Kanagawa, Japan) 1.0% in distilled water (pH 7.0). Flasks were shaken on a rotary shaker (200 rpm) at 30°C for 13 days.

Extraction and isolation

After cultivation, the whole culture broth was agitated with 1-butanol (100 ml for each flask) at 100 rpm for 1 h. The resulting mixture was centrifuged at 6000 rpm for 10 min. The organic layer was collected and concentrated *in vacuo* to give 9.0 g of an extract from 5 l of culture. The extract was chromatographed on silica gel with a stepwise gradient elution of $CHCl_3$-MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1, and 0:1 v/v). The 3rd fraction ($CHCl_3$:MeOH = 10:1) was evaporated to give 450 mg of brownish residue, which was further fractionated by ODS flash chromatography with a stepwise gradient elution of

MeCN–0.1% HCO_2H aqueous solution (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 v/v). The 3rd fraction (4:6) was concentrated *in vacuo* to give a semipure material (49 mg). Purification by reverse-phase HPLC (Cosmosil $5C_{18}$ MS-II column, Nacalai Tesque Inc., 10 × 250 mm, 4 ml min^{-1}, UV detection at 254 nm) with an isocratic elution (MeCN:0.1% HCO_2H aqueous solution = 32:68) afforded acroindole A (**1**, 8.0 mg, t_R 30.5 min), acroindole B (**2**, 3.5 mg, t_R 52.0 min), and acroindole C (**3**, 1.0 mg, t_R 32.5 min).

Acroindole A (**1**): white amorphous solid; UV (MeOH) λ_{max} (log ε) 290 (3.84), 263 (3.90), 239 (3.95), 207 (4.25) nm; IR (ATR) ν_{max} 3303, 3169, 1630, 1568, 1516, 1423, 1370, 1289, 1162, 1060, 1022, 926, 908, 862, 815, 779, 714 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HR-ESITOFMS *m/z* 249.0432 $[M - H]^-$ (calcd for $C_{12}H_{10}{}^{35}ClN_2O_2$, 249.0431).

Acroindole B (**2**): white amorphous solid; UV (MeOH) λ_{max} (log ε) 289 (3.88), 262 (3.95), 239 (4.00), 208 (4.30) nm; IR (ATR) ν_{max} 3298, 3169, 1618, 1514, 1424, 1332, 1249, 1158, 1058, 925, 857, 811, 777 cm^{-1}; ^1H and ^{13}C NMR data, Table 2; HR-ESITOFMS *m/z* 263.0589 $[M - H]^-$ (calcd for $C_{13}H_{12}{}^{35}ClN_2O_2$, 263.0587).

Acroindole C (**3**): white amorphous solid; UV (MeOH) λ_{max} (log ε) 290 (3.68), 263 (3.75), 238 (3.80), 208 (4.11) nm; IR (ATR) ν_{max} 3428, 3284, 3112, 2917, 1620, 1525, 1415, 1384, 1290, 1062, 921, 855, 795, 726 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; HR-ESITOFMS *m/z* 208.0168 $[M - H]^-$ (calcd for $C_{10}H_7{}^{35}ClNO$, 208.0165).

Antimicrobial and cytotoxicity assay

Antimicrobial assay was carried out according to the procedure previously reported [20] against *Kocuria rhizophila* ATCC9341, *Staphylococcus aureus* FDA209P JC-1, *Escherichia coli* NIHJ JC-2, and *Candida albicans* NBRC0197. Cytotoxicity was tested against P388 murine leukemia cells according to the reported protocol [21].

Seed germination assay

The assay was carried out according to the standard protocol previously reported [22].

Declarations

Author Contribution

MMAR designed the study, performed the experiments, isolated and purified the compounds, analyzed the spectroscopic data, determined the structure, performed the antimicrobial, seed germination, and cytotoxicity assays, and drafted the manuscript. TN contributed the MS analysis. YI supervised the

research, provided guidance on the experimental design and data interpretation, revised the manuscript, and approved the final version for submission.

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Figures

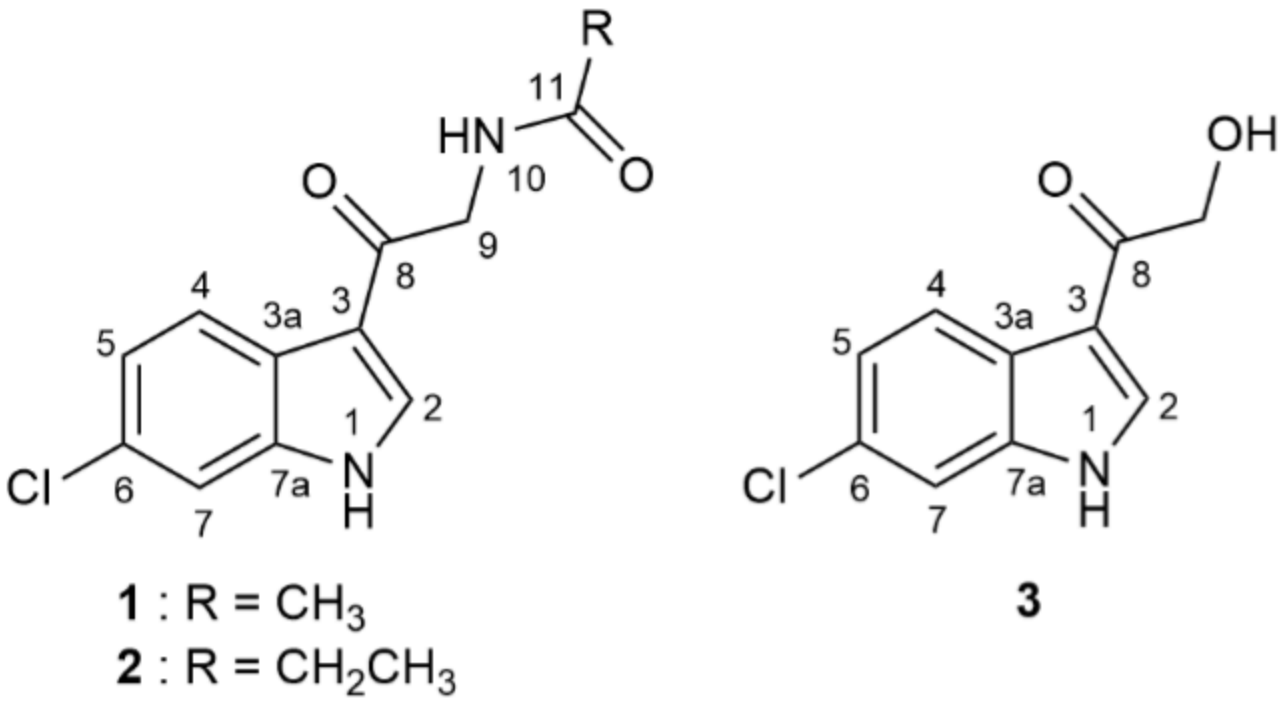

Figure 1

Structures of **1**–**3**.

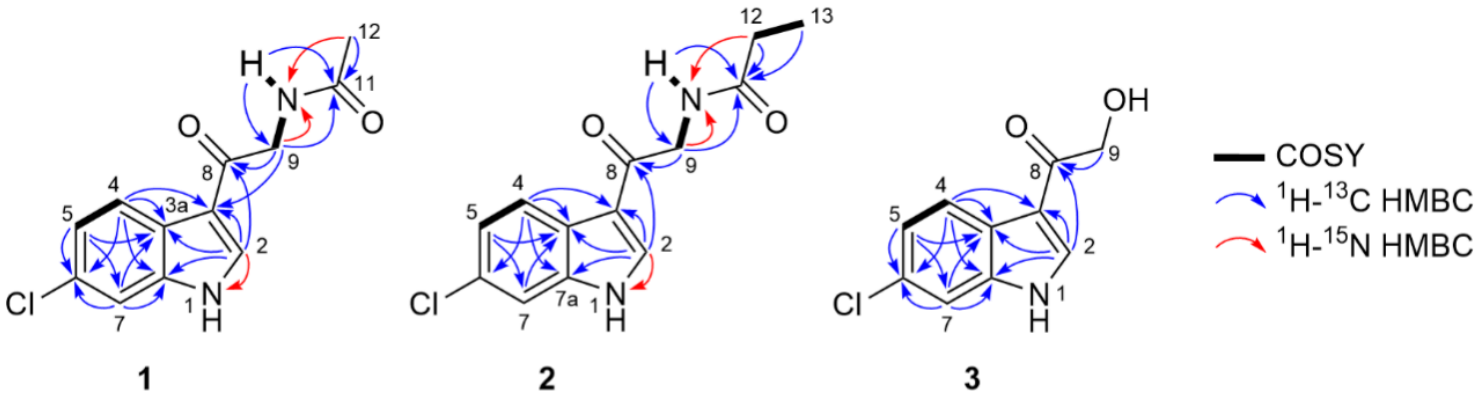

Figure 2

Selected 2D NMR correlations for **1**–**3**.

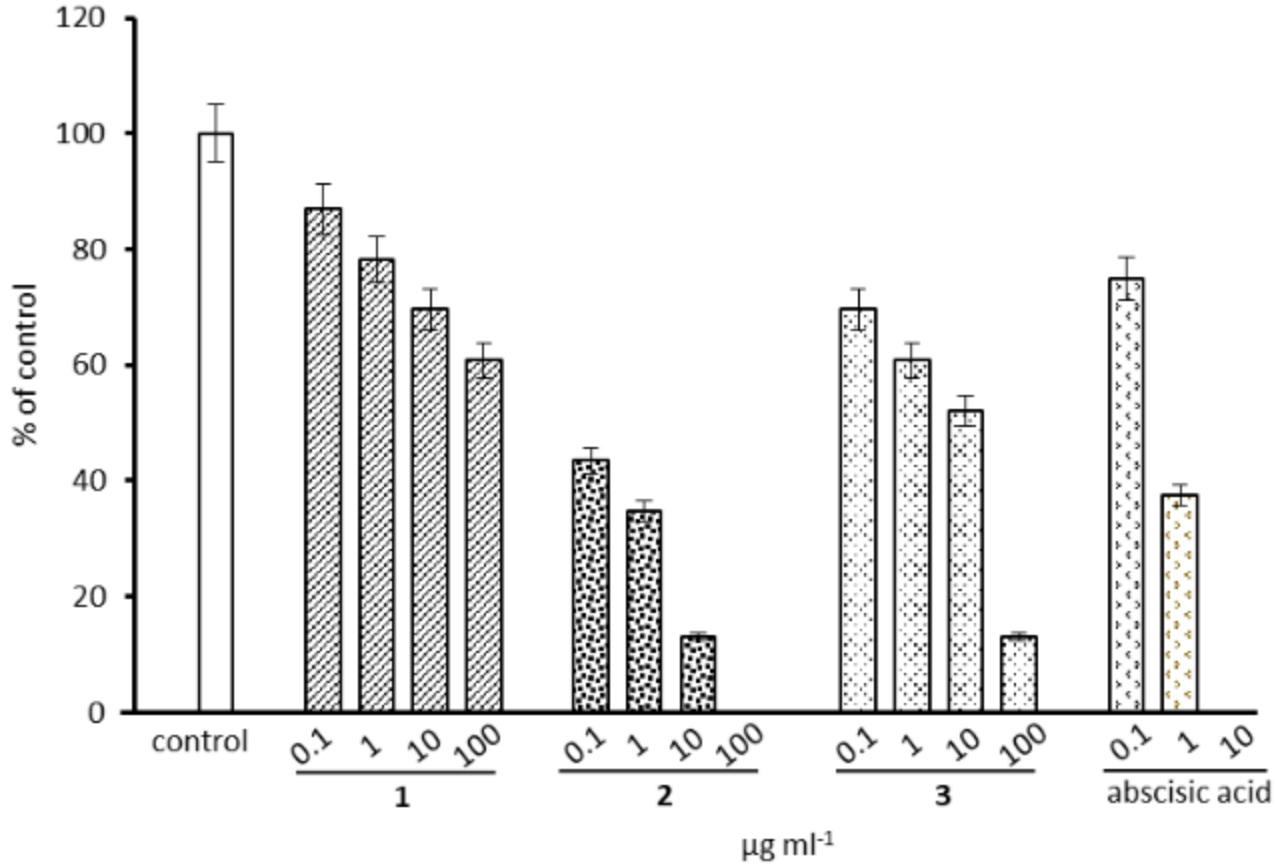

Figure 3

Inhibition of radicle elongation of lettuce seeds by **1**–**3**.

Supplementary Files

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- AcrocarposporaSI.pdf