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Isolation and identification of herbicidal active substances of *Botrytis* strain HZ-011

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Abstract: The herbicidally active *Botrytis* strain HZ-011 was isolated from naturally infected leaves of *Rumex patientia* L., and previous studies found that strain HZ-011 exhibits highly effective herbicidal effects against the broadleaf weeds *Amaranthus retroflexus* L., *Elsholtzia densa* Benth, *Malva crispa*, and *Chenopodium album* L. In this study, the active components of strain HZ-011 were isolated, purified, and structurally characterised using silica gel column chromatography, thin-layer chromatography, and high-performance liquid chromatography. The extraction test was carried out using four organic solvents with different polarities, and ethyl acetate was ultimately used as the extractant for the bulk preparation of the crude product of the active substances. Thin-layer chromatography yielded 10 different fractions and 26 components, and components B_3 and E_1 showed pathogenic effects on *C. album*. High-performance liquid chromatography (HPLC) of compound components B_3 and E_1 yielded three fractions with larger signal values and peak areas, and raw measurements of them revealed that component 3 was active. By analysing and comparing the spectral data of component 3 from ^{1}H-NMR, ^{13}C-MNR, ESIMS, and EIMS, component 3 was identified as dibutyl phthalate. The activity of the individual compounds was verified. The results of this study lay a theoretical foundation for the discovery and exploration of lead compounds. They will also provide a theoretical basis for developing this strain into a microbially-derived herbicide.

Keywords: herbicidal effects; active components; structural identification

Weed control is an important issue in the agricultural ecosystem and one of the important links in the agricultural production process (Oerke 2006; Anderson 2007). The growth of weeds seriously affects the growth and development of crops (Stokstad. 2013). Also, weeds are the main medium for spreading and hosting field pests and diseases (Ellison & Barreto 2004). Weeds increase the labour burden of farmland management; at present, the most widely used weed control methods are manual weed control and chemical control (Lake & Minteer 2018). There are about 600 kinds of chemical herbicides that can be used to control various types of weeds worldwide, such as glyphosate, bisglycyrrhizin and other compounds that control the germination and growth of weeds (Green & Owen et al. 2011; Duke et al. 2024). The long-term use of chemical herbicides has increased the resistance of weeds and soil pesticide residues, killed many beneficial insects, caused serious harm

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to the ecological environment, and had irreversible effects on both humans and livestock (Duke et al. 2002; Cordeau et al. 2016). Therefore, there is an urgent need to seek new environmentally friendly weed control methods. The rapid development of biogenic herbicides has been incorporated into comprehensive weed management strategies to prevent the sustained development of resistant weeds (Schwarzländer et al. 2018; Charudattan 2001). New biocontrol herbicides of microbial origin must be developed to replace traditional manual, mechanical, and chemical weeding strategies (Dayan et al. 2014).

Fungi of the genus *Botrytis* usually infest plants as pathogens, and they are species of several plant grey mould pathogens and microorganisms with potent herbicidal activity. Recently, fungi of the genus *Botrytis* have also been used in biocontrol and weed control studies. For example, Li (2003) reported *Botrytis ricini* Buchwald (N) as a potential weed biocontrol agent against *Euphorbia heterophylla* and *E. hirta* under Brazilian conditions. Mycoherbicide development is probably the best approach for controlling weeds, and *Botrytis* has merited attention for its possible exploitation as a mycoherbicide (Skèkacs 2024). Li (2003) isolated *B. cinerea* from *Botrytis* disease samples from different regions and reported that its metabolites strongly inhibited the leaves and seedlings of several weeds. Zhang et al. (2011) showed that the fermentation filtrate of *B. cinerea* strain BC1 isolated from *Tagetes erecta* L. significantly inhibited the growth of potted *Amaranthus retroflexus*. Wang et al. (2003) showed that the metabolites of *B. cinerea* had significant inhibitory effects on the growth of both broadleaf weeds and the seeds of graminaceous crops and that all weed seedlings such as *A. retroflexus* and *Pharbitis nil* (Lapisardi et al. 2005) Choisy were killed within 24 h after the plant seedlings were sprayed with the active substance.

Botrytis strain HZ-011 is a weed pathogenic fungus with broad-spectrum and high-efficiency herbicidal activity against farmland weeds in Qinghai, such as *A. retroflexus*, *E. densa*, *M. crispa*, and *C. album* (Zhu et al. 2023). To identify the herbicidal active substances in the fermentation broth of *Botrytis* strain HZ-011, chromatographic techniques (silica gel column chromatography, thin-layer chromatography and liquid chromatography) were used in this study to isolate and purify the active substances of the strain to obtain the components of the strain's active substances, and to characterise the individual compounds structurally. The results of this study provide a theoretical basis for further development of this strain into microbially-sourced herbicides.

In recent years, countries worldwide have vigorously advocated for "green pesticides", using plant pathogens or secondary metabolites to develop bio derived herbicides, which is the main direction for developing new bio-derived pesticides. Microbial herbicides are a demand for the development of ecological agriculture and a trend in the future development of herbicides. With the implementation of the dual reduction policy, it has gradually become a global consensus to vigorously develop the biopesticide industry and strive to develop biological weeding technology. Studying the active ingredients of *Botrytis* strain HZ-011 can provide a basis for accelerating the creation of new biological weeding technologies and products with independent intellectual property rights.

MATERIAL AND METHODS

Strain Isolation

The *B. euroamericana* HZ-011 strain was deposited on December 8, 2021, at the China General Microbiological Culture Collection Center of China, Microbe Preservation Management Committee. The deposit address is No. 1 West Beichen Road, Chaoyang District, Beijing, and the deposit number is CGMCC No.23894.

Fermentation Culture of Strain HZ-011 and Preparation of the Fermentation Filtrate

The strain HZ-011 stored in a test tube on a PDA plate was activated and cultivated at 27 °C for 8 days. A punch was used to take the mushroom cake (Φ = 8 mm), which was inoculated with 50 mL/block of inoculum in a sterilised PDB triangular flask, 250 mL per triangular bottle, and incubated for 7 days on a shaking table, with shaking at 27 °C and 180 r/min. The total volume of the fermentation was 80 L. After fermentation, the culture was centrifuged at a low temperature for 20 min to remove the mycelium, and the supernatant was collected for use.

Screening of Crude Extracts of Strain HZ-011

Organic solvents with different polarities were selected as the extractants, including n-butanol, ethyl

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acetate, dichloromethane and petroleum ether. The samples were extracted three times with equal volumes of strain HZ-011 fermentation filtrate, and the organic phases were collected after standing for 6 h. The crude extracts of the four organic phases were concentrated separately by vacuum concentration with a rotary evaporator at 45 °C. Leaves of *C. album* were selected as the target and spread on filter paper in a 9 cm petri dish. The crude extract for the crude organic phase was dissolved with methanol prepared into 3 mg/mL crude extract solution, the dissolved crude product was dropped onto the leaf, pure methanol was used as the control, and each experimental treatment was repeated three times. The treated leaves were cultured at room temperature, and the degree of infection was determined by observing the symptoms of leaf curling, chlorosis, and disease spots.

Silica Gel Column Chromatography

Column loading. First, pour 200–300 mesh silica gel powder into a beaker, add the appropriate amount of low-polarity petroleum ether, and add silica gel while stirring when presented with a bubble-free transparent paste. Add the stirred silica gel liquid into the glass column (column height 120 cm, diameter 5 cm), gently tap the column with a plastic object to drive away the air bubbles generated, and stop loading the column when the silica gel liquid is 15 cm away from the mouth of the column. After 24 h, the column was flushed with pure petroleum ether at a 1–2 drops/s flow rate. The silica gel would slowly fall down inside the column until the silica gel inside the column was stationary, indicating that the silica gel inside the glass column had been compacted, and the column flushing was stopped.

Sample loading. The active crude extract obtained by extracting 80 L of fermentation broth was dissolved in methanol. Silica gel powder was added to thoroughly mix the sample with silica gel powder (methanol : silica gel powder = 2 : 1). Then, the sample was concentrated under reduced pressure to adsorb the sample onto the silica gel powder. The agglomerated sample was ground and added to a glass column (with a sample loading height of 5 cm). The sample was added slowly so that the surface remained flat and was not exposed to air to prevent inconsistent rates of decline of active substances during elution.

Elution. The lower piston was opened, and the eluent flow rate was maintained at 1 drop/s. The elution gradient sequence was petroleum ether : chloroform : ethyl acetate (10 : 3 : 2), chloroform : ethyl acetate (8 : 7), acetone : ethyl acetate (1 : 1), dichloromethane : methanol (10 : 1), dichloromethane : methanol (5 : 1) and dichloromethane : methanol (1 : 1). These six mixed solutions were used to perform the elution and each was collected in a 160 mL bottle.

Preparative Thin Layer Chromatography

Sampling. Using a glass knife, a preparation type HSGF254 silicone adhesive board (200 × 200 mm, thickness 0.4–0.5 mm) was cut into 2.5 cm × 5 cm small boards. A straight line was drawn as the baseline at a distance of 1 cm from the bottom of the silicone board, and another straight line was drawn as the top line at a distance of 0.5 cm from the top of the board. Using glass sampling capillary tubes (0.5 × 100 mm), the sample was spotted on the baseline, and this was repeated multiple times until the spots showed a colour under UV light.

Deployment. The deployment steps need to be carried out in a sealed chromatography cylinder, and the size of the chromatography cylinder should be selected based on the size of the silicone board. The proportion of the elution mixed solution was selected as the developing agent, and the proportion was adjusted slightly according to the colour development situation. The developing agent proportions were as follows: petroleum ether : chloroform : ethyl acetate (10 : 3 : 2), chloroform : ethyl acetate (8 : 7), acetone : ethyl acetate (1 : 1), dichloromethane : methanol (10 : 1), dichloromethane : methanol (5 : 1) and dichloromethane : methanol (1 : 1). It should be noted that the amount of developer added cannot exceed the baseline or the top line.

UV colour development. After the developer resolved the sample bands, a wavelength of 254 nm was selected for colour development under the UV analyser. Different bands represent different substances, and samples with the same bands were combined. The merged samples were spotted again and developed as described above, and the different bands were circled with a pencil as fractions. The strips on the silicone board were scraped off along with the silicone powder, and they were soaked and dissolved in a small amount of methanol for further testing.

Bioactivity determination of herbicide active ingredients. Fresh and disease-free leaves of *C. album*

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plants were selected, cleaned with sterile water, and dried naturally. Then, the leaves were placed in a sterilised Petri dish (Φ = 9 cm), and the Petri dish was covered with filter paper that was soaked with sterile water. Each distillate was dissolved in methanol and diluted to 100 μg/mL, and 20 μL was added dropwise to each blade for treatment, with methanol as the control. Each treatment was repeated three times. Treated leaves were placed in a 25 °C light incubator for light cultivation, and experimental results were observed after 3 days.

High-Performance Liquid Chromatography (HPLC) analysis. Using high-performance liquid chromatography, a C18 reverse phase column Agilent 7890A/5975C GC-MS System, 4.6 mm × 250 mm) was selected to explore the separation conditions, with a flow rate of 1 mL/min and detection wavelength of 220 nm. The separation conditions used an initial mobile phase ratio of methanol : water = 95 : 5. Then, the methanol ratio was reduced in sequence with a gradient of 5% to increase the water ratio until the optimal mobile phase ratio of the peak type was determined. Finally, the optimal mobile phase ratio was selected as the condition for separating and preparing the crude extract. Then, based on the optimal conditions from the analysis and exploration above, the C18 reverse phase column (30 mm × 250 mm) was used to continue to explore the optimal conditions for separation and preparation, and the chromatographic peaks obtained from the analysis were collected for further analysis of the active components.

HZ-011 pure product structure identification. Nuclear magnetic resonance analysis: The individual compounds were dissolved in a suitable deuterated solvent and transferred to a nuclear magnetic resonance tube for nuclear magnetic resonance detection. The (Zhu 2023) H-NMR and (Barreto 1998)C-NMR NMR spectra were obtained. Through the chemical shift values of (Barreto 1998) C and hydrogen nuclei, the microspectral data were queried, and the structures of the compounds were preliminarily judged through comparison.

Mass spectrometry analysis of the molecular weight of each compound: The sample to be tested was dissolved with chromatographic methanol and injected into the mass spectrometer. The electrospray ionisation source (ESI) was selected for mass spectrometry detection, and the compound's molecular weight was calculated.

Nuclear magnetic resonance (NMR) analysis conditions: The NMR analysis used a Brooke ADVANCE III 400 MHz instrument, the solvent was deuterated methanol, the hydrogen spectrum resonance frequency was 400 MHz, the resolution was 0.244532 Hz, the carbon spectrum resonance frequency was 100 MHz, and the carbon spectrum resolution was 0.733596 Hz.

The structures of the compounds were determined by analysing their hydrogen, carbon, and mass spectra, combined with literature research.

The herbicidal activity of the isolated individual compounds was also tested using the leaf inoculation method. Each treatment was repeated three times.

RESULTS

Screening of Crude Extract Extractants of Strain HZ-011. As shown in Table 1, the organic phase and aqueous phase in dichloromethane, petroleum ether, ethyl acetate and n-butanol after liquid-liquid extraction were used to treat the isolated leaves of *C. album*. The results showed that the dichloromethane organic phase had no disease symptoms on the leaves of *C. album*, while the aqueous phase had small disease spots on the leaves of *C. album*. It was indicated that dichloromethane was used as the extractant, and the active substances were in the aqueous phase. In contrast, the petroleum ether organic phase had slight yellowing of the *C. album* leaves, while the aqueous phase of the petroleum ether extract caused small lesions in the leaves, indicating that the active substances were in the aqueous phase. The organic phase of ethyl acetate caused large disease spots on the leaves of *C. album*. In contrast, the aqueous

Table 1. Pathogenicity of crude extracts of four polar organic solvents on isolated leaves of *Chenopodium album*

	Dichloromethane	Petroleum ether	Ethyl acetate	n-Butanol
Organic phase	–	–	++	–
Aqueous phase	+	+	–	–

– no symptoms; + mild symptoms, with the lesion area accounting for about 15% of the entire leaf area; ++ moderate symptoms, where the lesion area accounts for 16% to 59% of the entire leaf area; and +++ severe symptoms, where the lesion area accounts for 60% to 80% of the entire leaf area

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Table 2. Selection of the eluting agent ratio system for column chromatography

Serial No.	Elution ratio	Bottle No.	Total volume (mL)
1	petroleum ether : chloroform : ethyl acetate = 10 : 3 : 2	1–14	2 520
2	chloroform : ethyl acetate = 8 : 7	15–24	1 800
3	acetone : ethyl acetate = 1 : 1	25–34	1 800
4	dichloromethane : methanol = 10 : 1	35–44	1 800
5	dichloromethane : methanol = 5 : 1	45–54	1 800
6	dichloromethane : methanol = 1 : 1	55–65	1 980

phase of the ethyl acetate extract did not produce pathogenic symptoms on *C. album* leaves, indicating that the active substances are present in the organic phase. The organic and aqueous phases of n-butanol had no pathogenic effects on *C. album* leaves, indicating that n-butanol could not extract the herbicidal active substances of strain HZ-011. Therefore, the order of phytotoxicity effects on *C. album* leaves was ethyl acetate phase > petroleum ether phase > dichloromethane phase > n-butanol phase, so ethyl acetate was selected as the best extractant.

Silica gel column chromatography. Table 2 shows six organic solvents with different polarities, namely petroleum ether, chloroform, ethyl acetate, acetone, dichloromethane, and methanol, which were selected and mixed to prepare eluents with different polarities. The crude extract with herbicidal activity was separated by silica gel column chromatography, and six different samples were obtained.

Table 3. Thin Layer Chromatography analysis of each component

Serial No.	Distillate No.	Elution ratio	Development ratio	Composite component name	Rf value
1	B	petroleum ether : chloroform : ethyl acetate = 10 : 3 : 2	petroleum ether : chloroform : ethyl acetate = 10 : 3 : 2	B_1	0.265 ± 0.0019
				B_2	0.613 ± 0.0125
				B_3	0.764 ± 0.0142
	L	petroleum ether : chloroform : ethyl acetate = 10 : 3 : 2	chloroform : ethyl acetate = 2 : 1	L_1	0.499 ± 0.0296
				L_2	0.672 ± 0.0136
2	E	chloroform : ethyl acetate = 8 : 7	chloroform : ethyl acetate = 1 : 1	E_1	0.360 ± 0.0164
				E_2	0.582 ± 0.0057
				E_3	0.756 ± 0.0096
	N	chloroform : ethyl acetate = 8 : 7	dichloromethane : methanol = 8 : 1	N_1	0.312 ± 0.0488
				N_2	0.431 ± 0.0215
				N_3	0.675 ± 0.0080
	M	chloroform : ethyl acetate = 8 : 7	dichloromethane : methanol = 6 : 1	M_1	0.575 ± 0.0133
				M_2	0.690 ± 0.0144
3	O	acetone : ethyl acetate = 1 : 1	dichloromethane : methanol = 10 : 1	O	0.463 ± 0.0030
	F	acetone : ethyl acetate = 1 : 1	dichloromethane : methanol = 6 : 1	F_1	0.479 ± 0.0145
				F_2	0.626 ± 0.0157
				F_3	0.785 ± 0.0374
3 and 4	G	acetone : ethyl acetate = 1 : 1 and dichloromethane : methanol = 10 : 1	dichloromethane : methanol = 6 : 1	G_1	0.422 ± 0.0175
				G_2	0.523 ± 0.0191
				G_3	0.648 ± 0.0112
				G_4	0.700 ± 0.0167
				G_5	0.806 ± 0.0129
5	P	dichloromethane : methanol = 10 : 1	dichloromethane : methanol = 5 : 1	P	0.630 ± 0.0257
6	H	dichloromethane : methanol = 1 : 1	dichloromethane : methanol = 10 : 1	H_1	0.432 ± 0.0085
				H_2	0.589 ± 0.0184
				H_3	0.698 ± 0.0218

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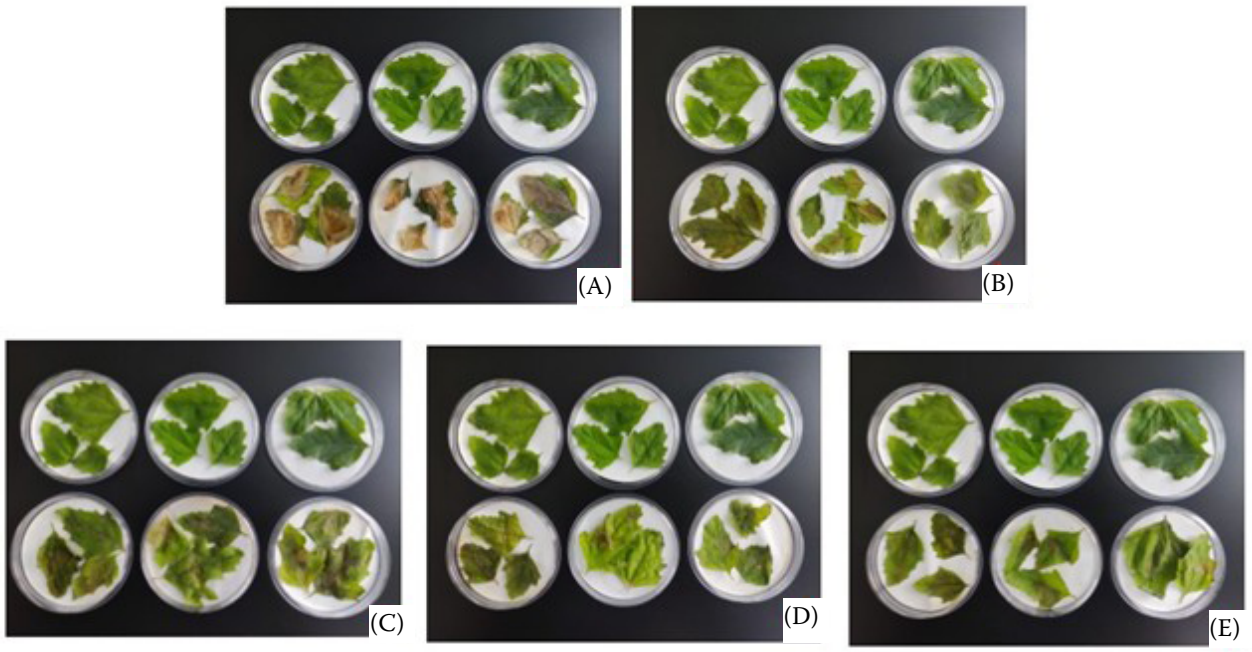

Figure 1. Pathogenic effect of herbicidal active distillates on *Chenopodium album* leaves
(A) distillate B; (B) distillate E; (C) distillate F; (D) distillate G; (E) distillate H; in A–E, the first row is the control group, and the second row is the experimental group

Preparative Thin Layer Chromatography (Prep TLC). The six different samples obtained above were subjected to thin-layer chromatography. Ten different distillates labelled B, E, F, G, H, L, M, N, O, and P were obtained. The substances developed by UV light were analysed using Rf values (Rf = 0.2–0.8), as shown in Table 3. Twenty-six composite components met the conditions of the qualitative analysis, namely B1, B2, B3, E1, E2, E3, L1, L2, H1, H2, H3, O, N1, N2, N3, G1, G2, G3, G4, G5, M1, M2, F1, F2, F3, and P.

Determination of bioactivity of the herbicidal active ingredients. The activities of 10 distillates were measured, and the results (Figure 1) showed that distillate B caused chlorosis of *C. album* leaves, resulting in yellow spots and severe symptoms; distillate E caused yellowing of *C. album* leaves, with some leaves exhibiting yellowish-brown lesions and severe symptoms; distillate F caused brown spots near the main veins of *C. album* leaves, and some leaves wilted, exhibiting severe symptoms; distillate G caused some *C. album* leaves to turn green or

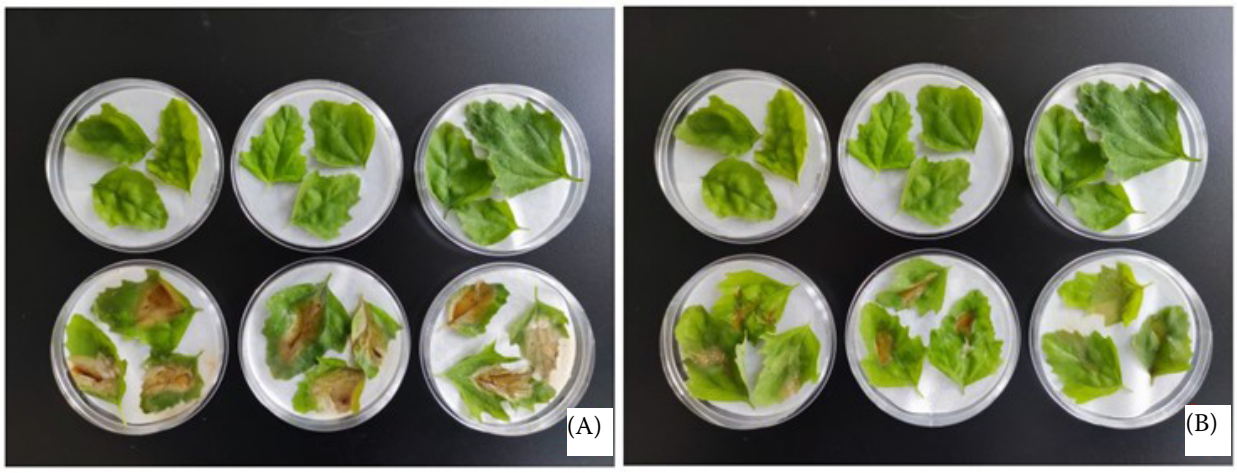

Figure 2. Pathogenic effects of composite component B_3 and composite component E_1 on *Chenopodium album* leaves
(A) composite component B_3; (B) composite component E_1

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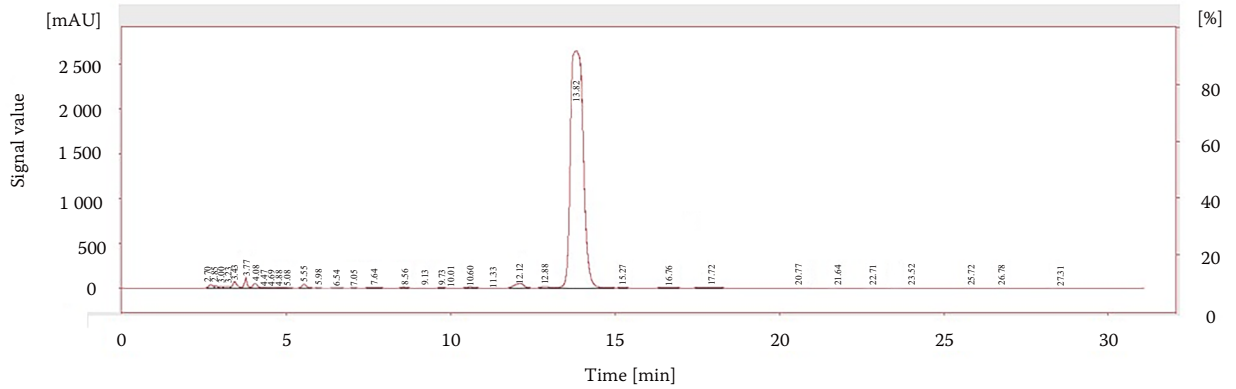

Figure 3. HPLC chromatogram of composite component B_3

yellow, and produce yellow spots, exhibiting moderate symptoms; and distillate H caused leaf spots and some leaves appeared wilted, presenting as moderate symptoms; while the other distillates L, M, N, O, and P were not pathogenic to *C. album* leaves.

Activity measurements were conducted for 11 components in distillates B, E, and G, and the results showed that composite components B_3 and E_1 exhibited pathogenic effects. As shown in Figure 2, composite component B_3 caused yellow spots on *C. album* leaves, resulting in death of all parts in contact with the components and severe symptoms; while composite component E_1 caused yellow-brown spots on the leaves of *C. album*, with slight chlorosis and mild symptoms. The other nine composite components (B_1, B_2, E_2, E_3, G_1, G_2, G_3, G_4 and G_5) all showed pathogenic effects, while the active substance in distillate G was probably undeveloped by the unfolding agent, which has to be explored further.

High-Performance Liquid Chromatography (HPLC) analysis. Using high-performance liquid chromatography (P230p Dalian Yilite Analytical Instrument Co., Ltd., P. R. China), with a C18 reverse phase column (4.6 mm × 250 mm) and a methanol-water system as the mobile phase, the sample concentration was 20 μg/mL, the flow rate was 1 mL/min, the injection volume was 10 μL, and the detection wavelength was 220 nm. The composite component B_3 was examined under these conditions. The results are shown in Figure 3. Under the conditions of mobile phase methanol : water = 80 : 20, 19 different peaks were obtained, with RT 13.82 having the highest signal value of 2 644.624 mAU and the largest peak area of 75 651.626 mAU/s. This peak was named "Component 1" for the subsequent activity determination.

The results of using high-performance liquid chromatography to analyse the composite component E1, with the mobile phase of methanol : water = 30 : 70, are shown in Figure 4. Twelve peaks were obtained, with the peak RT 3.15 having the largest area of 8 733.237 mAU/s and a peak height of 385.507 mAU, named "Component 2". The peak with RT 12.88, with a symmetrical peak shape, a peak area of 1 683.267 mAU/s, and a peak height of 56.544 mAU, was named "Component 3".

The activities of components 1, 2, and 3 were determined; component 1 caused some leaves to lose their green colour and turn yellow, while

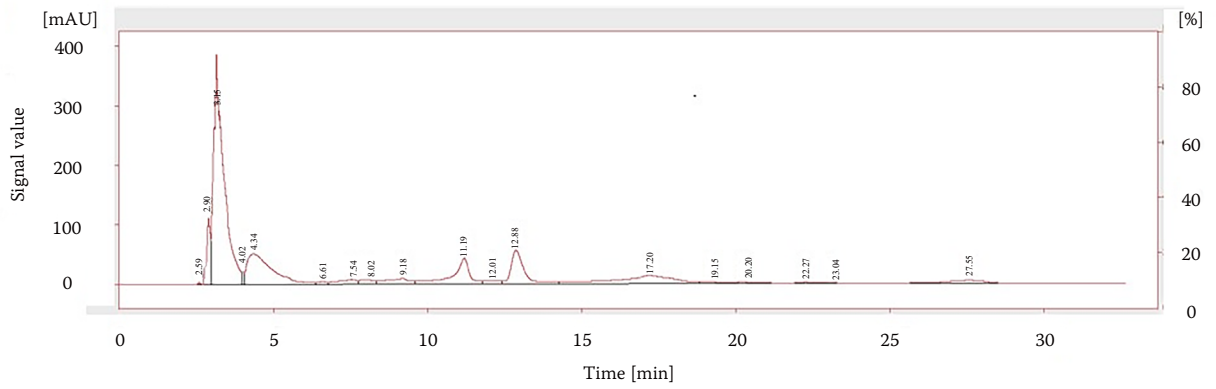

Figure 4. HPLC chromatogram of composite component E_1

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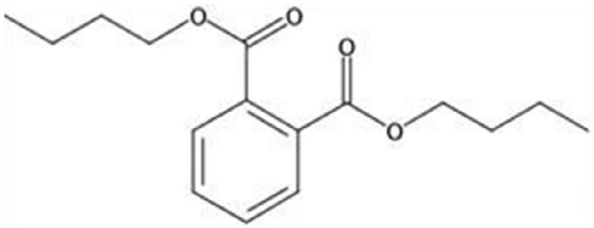

Figure 5. The structural formula of dibutyl phthalate

a small number of leaves developed disease spots and exhibited wilting symptoms. Component 2 and component 3 had no pathogenic effects on *C. album* leaves.

Structural identification of the individual compounds. The mass spectrum of "Compound 1" shows that the molecular ion peaks of [M + H] + 279.1550 and [M + Na] + 301.1356 infer that its molecular formula is $C_{16}H_{22}O_4$. ^{1}H-NMR (400 MHz, CDCl3) δ: 7.48 (2 H, dd, J = 8 Hz, 4 Hz), 7.25 (2 H, d, J = 8 Hz, 4 Hz), 4.07 (4 H, t, J = 7 Hz), 1.45 (4 H, m), 1.22 (4 H, m), 0.71 (6 H, t, J = 2 Hz). ^{13}C-NMR (400 MHz, CDCl3) ä: 166.78, 131.91, 130.31, 128.21, 64.69, 30.04, 18.61, 13.05. Through the analysis and comparison of the ^{1}H-NMR, ^{13}C-NMR, ESIMS, EIMS and other spectral data, the compound was determined to be dibutyl phthalate, and the structural formula is in Figure 5.

The pathogenicity of compound 1 at a concentration of 200 μg/L on isolated leaves of *C. album* was determined by bioactivity determination of detached leaves. After inoculation, the leaves showed yellowing and wilting phenomena (Figure 6).

DISCUSSION

In this study, the herbicidal active substances of *Botrytis* strain HZ-011 were isolated and purified step-by-step using column chromatography, thin-layer chromatography and high-performance liquid chromatography. Most of the active substances among the secondary metabolites of fungi can be extracted after several solvent extractions (Sun et al. 2020). Dichloromethane, petroleum ether, ethyl acetate and n-butanol, organic solvents with different polarities, were selected to extract the secondary metabolites of strain HZ-011 separately, and isolated *C. album* leaves were used as the target. The results showed that ethyl acetate was the best in pathogenicity to *C. album* leaves, so ethyl acetate was selected as the extractant for the bulk preparation of the crude product of the active substance. Since ethyl acetate was selected as the extractant through the extractant screening test, this indicated that the herbicidal active substances of strain HZ-011 had low polarity.

Isolation and purification are prerequisites for subjecting the target products to structural and property studies (Lapisardi 2005). Through column chromatography and thin-layer chromatography, then using six mixed solutions for elution in the

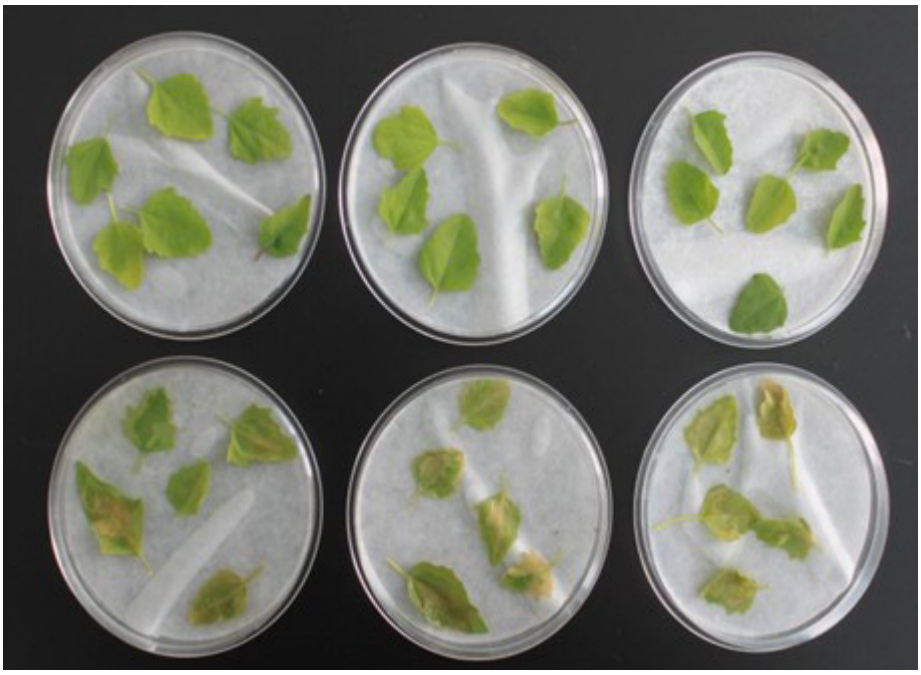

Figure 6. Pathogenic effect of the individual compound 1 on *Chenopodium album* leaves The first row is the control group, and the second is the experimental group

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thin-layer chromatography separation – petroleum ether : chloroform : ethyl acetate (10 : 3 : 2), chloroform : ethyl acetate (8 : 7), acetone : ethyl acetate (1 : 1), dichloromethane : methanol (10 : 1), dichloromethane : methanol (5 : 1), and dichloromethane : methanol (1 : 1), with ultraviolet light colour development and observation, the identical bands were combined. The solutions with the same bands were analysed by ultraviolet light, and 10 different fractions and 26 components were obtained. Herbicidal activity assay tests were carried out on the 10 fractions, and the results showed that Fractions B, E, F, G, and H had different degrees of pathogenicity on *C. album* leaves. Fractions B, E and G were selected for thin-layer chromatographic separation, and 11 different fractions were obtained, including B_1, B_2, B_3, E_1, E_2, E_3, G_1, G_2, G_3, G_4 and G_5. The results of the activity assays showed that B_3 and E_1 had pathogenic effects on *C. album* leaves, while the active ingredients of fractions F, G and H need to be further explored. HPLC analysis of composite fraction B_3 and composite fraction E_1 was used to obtain the signal values and peak areas of the three components, and the bioassay test of the components with the larger values found that fraction 1 was active. By analysing and comparing the spectral data of component 1 with (He et al. 2024) H-NMR, (Wang et al. 2024) C-NMR, ESIMS, EIMS, etc., the compound of component 1 was identified as dibutyl phthalate. The activities of the individual compounds were verified.

Dibutyl phthalate (DBP) is a phthalate ester that is widely used and representative in life (Mankidy et al. 2013) and has become a widely used plasticiser because of its excellent chemical properties (Zhao et al. 2016). In addition, it is an important root secretion. The existence of this substance has been detected in many plants, such as in the root secretion of *Zea mays* L, *Glycine max* L, *Capsicum annuum* L, as well as in the composition of *Solanum lycopersicum* L. plants, which has been isolated and identified. This substance can inhibit the plant itself, damaging plants such as *Cucumis sativus* L. and *Brassica rapa* var. (Li et al. 2016). DBP can accumulate in the plant and affect its growth. By affecting the morphological changes of the plant, it damages the plant cells and interferes with normal physiological metabolism (Wang et al. 2015). However, it has not been reported in weed control. This paper reported for the first time that DBP has herbicidal activity, laying the foundation for developing and large-scale production of new herbicides.

CONCLUSION

In this study, ethyl acetate was the most suitable active substance extractant for Botrytis strain HZ-011, which was screened using four organic solvents with different polarities. The activity of individual compounds was verified by determining that fractions B_3 and E_1 had the best effect on the pathogenicity of *C. album* leaves by thin-layer chromatography and liquid chromatography. Component 1 was found to be active and identified as dibutyl phthalate, indicating that *Botrytis* strain HZ-011 can synthesise and secrete the precursor compounds that inhibit the growth of weeds, thus achieving the purpose of weed control. The next step will be to investigate the synthetic pathway and regulatory genes of the compound at the molecular level, further clarify the herbicidal mechanism of this strain, and provide a theoretical basis for researching and developing this bacterium into microbial herbicides. The next step will be to clarify the mechanism of herbicidal action of this strain to provide a theoretical basis for developing this strain into microbial herbicide.

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