Endophytic *Streptomyces* sp. LRE541 isolated from *Lilium davidii var.* Unicolor Cotton as a Cell Factory for Antimicrobials and Anticarcinogens with Inducing Apoptosis and Cell Cycle Arrest Properties

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Abstract

Background: Endophytic actinomycetes, as emerging sources of bioactive metabolites, play a vital role in pharmaceutical development. Recent reports demonstrated that endophytic *Streptomyces* isolates could yield compounds with potent anticancer and antimicrobial properties that may be developed into chemotherapeutic drugs. Our study displayed that *Streptomyces* sp. LRE541 obtained from the root tissues of *Lilium davidii* var. unicolor Cotton, could be a potential source of anticarcinogens and antimicrobials.

Results: Isolate LRE541 was characterized and identified as belonging to the genus *Streptomyces* based on the 16S rDNA sequence analysis, with highest sequence similarity to *Streptomyces* tauricus JCM4837^T (98.81%). It produced extensively branched red substrate and vivid pink aerial hyphae that changed into amaranth, with elliptic spores sessile to the aerial mycelia. The secondary metabolites (EtOAc extract) produced by isolate LRE541 exhibited significant anticancer activities with IC_{50} values of 0.021, 0.2904, 1.484, 4.861, 6.986, 8.106, 10.87, 12.98, and 16.94 μg/mL against cancer cells RKO, 7901, HepG2, CAL-27, MCF-7, K562, Hela, SW1190 and A549, respectively, evaluated by the MTT assay. In contrast, the EtOAc extract showed less cytotoxicity activity against the normal human pulmonary artery endothelial cell (HPAEC) with an IC_{50} value of > 20 μg/mL than that of the cancer cells. To further explore the mechanism underlying the decrease in viability of cancer cells following the EtOAc extract treatment, cell apoptosis and cell cycle arrest assays were performed using two cancer cell lines, RKO and 7901. The result demonstrated that the EtOAc extract inhibited cell proliferation of RKO and 7901 cells by causing cell cycle arrest both at the S phase and inducing apoptosis in a dose-dependent manner. Moreover, the EtOAc extract of isolate LRE541 with the concentrations within 100 μg/mL also possessed the antagonistic activities against *E. coli* ATCC 25922, MRSA ATCC 25923, *P. aeruginosa* and *C. albicans* ATCC 66415, and the antagonistic potent against the tested pathogens all displayed a dose-dependent manner. The UHPLC-MS/MS analysis of the EtOAc extract revealed that the presence of antitumor, potential antitumor and antimicrobial compounds could account for the potent antineoplasmic and antagonistic properties of the extract.

Conclusion: This study provides the potential therapeutic applications of the bioactive metabolites from *Streptomyces* sp. LRE541 as novel antimicrobial and anticancer agents.

Introduction

Although major progress has been achieved in the field of cancer therapy for the past few decades, cancer remains a serious public health threat (1, 2). Chemotherapy is one of the common therapeutic approaches for controlling cancers. Unfortunately, most patients eventually relapse and develop drug resistance (3, 4). Furthermore, patients, especially those undergoing cancer chemotherapy, joint replacement surgery and organ transplantation, starve for effective antibiotics to prevent postoperative infection (5). On this account a continuous supply of novel drugs with high effectiveness and safety is urgently needed. Besides, druginduced apoptosis of malignant cells is a promising antitumor strategy

with emerging evidence supporting its efficacy against various cancer types (6–8), thus, extensive and intensive studies on the underlying anti-tumor mechanism of the drugs are also required.

Streptomyces, with a vast distribution and innate capability of producing diverse bioactive secondary metabolites, has served as an important source of novel antibiotic candidates for decades (9–11). Nowadays, bioprospecting for *Streptomyces* sp. from untapped or unique ecosystems may be an effective way to meet the everlasting demand for novel drugs and other biomolecules, which have been preferred attributing to their potent therapeutic applications and desired pharmacokinetic properties for clinical uses and served as precursors of drug semi-synthesis or the template of drug chemical synthesis (12–14). Over the past decade, endophytic *Streptomyces* spp. from medicinal plants in various ecotopes, as relatively unexploited fascinating sources of novel natural products, have been explored extensively and gained some remarkable results. For example, reports covering new endophytic *Streptomyces* species and their novel secondary metabolites along with antimicrobial and antioxidant activities have sprung up (14–17). Moreover, anticancer and cytotoxic compounds have also been discovered in endophytic *Streptomyces* spp., although such reports are sporadic compared to marine actinomycetes; however, their anticancer effects or cytotoxic activities are comparable to those of their marine counterparts, even stronger (18, 19). In addition, it is widely accepted that medicinal plants are rich sources of precious bioactive compounds, and increasing evidence indicates that endophytic actinomycetes may participate in the metabolic pathways of their host plants, and obtain some genetic information to yield bioactive compounds similar to their host plants (5, 20). These findings suggest that endophytic *Streptomyces* sp. of medicinal plants may be a good choice for anticarcinogens and antimicrobials.

Lilium davidii var. unicolor Cotton, a famous health-care edible medicinal plant, rich in amino acid, vitamins, glycosides, alkaloids and polysaccharides, possesses antioxidant activities (21). Accordingly, the actinomycetes from the plants may develop adaptive strategies and yield chemically unique secondary metabolites. However, there is no report concerning the antimicrobial and anticancer activities of *Streptomyces* sp. from *Lilium davidii* var. unicolor Cotton *in vitro*. Given the immense potential of the secondary metabolites of endophytic *Streptomyces* sp. for pharmaceutical applications, we isolated and characterized a *Streptomyces* isolate from the root tissues of *Lilium davidii* var. unicolor Cotton, and investigated the antimicrobial and antitumor effects of the secondary metabolites of the isolate, as well as the inductive effects on apoptosis and cell cycle arrests of tumor cells.

Results

Phenotypic characteristics of Isolate LRE541

The isolate LRE541 obtained from the root tissues of *Lilium davidii* var. unicolor Cotton is Gram-positive and aerobic. The cultural characteristics of LRE541 on various media were shown in Table 1. It grew well on all the tested media (ISP2 ~ ISP7 and Gao's No. 1) with varying colors of aerial and substrate myceliums on different medium. Compared with ISPs, the diffusible pigment was only produced on Gao's

No. 1 medium. As presented under the scanning electron microscopy in Fig. 2, isolate LRE541 produced elliptic spores sessile to the aerial hyphae, which extensively branched and grew in segments with verrucous protrusions. As shown in Table 2, the growth of LRE541 was observed at the temperature range of 18–37 ℃ (optimum at 23 ℃), and pH range of 4–12; however, at NaCl concentration above 6% (w/v), no growth was observed. Isolate LRE541 was positive for cellulose utilization but negative for both methyl red test and H_2S production. In the extracellular enzyme activity tests, the isolate demonstrated to produce various enzymes such as urease, catalase, amylase, protease, and lipase. Furthermore, it was found that LRE541 had broad utilizations of carbon and nitrogen sources (Table 2). These phenotypic properties of LRE541 were congruent with the genus *Streptomyces* as depicted by E. A. Barka et al. (22) in that they are prolific aerobic Gram-positive bacteria possessing extensively branched vegetative form and aerial hyphae and produce various water-soluble pigments.

Table 1
Cultural characteristics of *Streptomyces* sp. LRE541.

Media	Growth	Color of colony mycelium		Diffusible pigment
		Aerial	Substrate	
ISP2	Good	light pink to red	orange-yellow to red	-
ISP3	Good	bright red	red	
ISP4	Good	pinky white	pinky white	
ISP5	Good	red in white	red in white	
ISP6	Good	transparent to pale violet red	pale violet red	
ISP7	Good	brick red	vivid red	
Gao's No. 1	Good	vivid pink	red	claret-colored pigment
–, absent				

Table 2
Physiological properties of *Streptomyces* sp. LRE 541

Tests	Results	Tests	Results
cellulose utilization	+	**Nitrogen sources utilization**	
MR test	−	Urea	+
H_2S production	−	Glycine	+
Extracellular enzyme activity		Peptone	+
Urease	+	Maizena	−
Catalase	+	Tyrosine	+
Starch hydrolysis	+	Aspartic acid	−
Gelatin hydrolysis	+	Soybean meal	+
Degradation of		Ammonium sulfate	+
Tween 20	−	L- Proline	+
Tween 40	+	L-Arginine	+
Tween 80	+	L-Cysteine	Nd
Carbon sources utilization		L-α- Alanine	−
Xylose	++	**Growth at pH**	
Starch	+	pH 2	−
Glucose	++	pH 4	+
Maltose	+++	pH 6	+
Lactose	+++	pH 7	+++
Sucrose	++	pH 8	++
Fructose	+	pH 10	++
Mannose	++	pH 12	+++
Trehalose	−	**Growth at Temp**	
Raffinose	−	4 ℃~16 ℃	−
Arabinose	+	18℃~20 ℃	+
Rhamnose	+	23 ℃	+++

Note: −, negative test; Nd, not determined; +, slight development/positive; ++, good development; +++: very good development

Tests	Results	Tests	Results
Growth at NaCl (w/v)		28 ℃	++
0 ~ 6%	++	37 ℃	+
Gram staining	+		
Note: –, negative test; Nd, not determined; +, slight development/positive; ++, good development; +++: very good development			

16S rDNA-Based Phylogenetic analysis

The almost complete 16S rDNA sequencing revealed that isolate LRE541 comprises 1471 bp, which was submitted in GenBank/EMBL/DDBJ under the accession number MK138546 (https://www.ncbi.nlm.nih.gov/nuccore/MK138546). The 16S rDNA sequence of LRE541 was aligned with those of the type strains retrieved from GenBank/EMBL/DDBJ databases. As presented in Fig. 1, the phylogenetic tree demonstrated that LRE541 formed a distinct phyletic line with the type strain *Streptomyces tauricus* JCM4837^T at bootstrap value of 95%, displaying the highest 16S rDNA sequence similarity value with *Streptomyces tauricus* JCM4837^T (98.81%), and followed by *Streptomyces ederensis* NBRC15410^T (98.45%), *Streptomyces aurantiacus* NBRC13017^T (98.18%), *Streptomyces glomeroaurantiacus* NBRC15418^T (98.12%).

Cytotoxicity of the EtOAc extract toward various cells

We had determined the cytotoxicity of EtOAc extract from *Streptomyces* sp. LRE541 towards a panel of cancer cell lines (A549, SW1190, HepG2, CAL-27, MCF-7, 7901, RKO, Hela and K562). As illustrated in Table 3, the EtOAc extract exhibited cytotoxic activity against six cancer cells with IC_{50} values < 10 μg/mL, and against all of the cancer cells with IC_{50} values (0.021–16.94 μg/mL) < 20 μg/mL. Among all of the nine tested cancer cells, the EtOAc extract demonstrated the most potent efficacy towards human colon cell RKO, followed by human gastric adenocarcinoma cell 7901 and human liver carcinoma cell HepG2 with IC_{50} values of 0.021, 0.29 and 1.484 μg/mL, respectively, after 48 h treatment. To further evaluate if the EtOAc extract had selectivity towards cancer cells, we investigated the cytotoxicity of the EtOAc extract toward one normal cell, human pulmonary artery endothelial cell (HPAEC). Compared to the cytotoxicity against the normal cell with IC_{50} value > 20 μg/mL, the EtOAc extract displayed greater cytotoxicity towards cancer cells *in vitro*. In conclusion, the EtOAc extract potently inhibits various types of cancer cells with high preference for RKO and 7901. Thus, RKO and 7901 cells were opted to furtherly investigate the effect of EtOAc extract on cancer cells. As illustrated in Fig. 3, compared to HPAEC, the cell viability of RKO and 7901 cells were dramatically decreased when the concentration of the EtOAc extract was within 10 μg/mL, and below 30% when reached 10 μg/mL.

Table 3
IC_{50} value of the EtOAc extract against various cells (μg/mL)

Cell types	IC_{50}
Human colon cell RKO	0.02127
Human gastric adenocarcinoma 7901	0.2904
Human liver carcinoma cell HepG2	1.484
Human tongue cancer cell CAL-27	4.861
Human breast carcinoma cell MCF-7	6.986
Human chronic promyelocytic leukemia cell K562	8.106
Human cervical cancer cell Hela	10.87
Human pancreatic cancer cell SW1190	12.98
Human non-small cell lung cancer A549	16.94
Human pulmonary artery endothelial cell HPAEC (Human normal cell)	20.14

Induction of apoptosis in 7901 and RKO cells

Inducing apoptosis and necrosis of tumor cells is the main mechanism of chemotherapy drugs inhibiting tumors, and it is also one of the main indicators for evaluating the efficacy of chemotherapy drugs (23, 24). Herein, we quantitatively detected the cell death type triggered by the EtOAc extract in 7901 and RKO cells using the annexin V-FITC and PI double staining, which were presented in Fig. 4a. After the cells were processed with the EtOAc extract (2 μg/mL) for 48 h, FITC-positive cells accounted for ~ 50% and ~ 40% of the total cells in 7901 and RKO cells, respectively, suggesting that apoptosis was a major mechanism of the cytotoxicity of the EtOAc extract whether in 7901 or RKO cell, and the EtOAc extract induced apoptotic cell death in a dose-dependent manner in both two cells (Fig. 4b). However, the apoptosis patterns of the RKO and 7901 cells were distinctly diverse. As demonstrated in Fig. 4c, for the 7901 cell, the proportion of early apoptotic cells was higher than that of late apoptotic cells at low concentration of the EtOAc extract; however, the number of early apoptotic cells gradually decreased as the concentration increased, while the number of late apoptotic cells sharply increased with the increased concentration of EtOAc extract. In contrast, for the RKO cell, the late apoptotic cells were dominant at first and displayed a dose-dependant manner, while the number of early apoptotic cells slightly increased with the increasing of concentration.

The EtOAc extract inhibits the cell cycles of 7901 and RKO cells

Flow cytometric analysis of DNA showed a dose-dependent accumulation of cells in the S phase of the cell cycle both in 7901 and RKO cells, with a concomitant decrease in the proportion of cells in the G_0/G_1 phase when treated with a concentration gradient of the EtOAc extract for 48 h, indicating the EtOAc extract blocked the cell cycle of 7901 and RKO in S phase (Fig. 5a and 5b).

Antimicrobial activity of the EtOAc extract

The antimicrobial potential of EtOAc extract from *Streptomyces* sp. LRE541 was investigated against four human pathogens. As presented in Fig. 6, the EtOAc extract showed antagonistic activities against all of the tested microorganisms, and the inhibition zone diameters of the four human pathogens displayed a dose-dependent manner. However, the antimicrobial potent of the EtOAc extract against the four pathogens varied. For example, when the concentration of the EtOAc extract was 100 μg/mL, the maximum activity was found against *E. coli* ATCC 25922 (inhibition zone of 10.94 ± 0.45361 mm diameter), followed by MRSA ATCC 25923 (10.7567 ± 0.92716 mm), *P. aeruginosa* (10.4533 ± 0.47154 mm) and *C. albicans* ATCC 66415 (7.665 ± 0.78421 mm).

Chemical Profiling of the EtOAc extract Using UHPLC-MS/MS Analysis

To examine the compounds that may be responsible for its antineoplastic and antimicrobial properties, the EtOAc extract of isolate LRE541 was subjected to ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis (Additional file 1: Fig. S1). Based on the UHPLC-MS/MS analysis, we successfully detected thirty-nine antitumor compounds, ten potential antitneoplastic compounds, sixteen antimicrobial compounds and thirty compounds without specific literatures to support their biological activities, but with a relative ratio above 1% in the EtOAc extract. The detailed information of the ninety-five chemical compounds, including retention time, molecular formula, molecular weight and relative ratio, are listed in Table 4 and Additional file 1: Table S1, respectively, and their chemical structures are presented in Fig. 7 and Additional file 1: Fig. S2.

Table 4
Chemical constituents of antitumor, potential antitumor, and antimicrobial compounds identified in the EtOAc extract of LRE541.

Mode	No.	Constituents	Molecular formula	*m/z*	Retention time (min)	Relative ratio (%)
ESI (−)	1	Ferulic acid	$C_{10}H_{10}O_4$	194.05786	8.241	15.3965
	2	4-Hydroxybenzylalcohol	$C_7H_8O_2$	124.05242	8.554	0.6811
	3	Formononetin	$C_{16}H_{12}O_4$	268.07325	13.577	0.5133
	4	Sinapinic acid	$C_{11}H_{12}O_5$	224.06869	8.077	0.2469
	5	(+/-)-Equol	$C_{15}H_{14}O_3$	242.09479	9.867	0.1788
	6	4-Hydroxycoumarin	$C_9H_6O_3$	162.03198	6.413	0.0484
	7	Resveratrol	$C_{14}H_{12}O_3$	228.07894	9.699	0.015
	8	Catechin	$C_{15}H_{14}O_6$	290.07954	10.018	0.009
	9	Naringenin	$C_{15}H_{12}O_5$	272.06889	9.903	0.003
	10	Ursolic acid	$C_{30}H_{48}O_3$	456.36082	14.59	0.0002
	40	4-Hydroxyphenylacetic acid*	$C_8H_8O_3$	152.047	8.944	5.77495
	41	Eugenol*	$C_{10}H_{12}O_2$	164.084	11.048	1.73939
	42	Rosmarinic Acid*	$C_{18}H_{16}O_8$	360.085	9.712	0.0009
	43	Eriodictyol*	$C_{15}H_{12}O_6$	288.064	7.485	0.0008
	44	7-Methylguanosine*	$C_{11}H_{17}N_5O_5$	299.124	11.823	0.0003
	50	Lauric acid**	$C_{12}H_{24}O_2$	200.178	13.361	1.1963
	51	trans-Cinnamic acid**	$C_9H_8O_2$	148.05252	8.859	0.7326
	52	Coniferyl alcohol**	$C_{10}H_{12}O_3$	180.07876	9.483	0.4579
	53	Citrinin**	$C_{13}H_{14}O_5$	250.08446	8.533	0.1862
	54	Mycophenolic acid**	$C_{17}H_{20}O_6$	320.12639	9.934	0.0751
	55	Undecanoic acid**	$C_{11}H_{22}O_2$	186.16217	12.79	0.0358

* denotes potential antitumor compounds; ** denotes antimicrobial compounds.

Mode	No.	Constituents	Molecular formula	*m/z*	Retention time (min)	Relative ratio (%)
	56	2-Hydroxy-1,4-naphthoquinone**	$C_{10}H_6O_3$	174.03177	6.412	0.0058
ESI (+)	11	4-Methylumbelliferone	$C_{10}H_8O_3$	176.04741	8.625	2.2697
	12	7-Hydroxycoumarine	$C_9H_6O_3$	162.03168	7.999	0.4477
	13	Citral	$C_{10}H_{16}O$	152.12002	10.117	0.2339
	14	2-Oxindole	C_8H_7NO	133.05268	8.707	0.2231
	15	Alternariol	$C_{14}H_{10}O_5$	258.05156	10.075	0.2003
	16	4-Phenylbutyric acid	$C_{10}H_{12}O_2$	164.08382	11.336	0.1944
	17	Coumarin	$C_9H_6O_2$	146.03678	8.096	0.1679
	18	Genistein	$C_{15}H_{10}O_5$	270.05259	10.735	0.1268
	19	Cuminaldehyde	$C_{10}H_{12}O$	148.08881	13.689	0.095
	20	Isorhapontigenin	$C_{15}H_{14}O_4$	258.08888	9.087	0.0886
	21	3-Acetoxyurs-12-en-23-oic acid	$C_{32}H_{50}O_4$	476.38654	13.587	0.0639
	22	Shikonin	$C_{16}H_{16}O_5$	288.09939	7.453	0.0321
	23	Glycocholic acid	$C_{26}H_{43}NO_6$	465.30892	13.916	0.0291
	24	18-β-Glycyrrhetinic acid	$C_{30}H_{46}O_4$	470.33931	12.8	0.0236
	25	(+)-ar-Turmerone	$C_{15}H_{20}O$	216.15145	10.875	0.0199
	26	Betulin	$C_{30}H_{50}O_2$	442.38092	14.295	0.0172
	27	5'-S-Methyl-5'-thioadenosine	$C_{11}H_{15}N_5O_3S$	297.08953	6.514	0.0142
	28	D-(+)-Camphor	$C_{10}H_{16}O$	152.12003	12.35	0.014
	29	D-Sphingosine	$C_{18}H_{37}NO_2$	299.28214	12.019	0.0101
	30	Viramune	$C_{15}H_{14}N_4O$	266.11528	10.455	0.0083
	31	Diosmetin	$C_{16}H_{12}O_6$	300.06322	10.742	0.0066

* denotes potential antitumor compounds; ** denotes antimicrobial compounds.

Mode	No.	Constituents	Molecular formula	*m/z*	Retention time (min)	Relative ratio (%)
	32	1-Caffeoylquinic Acid	$C_{16}H_{18}O_9$	354.09499	7.112	0.0062
	33	Cryptotanshinone	$C_{19}H_{20}O_3$	296.1418	11.047	0.0015
	34	β-Lapachone	$C_{15}H_{14}O_3$	242.09442	11.613	0.0013
	35	Diosgenin	$C_{27}H_{42}O_3$	396.30347	12.826	0.0011
	36	Nobiletin	$C_{21}H_{22}O_8$	402.13108	12.035	0.0006
	37	Fisetin	$C_{15}H_{10}O_6$	286.04747	10.069	0.0005
	38	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.0735	13.644	0.0004
	39	Veratramine	$C_{27}H_{39}NO_2$	409.29756	11.481	0.0003
	45	Daidzein*	$C_{15}H_{10}O_4$	254.058	10.19	3.09282
	46	Apocynin*	$C_9H_{10}O_3$	166.063	8.871	2.2817
	47	Glycitein*	$C_{16}H_{12}O_5$	284.068	10.314	0.4888
	48	(-)-Caryophyllene oxide*	$C_{15}H_{24}O$	220.183	13.938	0.2325
	49	Cynaropicrin*	$C_{19}H_{22}O_6$	346.141	9.674	0.0407
	57	Sorbic acid**	$C_6H_8O_2$	112.05235	6.724	14.8589
	58	3,5-Dimethoxybenzoic acid**	$C_9H_{10}O_4$	182.058	6.835	2.7591
	59	Methyl cinnamate**	$C_{10}H_{10}O_2$	162.06811	10.654	0.30
	60	trans-Cinnamaldehyde**	C_9H_8O	132.05749	5.453	0.2613
	61	2-Oxindole**	C_8H_7NO	133.05268	8.707	0.2231
	62	5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chromen-4-one **	$C_{17}H_{14}O_5$	298.08368	10.553	0.0333
	63	3,4-Dihydroxybenzaldehyde**	$C_7H_6O_3$	138.02959	1.666	0.0189
	64	Cefradine**	$C_{16}H_{19}N_3O_4S$	349.1028	13.521	0.0101
	65	Pleuromutilin**	$C_{22}H_{34}O_5$	378.24019	11.912	0.0069

* denotes potential antitumor compounds; ** denotes antimicrobial compounds.

Discussion

To date, despite cancer treatment has made great advancement and innovation based on the development of basic science, genetic engineering technology and big data analysis technology, cancer remains one of the most refractory diseases nowadays, and the number of patients has increased year by year, causing a heavy burden on families and society (2, 25, 26). Moreover, the frequently occurrence of multidrug resistant pathogenic microorganisms is another challenge for researchers (40). Therefore, the discovery and development of safer and novel drugs based on natural products has become one of the research hotspots (27–29). While as an emerging source of novel natural products, the endophytic actinomycete has attracted increasing attention to academics (30–32). Our study obtained an endophytic isolate, designated as LRE541 from the root tissues of *Lilium davidii* var. unicolor Cotton, and found that the secondary metabolites of LRE541 possess potent antitumor and antimicrobial potential by inhibiting a variety of malignancies and pathogens growth.

The isolate LRE541, well-characterized by the comparative analysis of 16S rDNA sequence, was assigned to *Streptomyces* sp. The phylogenetic relationship demonstrated that isolate LRE541 formed a distinct branch with the highest 16S rDNA sequence similarity of 98.81% to the type strain *Streptomyces tauricus* JCM4837^T. Phenotypically, isolate LRE541 grew well on all the tested media with diverse aerial and substrate myceliums, but produced red diffusible pigment only on Gao's No. 1 medium. The extracellular enzyme tests found that LRE541 had the potential to yield various enzymes such as protease, amylase and lipase, which are industrially important. In addition, LRE541 could tolerate high pH value up to 12.0, a salinity of 6% NaCl, and temperature up to 37 °C. In sum, these physiological traits are consistent with the characteristics of streptomycetes that they are prolific and possess high adaptive capability for surviving in many unique niches (33–35), what's more, reflecting the physiological flexibility of *Streptomyces* isolate to adverse environmental conditions (36, 37). Furthermore, the availability of a broad spectrum of carbon and nitrogen sources plays a vital role in the production of diverse secondary metabolites by *Streptomyces* sp. (38). Here, isolate LRE541 also exhibited the capability to utilize a wide range of carbon and nitrogen sources. This data provided an overview of the metabolite profile of LRE541, potentially serving as references for future research concerning fermentation optimization for higher yield of the desirable bioactive metabolites.

In view of the remarkable physiological capabilities above mentioned, the secondary metabolites (EtOAc extract) of isolate LRE541 was examined against nine representative human malignant tumors *in vitro*. The result revealed that the EtOAc extract showed cytotoxic activity towards all of the tumor cells with IC_{50} < 20 μg/mL, which is within the cut off point of cytotoxicity criteria recommended by the National Cancer Institute (NCI) for screening the cytotoxicity of crude plant extracts (39, 40). Moreover, the EtOAc extract exhibited cytotoxic activity against approximately 70% of the examined cancer cells with IC_{50} < 10 μg/mL, and marvelous antitumor potential against RKO, 7901 with IC_{50} value of only 0.021 and 0.29 μg/mL, respectively. However, it displayed a lower cytotoxic activity against human normal cell

HPAEC with IC_{50} > 20 μg/mL. These results suggested that the EtOAc extract exerted potent and selective cytotoxic activity towards cancer cells. When tested against HepG2 cell line, the EtOAc extract (IC_{50} = 1.484 μg/mL) was shown to be more toxic than shikonin (IC_{50} = 4.3 μg/mL), a chemotherapeutic drug candidate, which was extracted from *Lithospermum erythrorhizon* Siebold *&* Zucc. (41, 42). When tested against MCF-7, several extracts from fungal endophytes (7 μg/mL ≤ IC_{50} ≤ 20 μg/mL) (43) and chromomycin SA (IC_{50} = 12.6 μg/mL) from *Streptomyces* sp. KML-2 inhabiting halophilic mines (44) showed less cytotoxic than the EtOAc extract (IC_{50} = 6.986 μg/mL). However, the EtOAc extract against Hela cell with IC_{50} value of 10.87 μg/mL, displayed less cytotoxic than Chromomycin SA (IC_{50} = 8.9 μg/mL) (44), while more cytotoxic than 6-gingerol and 6-shogaol extract (IC_{50} = 20.9 μg/mL) from *Zingiber officinale* Roscoe (45). The cytotoxic activity of the EtOAc extract against A549 (IC_{50} = 16.94 μg/mL) was found to be the weakest compared to the other cancer cells in this study, but examined to be more toxic than the extract from the endophyte *Hypocrea* lixii R18 (IC_{50} = 20.5 μg/mL) (46). Besides, the EtOAc extract from *Streptomyces* sp. LRE541 performed excellent antitumor effects towards K562 (IC_{50} = 8.106 μg/mL), SW1190 (IC_{50} = 12.98 μg/mL) and CAL − 27 (IC_{50} = 4.861 μg/mL), which represent leading causes of cancer-related death. Similarly, N. Kim et al. (47) reported the cytotoxic activities of salaceyins A and B from endophytic *Streptomyces laceyi* MS53 against SKBR3 with IC_{50} values of 3.0 and 5.5 μg/mL, respectively. Naphtomycin A from endophytic *Streptomyces* sp. CS showed cytotoxic activity against P388 and A549 cells with IC_{50} of 0.07 and 3.17 mM, respectively (48, 49). The cytotoxicity of 3-acetonylidene-7-prenylindolin-2-one, 7-isoprenylindole-3-carboxylic acid against A549 presented IC_{50} values of 3.3 and 5.1 μg/mL, respectively (50). Taken together, these results suggested that the endophytic isolate LRE541 colonizing the root tissues of *Lilium davidii* var. unicolor Cotton, could produce secondary metabolites with antitumor activities *in vitro*, but exhibit different anticancer potent against different cancer cells. This may be related to the specific molecular genetic background of different cancer cells, but further research will be needed.

It has been confirmed that apoptosis and necrosis are two patterns of cell death (51, 52). Compared to necrosis, an abnormal form of cell death, cell apoptosis regulated by various intra and extracellular signals and governed by several genes, plays a significant role in stress responses, control of normal cell proliferation and development of an organism (53, 54). While tumorigenesis is closely related to anti-apoptotic pathways (6). Thus, druginduced apoptosis of malignant cells is an efficient strategy in cancer therapy (6, 55). Our data presented that the EtOAc extract from the endophytic *Streptomyces* sp. LRE541 validly inhibited the cell viabilities of RKO and 7901 cells predominantly through the induction of apoptosis in a dose-dependant manner. Apparently, the apoptosis patterns between the two cells were greatly diverse, which suggested distinct mechanisms of the secondary metabolites actions occurring in the two cancer cells. Furthermore, previous studies have shown that cell cycle is likewise intimately associated with the tumorigenesis. Pathological or physiological apoptotic stimuli would greatly affect cell cycle progression, and disorder of cell cycle regulators is a common property of human cancer, which signifies that regulation of cell cycle progression in cancer cells is taken for an available method in the treatment of human malignancies (56, 57). This study, the EtOAc extract dramatically inhibited the cell

proliferation of RKO and 7901 in a dose-dependent manner through inducing S phase arrest of cell cycle and apoptosis *in vitro*. Several studies had discovered that chemotherapeutic drugs originating from marine actinomycetes could cause cancer cell cycle arrest as well. Echinosporin and 7-deoxyechinosporin produced by *Streptomyces albogriseolus* A2002 were exhibited to block the cell cycle of HCT-15, K562 and tsFT210 cells mainly at the G_0/G_1 phase and promote apoptosis in these cells (58). Proximicins obtained from two rare actinomycetes *Verrucosispora* sp. MG-37 and *Verrucosispora maris* AB-18-032 isolated from marine sediments were found to block human gastric adenocarcinoma AGS cells at G_0/G_1 phase. Likewise, thiocoraline produced by the marine actinomycete *Micromonospora* sp. L-13-ACM2-092 caused an arrest in G_1 phase of the cell cycle of human colon cancer cell lines (18). Collectively, chemotherapeutics with greater therapeutic efficiency and fewer side effects are of utmost desirability, and drug induced cancer cell death mode plays an important role in chemotherapy.

In the current study, the antagonistic activity of EtOAc extract associated with isolate LRE541 was screened *in vitro* condition by the agar well diffusion method against a panel of microbial pathogens. The results presented antimicrobial activities against Gram-positive, Gram-negative pathogens and yeast-like fugi with the concentration of EtOAc extract within 100 μg/mL. Interestingly, the antagonistic potent against the tested pathogens all displayed a dose-dependent manner, however, varied among different pathogens. This indicated diverse mechanisms of the EtOAc extract against those microorganisms. Similarly, the antagonism of endophytic *Streptomyces* sp. in this study has been discovered in other reports as well. For example, the endophytic *Streptomyces* sp. T3SB005 from root tissues of *Thymus roseus* exhibited antagonistic activities against three human pathogens (59); the culture filtrate of *Streptomyces* sp. HUST012 from *Dracaena cochinchinensis* Lour. was found against *E. coli* ATCC 25922 and MRSA ATCC 25923 with an inhibition zone of 18.9 mm (5). These findings suggested that endophytic streptomycetes may be exciting sources of bioactive compounds.

The antineoplastic and antimicrobial capacities of the EtOAc extract from isolate LRE541 suggest that the presence of antitumor and antimicrobial agents in the mixture of the extract may account for it. And we pursued this via ultra-high performance liquid chromatography-tandem mass spectrometry method. Based on the UHPLC-MS/MS analysis, the EtOAc extract was detected to contain thirty-nine compounds testified to exhibit various antitumor activities and cytotoxicity, besides, sixteen compounds proved to be antagonistic against diverse microorganisms. For example, ferulic acid, relative ratio of 15.3965% in the ESI (−) mode, as a new fibroblast growth factor receptor 1 (FGFR1) inhibitor, could inhibit the melanoma growth and angiogenesis using a melanoma model *in vivo* (60); similarly, 4-Hydroxybenzylalcohol, a promising anti-angiogenic agent, suppressed the vascularization and growth of newly developing CT26.WT tumors *in vivo* (61); while formononetin and catechin suppressed MCF-7 proliferation through blocking the cell cycle arrest or inducing apoptosis, respectively (62, 63); citral in combination with doxorubicin, and glycocholic acid with epirubicin, can dramatically increase the chemosensitivity of doxorubicin in human lymphoma Ramos cells, epirubicin in Caco-2 cells, respectively (64, 65). Sorbic acid and Lauric acid with relative ratios of 14.9% and 1.2% in the ESI (+)/(−) modes, respectively, have broad-spectrum antagonistic activities against most of the Gram-positive, Gram-negative pathogens and fungi

(66, 67). Cefradine and Pleuromutilin are common broad-spectrum, high effective and low toxic antibiotics. Moreover, trans-Cinnamaldehyde and Methyl cinnamate due to their antimicrobial activities have been used as preservatives in the food industry (68, 69). As a whole, these chemical constitutes detected by UHPLC-MS/MS may be directly responsible for the antineoplastic and antimicrobial properties of the EtOAc extract.

It is reported that oxidative stress and chronic inflammation can develop into cancerous lesions (70, 71). Here, several natural phenolic compounds (rosmarinic acid, 4-Hydroxyphenylacetic acid and eugenol) and flavonoids compounds (eriodictyol, daidzein, glycitein) detected from the EtOAc extract, have been reported to possess excellent antioxidant activity (72–76). (-)-Caryophyllene oxide and cynaropicrin present in the extract had been demonstrated to have potent anti-inflammatory effect (77, 78). A NADPH oxidase inhibitor, 7-Methylguanosine (79), and cNIIIB nucleotidase inhibitor, apocynin (80), were all detected in the extract. Taken together, the above compounds may play an indirectly role in the antitumor activity of the EtOAc extract through antioxidant or anti-inflammatory effects, although further experimental evidence is necessary to testify their antineoplastic activity. Moreover, considering the potent antineoplastic activity of the EtOAc extract in the present study, the antitumor activity of the thirty compounds with relative ratio above 1% deserved to be further research.

Conclusion

This is the first study to estimate the endophytic *Streptomyces* sp. LRE541 isolated from the root tissues of *Lilium davidii* var. unicolor Cotton and examine the antimicrobial and cytotoxic activities of secondary metabolites of the isolate against a panel of microbial pathogens and human malignant cells, further detecting of the cell apoptosis and cell cycle arrest of RKO and 7901 cells by flow cytometry revealed a preliminary mechanism underlying the biological action of the secondary metabolite and might shed light on the potential application of the metabolites in therapy of RKO and 7901 cells. The antitumor, potential antitneoplastic and antimicrobial compounds detected in the EtOAc extract may play directly or indirectly role in the antitneoplastic and antagonistic properties of secondary metabolites from isolate LRE541. Therefore, our study provides a strong push toward further exploration of endophytic *Streptomyces* sp. LRE541 as a cell factory for novel antitumor and antimicrobial agents.

Materials And Methods

Isolation and Purification of LRE541

During March 2017, thirty healthy roots of 3-year-old *Lilium davidii* var. unicolor Cotton were randomly selected from the lily planting farm of Shaojia Shan, (35°57'50.73" N, 103°48'39.69" E, H: 1868 m) located in Qilihe District, Lanzhou City, Gansu Province, China. The plant roots were dug out carefully to ensure its integrity, then kept in aseptic plastic bags at 4 ℃ and processed within 24 h after collection. After washed in running water, the surfaces of the roots were sterilized by sequential immersion in 0.1% Tween 20 for 5 min, following by 75% alcohol for 5 min, a solution of 2% sodium hypochlorite for 5 min, and

10% sodium bicarbonate solution for 5 min. Samples were washed in sterile distilled water at least three times to remove surface sterilization agents. Meanwhile, an aliquot (0.2 mL) of the last washing water was spread on agar plates, and incubated at 28 ℃ for 7 days to confirm surface sterilization. The surface-sterilized roots were then aseptically sectioned by commercial blender and spread onto the isolation media Gao's No. 1 medium supplemented with cycloheximide (25 mg/mL) and nystatin (10 mg/mL), followed by incubation at 23 ℃ for up to 2 weeks. Isolate LRE541 was purified with new Gao's No. 1 medium agar and maintained on slants of Gao' s No.1 agar at 23 ℃ and stored in 20% (v/v) glycerol suspensions at − 20 ℃.

Morphological and physiological characterization

To investigate the morphological and cultural characteristics of isolate LRE541, pure culture of LRE541 was examined every day grown on various international *Streptomyces* project (ISP) media. Micromorphology of the culture was observed under both light microscopy (Olympus IX71) and scanning electron microscopy (SEM) (Hitachi S-3400N) during 7 and 14 days. Physiological characteristics such as extracellular enzyme activity, carbon/nitrogen source utilization and temperature / pH tolerance were evaluated following the methods depicted in the Bergey's Manual of Systematic Bacteriology (81) and the ISP (82).

16S rDNA sequencing and phylogenetic analysis

The genomic DNA (gDNA) of isolate LRE541 was extracted as described by Orsini et al. (83) with minor adjustment. The universal bacterial primers targeted 16S rDNA, 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525 R (5'-AAGGAGGTGATCCAGCCGCA-3'), were used for polymerase chain reaction (PCR) amplification following the manufacture's protocol (Takara, Japan) with optimized adjustment. The checked PCR products were directly subjected to cycle sequencing using an ABI3100 automated sequencer (Beijing Sangon Biotech, Beijing, China). The sequenced 16S rDNA of isolate LRE541 was matched with the nearest gene sequences of *Streptomyces* spp. retrieved from a public database using the EzBioCloud tool with Clustal W program. The phylogenetic tree was constructed by using the neighbor-joining algorithms (84) and *p*-distance model with bootstrap analysis of 1,000 replicates (85) in the MEGA version 7.0 package.

The 16S rDNA sequence of isolate LRE541 has been submitted to the GenBank nucleotide sequence databases under accession no. MK138546.

Fermentation and Extraction of isolate LRE541

Isolate LRE541 cultured on a slant agar medium was inoculated into 500 mL Erlenmeyer flasks each containing 100 mL of the seed medium consisting of 15 g/L soluble starch, 10 g/L soybean powder, 1 g/L NaCl, 5 g/L glucose, 5 g/L tryptone and 5 g/L $CaCO_3$ (pH 7.3). The flasks were cultivated on a rotary shaker (150 rpm) at 28 °C for 3 days. Then the seed culture (11 mL) were transferred into 1,000-mL Erlenmeyer flasks each containing 500 mL of Gao's liquid medium and incubated at 28 ℃, 150 rev min^{-1} for 9 d. After the fermentation process, the biomass was discarded by centrifugation at 10000 g

for 20 min while the supernatant was harvested and extracted three times with an equal volume of ethyl acetate. Then the ethyl acetate fractions were concentrated at 40 ℃ in a rotary vacuum distillation apparatus, and dissolved in DMSO (1 mg/mL) for the investigation of antitumor and antimicrobial activities.

In Vitro **Cytotoxic Assay**

Cell cultures

The antitumor activity of the EtOAc extract was examined against a wide variety of cell lines, including nine human cancer cell lines (HepG-2, SW-1190, CAL-27, 7901, RKO, MCF-7, Hela, K562, A549) and one normal human pulmonary artery endothelial cell (HPAEC), which were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were incubated in RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine and 100 units/mL streptomycin–penicillin, then maintained in a humidified atmosphere of 5% CO_2 at 37 °C.

Cytotoxicity assay

The cell survival was evaluated using the MTT assay (42). In short, the cells were seeded at a density of 1×10^4 cells/well in 96-well plates for 24 h, then the medium was replaced with fresh medium containing different concentrations of ethyl acetate extract (EtOAc extract) for 48 h. Cells treated with DMSO alone were set as controls. Later, 10 μL MTT (5 mg/mL) reagent was added to each well and incubated for an additional 4 h at 37 °C. Absorbance (490 nm) of the medium was measured using a microplate reader (Thermo Scientific Multiskan GO, Finland).

Cell apoptosis analysis

RKO and 7901 cells were seeded in 6-well plates for 24 h, then incubated with EtOAc extract of various concentrations (0, 0.5, 1, and 2 μg/mL) for 48 h. Then, both of the cells were collected and washed with PBS three times. Afterwards, cell samples were stained with fluorescein 5-isothiocyanate (FITC)-conjugated annexin V and PI following the manufacturer's instructions (Zoman Biotech, Beijing, China). Data were obtained and analyzed using a FACS-Canto flow cytometer (BD Biosciences, San Jose, CA, USA) with FlowJo software.

Cell cycle analysis

Cell cycle analysis was also performed by flow cytometry (86, 87). In brief, RKO and 7901 cells were plated in 6-well plates for 24 h, and then incubated with EtOAc extract of various concentrations (0, 0.5, 1 and 2 μg/mL) for 48 h. Then, the cells were harvested and washed with PBS three times, and the percentages of cells in the G_0/G_1, S, and G_2/M phases were analyzed using the FACS-Canto flow cytometer (BD Biosciences, San Jose, CA, USA) in the presence of Propidium Iodide buffer with RNase (Zoman Biotech, Beijing, China).

Evaluation of antimicrobial activities

The antimicrobial activities of the secondary metabolites (EtOAc extract) from strain LRE541 were investigated against four human pathogens, including Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 25923 representing Gram-positive bacteria, *Escherichia coli* ATCC 25922 representing Gram-negative bacteria, a clinical isolated *Pseudomonas aeruginosa* strain representing freshly pathogenic multi-resistant bacterial strain and *Candida albicans* ATCC 66415 representing yeast-like fungi. The EtOAc extract was prepared in ten concentration gradients of 10–100 ug/mL for antimicrobial activity assays by the agar well diffusion method (40), and the antimicrobial activity was assessed by measuring the diameter (mm) of inhibition zone (59). Each test was conducted in sextuplicate, and the DMSO (0.5%) was used as the negative control.

Metabolite profiles by UHPLC-MS/MS Analysis

The EtOAc extract of strain LRE541 was subjected to a Vanquish UHPLC system equipped with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher) for metabolite profile analyse. The processed samples were injected onto a Hyperil Gold column (100 × 2.1 mm, 1.9 μm) using a 16-min linear gradient at a flow rate of 0.2 mL/min. The eluents for the positive polarity mode were eluent A (0.1% formic acid in Water) and eluent B (Methanol), and for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9.0) and eluent B (Methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2-100% B, 12.0 min; 100% B, 14.0 min: 100-2% B, 14.1 min; 2% B, 17 min. Q Exactive series mass spectrometer was operated in positive/negative polarity mode with spray voltage of 3.2 kV, capillary temperature of 320 °C, sheath gas flow rate of 35 arb and aux gas flow rate of 10 arb.

The raw data from UHPLC – ESI – Q – TOF - MS and MS/MS were analyzed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) to perform peak alignment, peak picking, and quantitation for each metabolite. The normalized data was used to predict the molecular formula based on additive ions, molecular ion peaks and fragment ions. And then peaks were matched with the mzCloud (https://www.mzcloud.org/)□mzVault and MassList database to obtained the accurate qualitative and relative quantitative results.

Statistical Analysis

Data are expressed as the means ± SD for at least three independent experiments. SPSS software was applied to perform the statistical analysis, and the statistical differences between two groups were assessed by Student's t test. $P < 0.05$ was used as the criterion for statistical significance.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this paper (and its additional file).

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Contributions

CZ and AM planned and designed the research. KJ, BC, XQ, JL and AM conducted the experiments and analyzed the data. AM wrote the manuscript. All authors were involved in revising the manuscript critically.

Consent for publication

All authors give consent to publish the research in the microbial cell factories.

Ethics approval and consent to participate

Not applicable.

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Figures

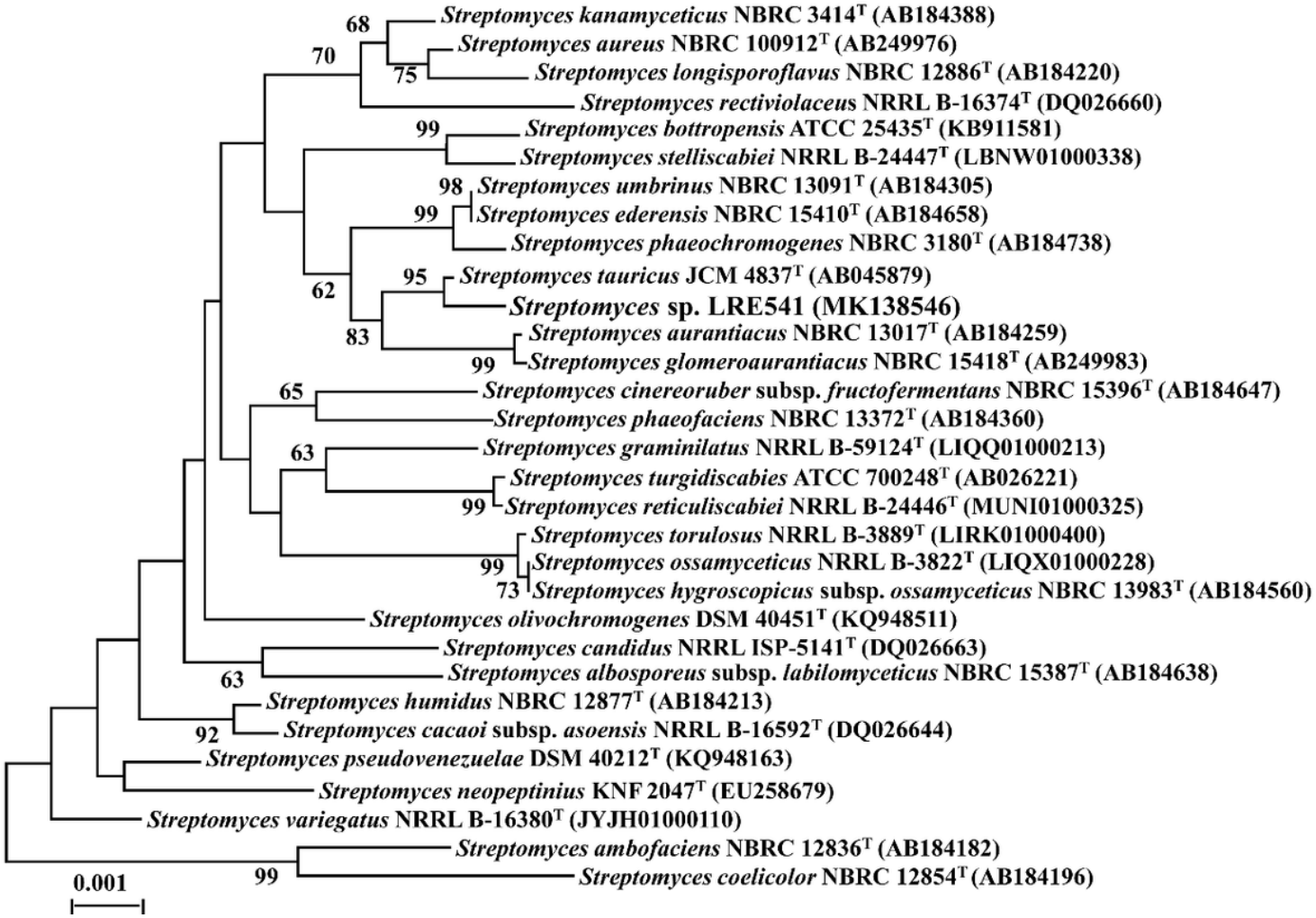
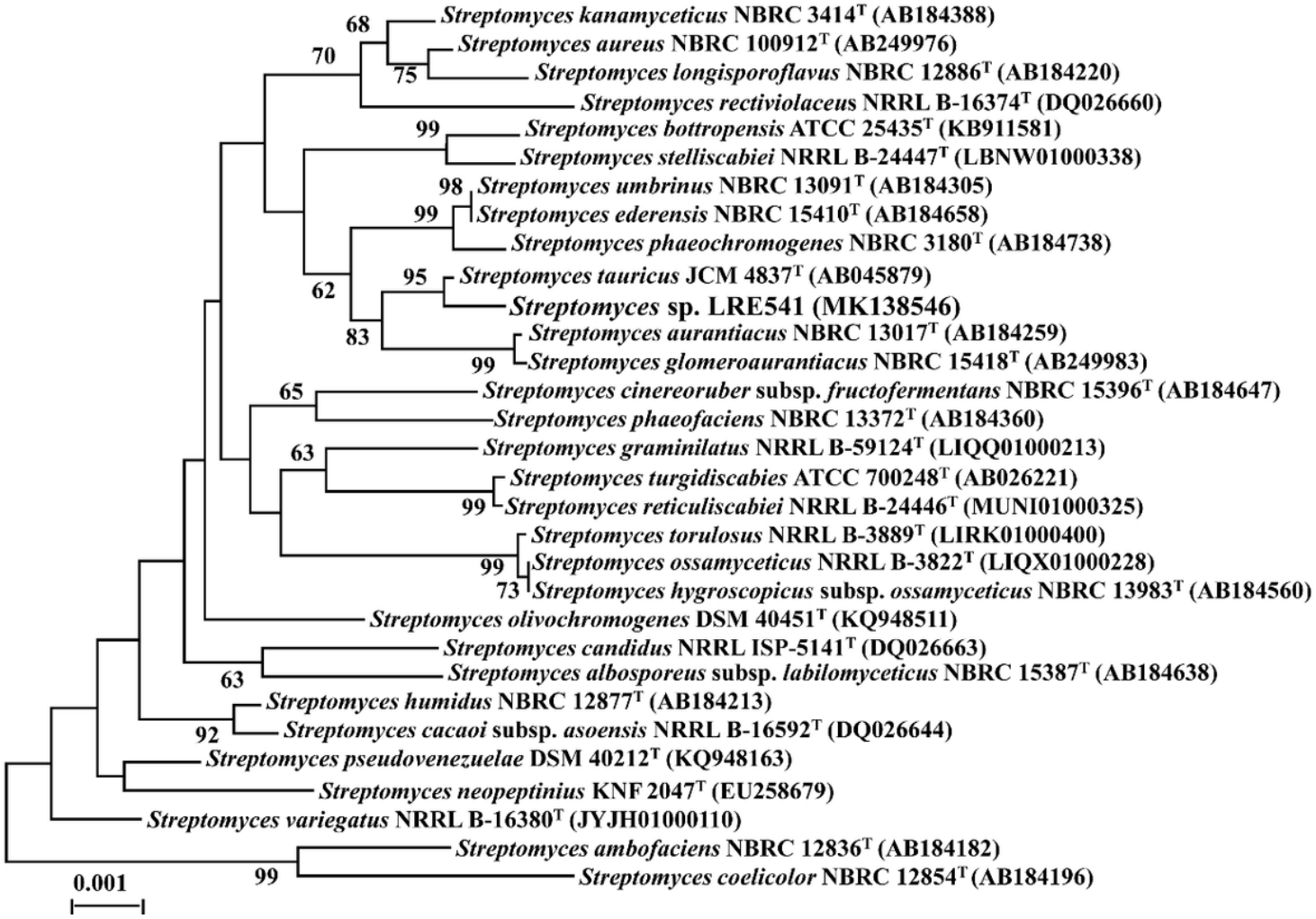


Figure 1

Neighbour-joining tree exhibiting phylogenetic relationship between isolate LRE541 and the closely related representatives of Streptomyces spp. based on almost complete 16S rDNA sequences. Only bootstrap values above 50% are present at the tree nodes. The scale bar denotes 0.001 substitutions per site.



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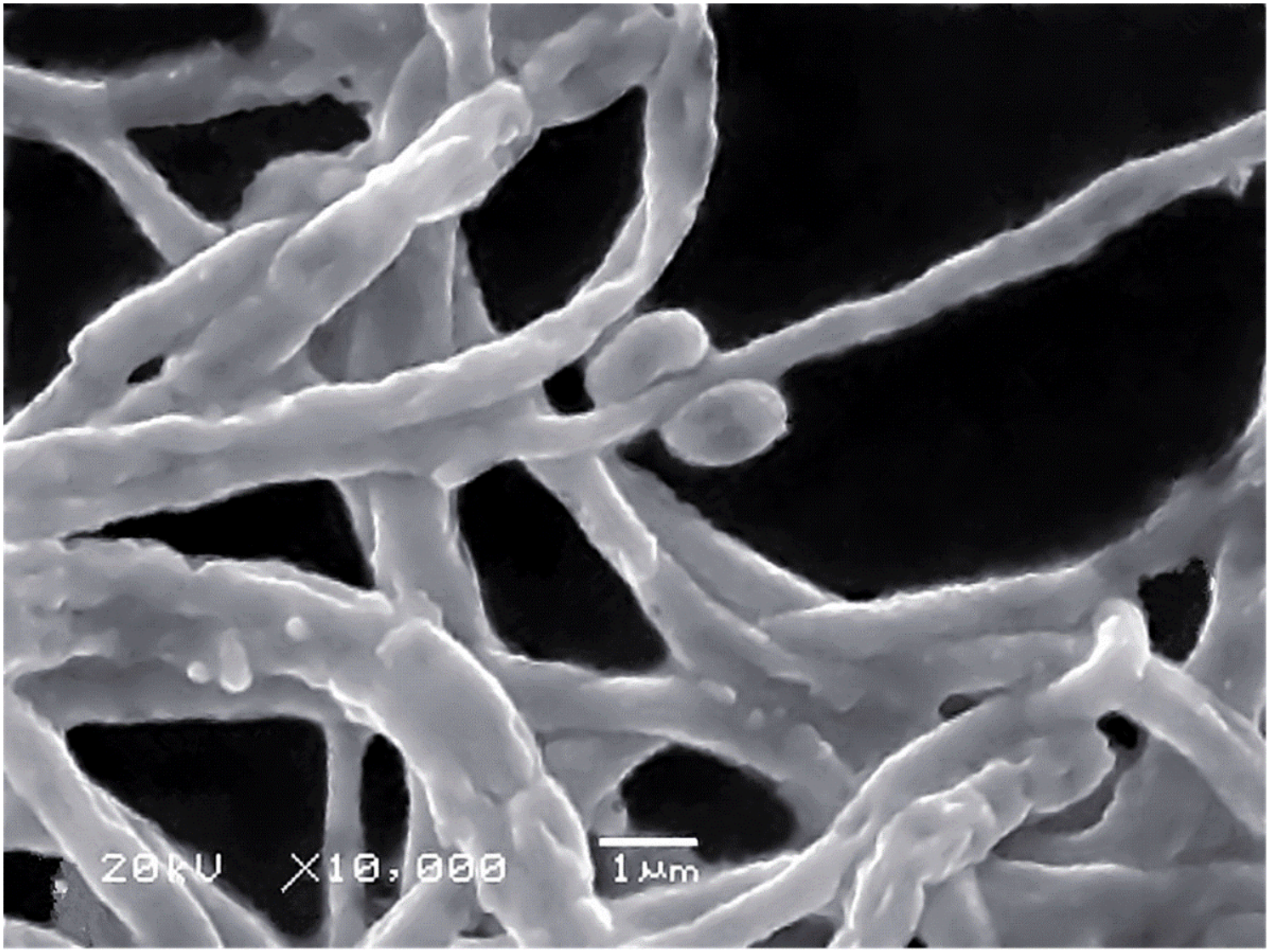
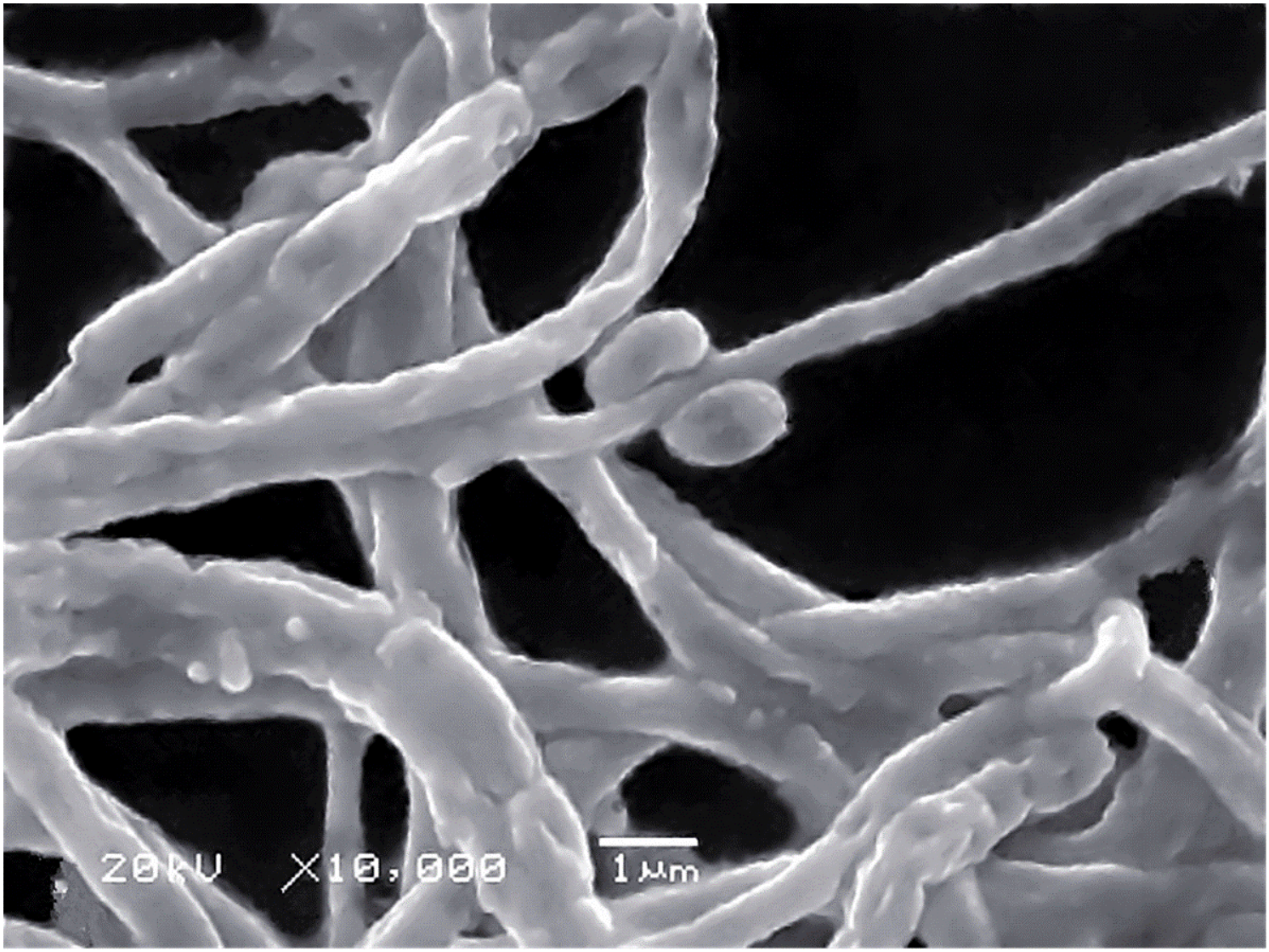


Figure 2

The scanning electron micrograph of Streptomyces sp. LRE541.



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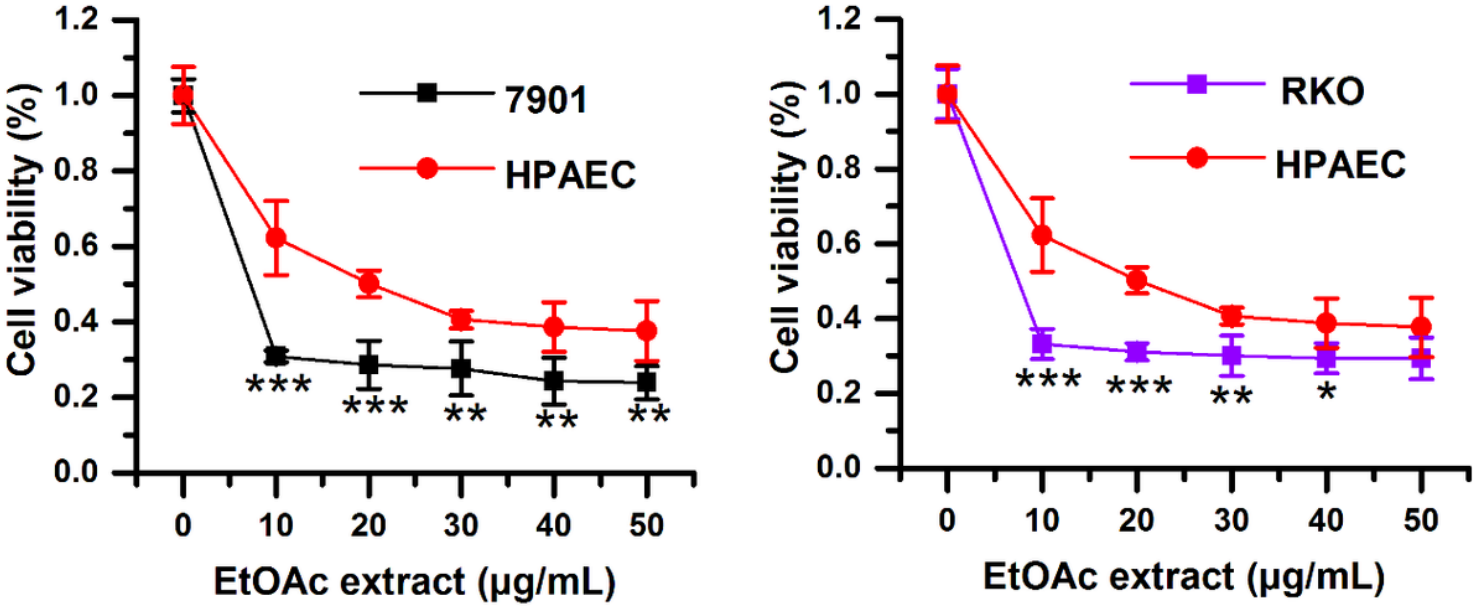
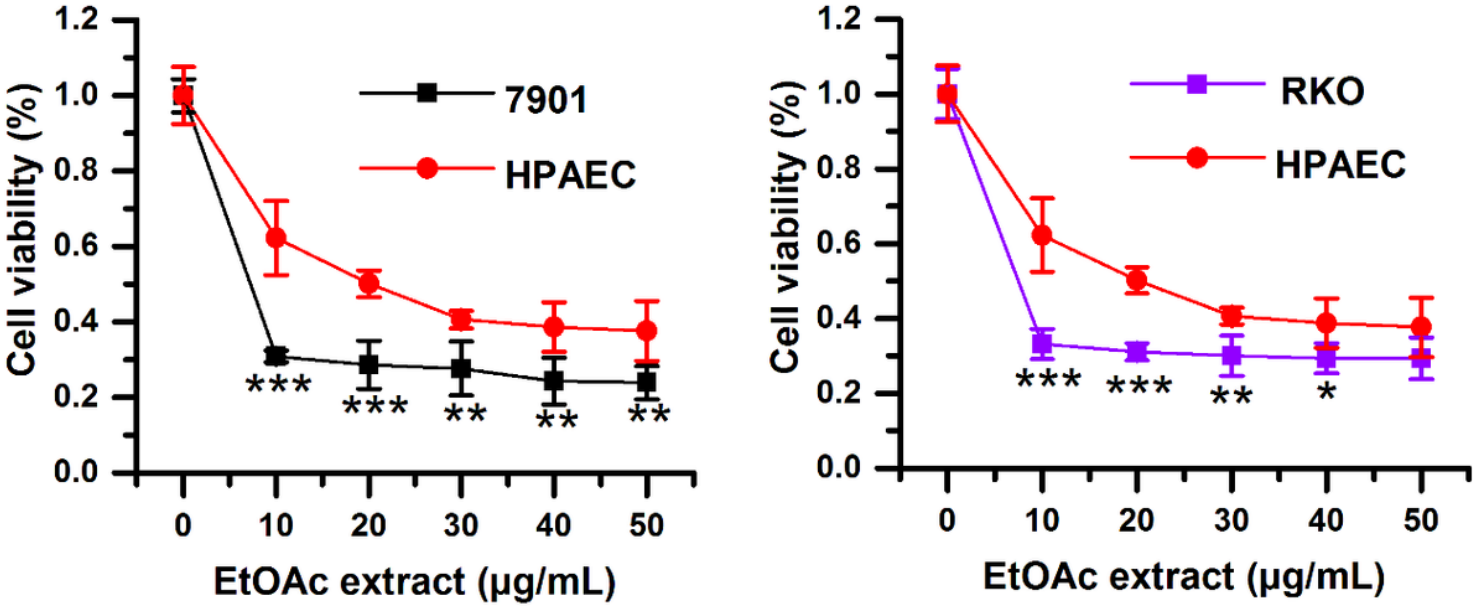


Figure 3

Sensitivity of various types of cells (7901, RKO and HPAEC) to the EtOAc extract. The three cells were incubated with increasing concentrations of the EtOAc extract for 48 h, and their viability were determined by the MTT method. *P < 0.05, **P < 0.01, ***P < 0.001 vs the HPAEC cell.



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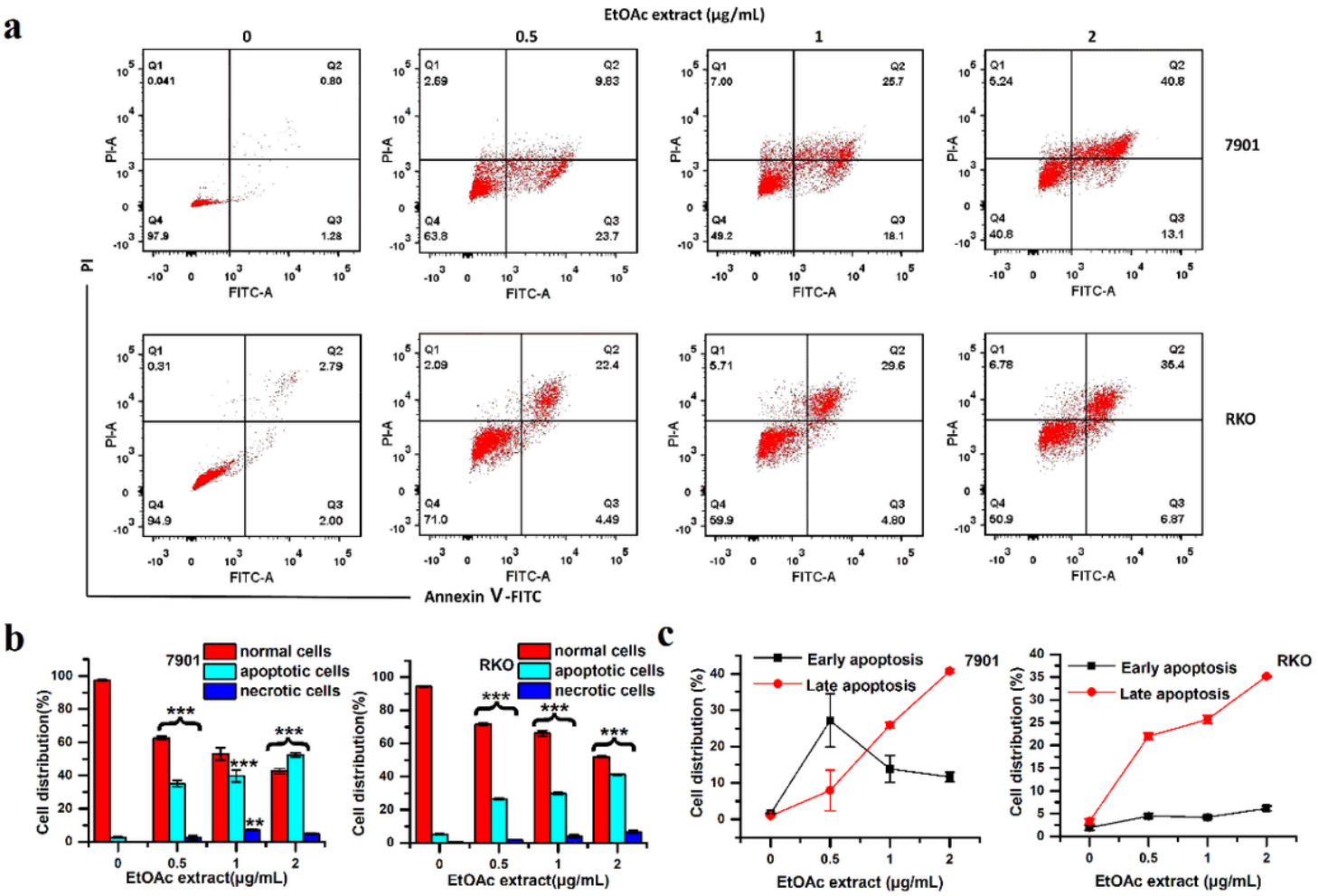
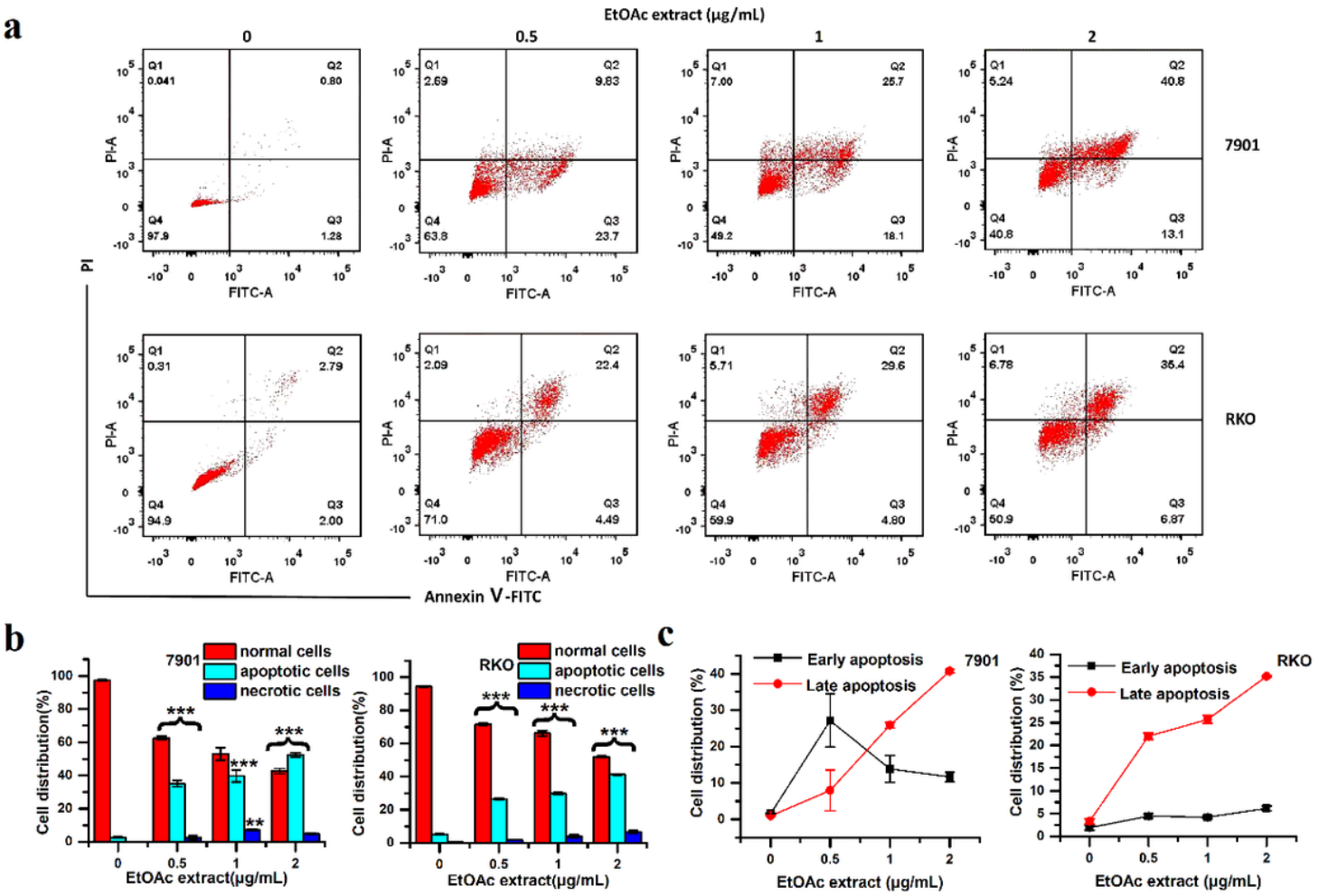


Figure 4

The EtOAc extract induces apoptosis in RKO and 7901 cells. (a) The apoptotic changes in RKO and 7901 cells when treated with increasing concentrations of the EtOAc extract for 48 h by Annexin V and PI double-staining assay. (b) The quantification of necrotic cells, apoptotic cells and normal cells. The datas are displayed as the mean ± SE of three independent samples. *P < 0.05, **P < 0.01, ***P < 0.001 vs the control groups. (c) Early and late apoptosis cells of 7901 and RKO cells treated with varying concentrations of the EtOAc extract for 48 h.



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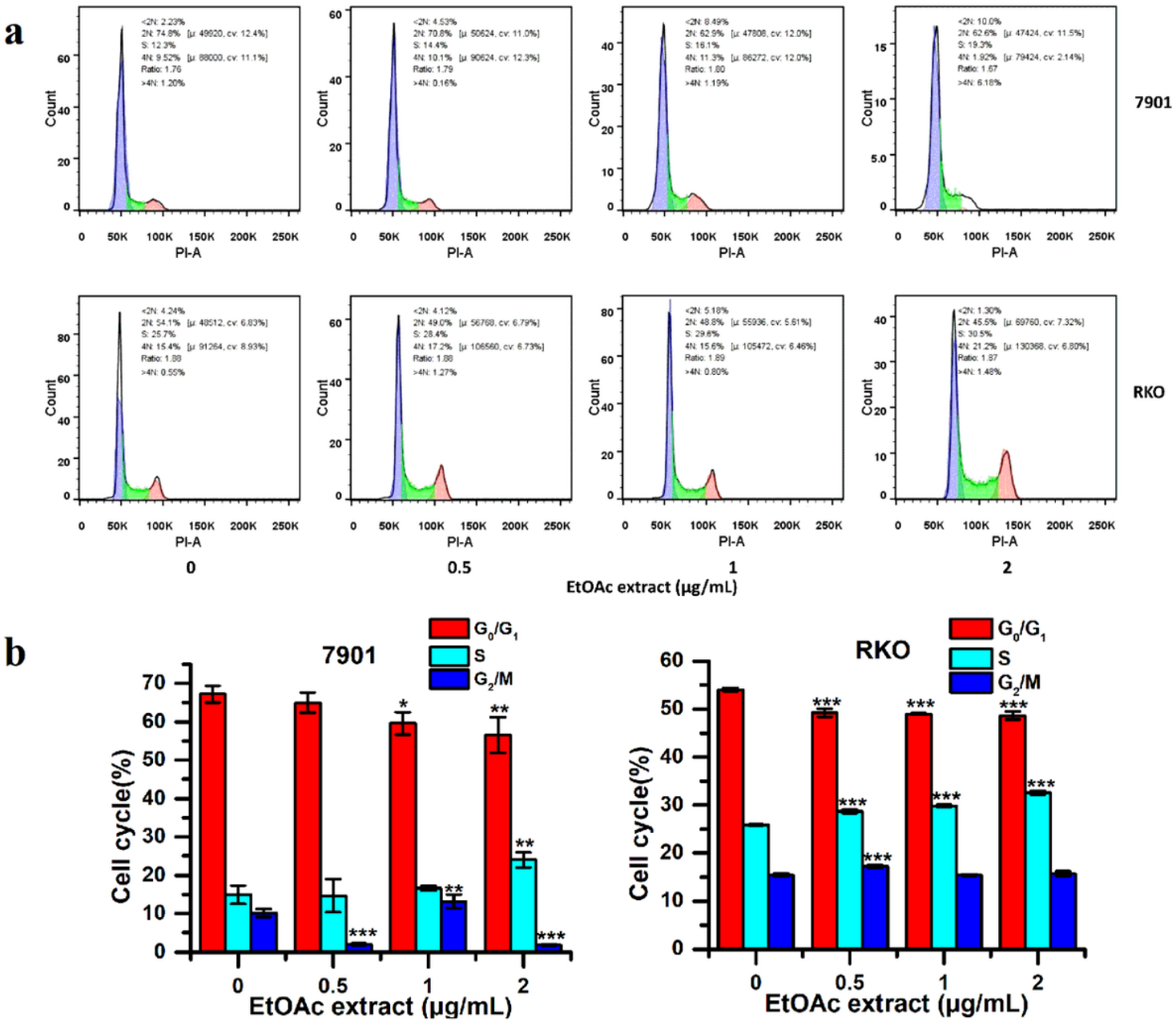

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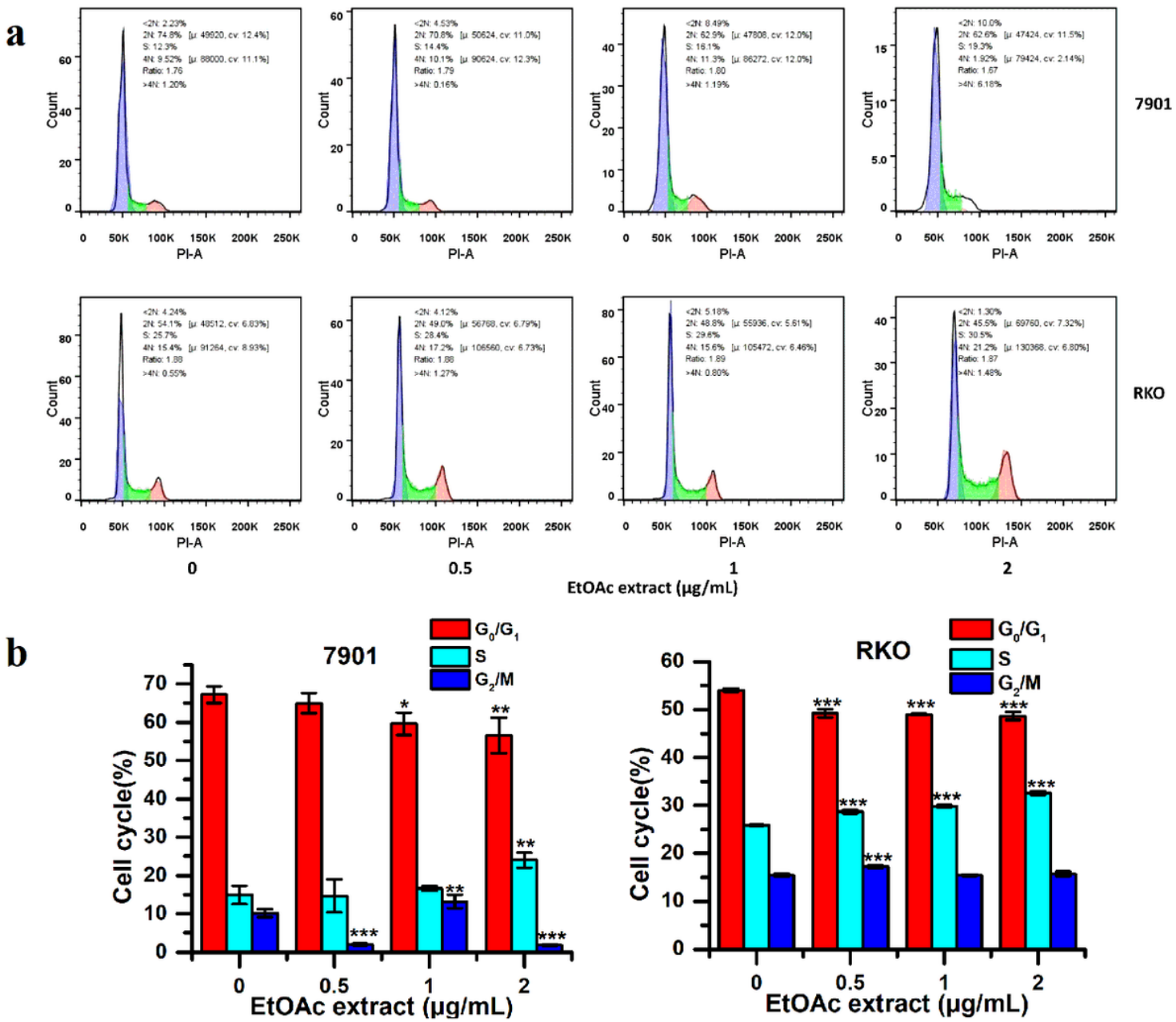

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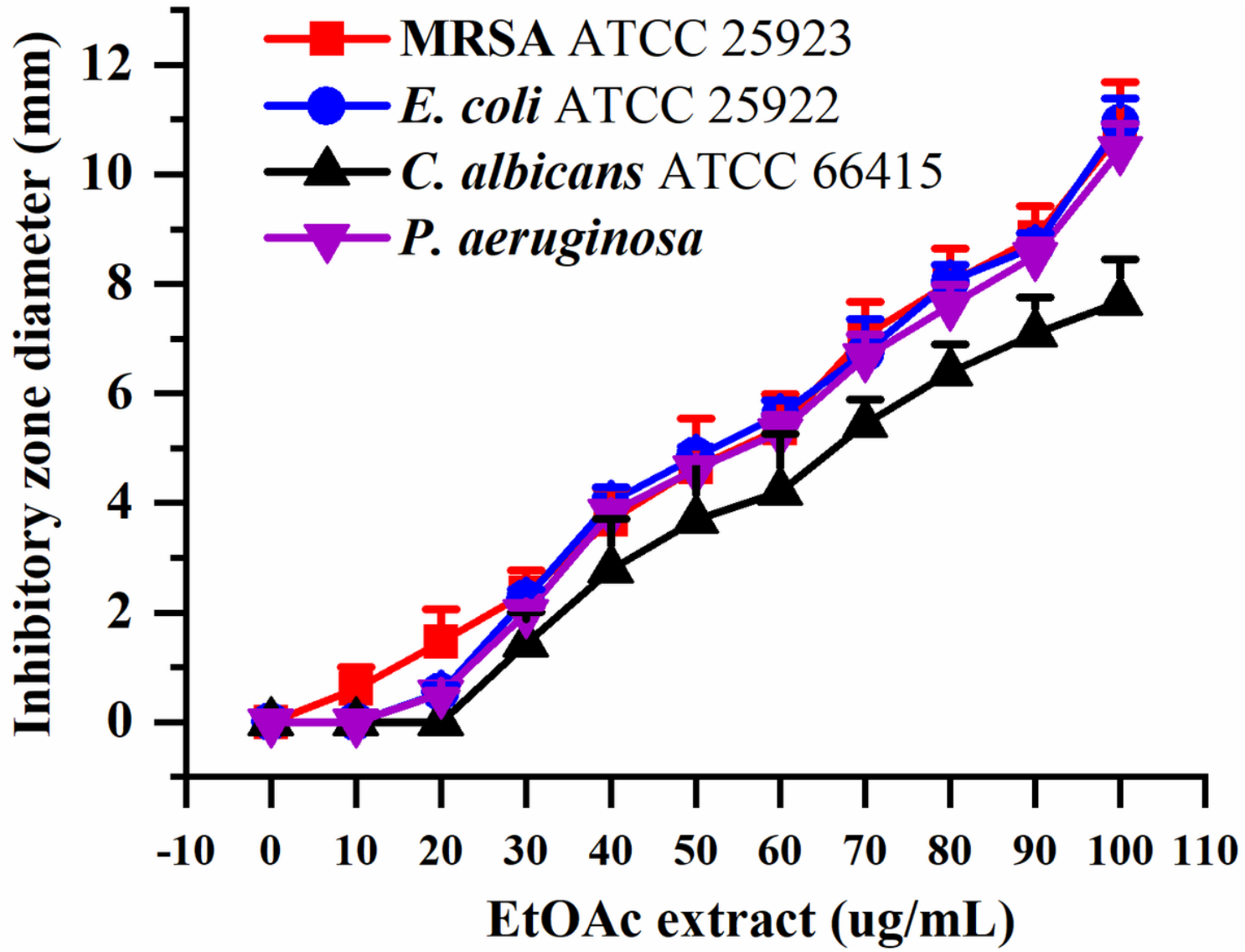
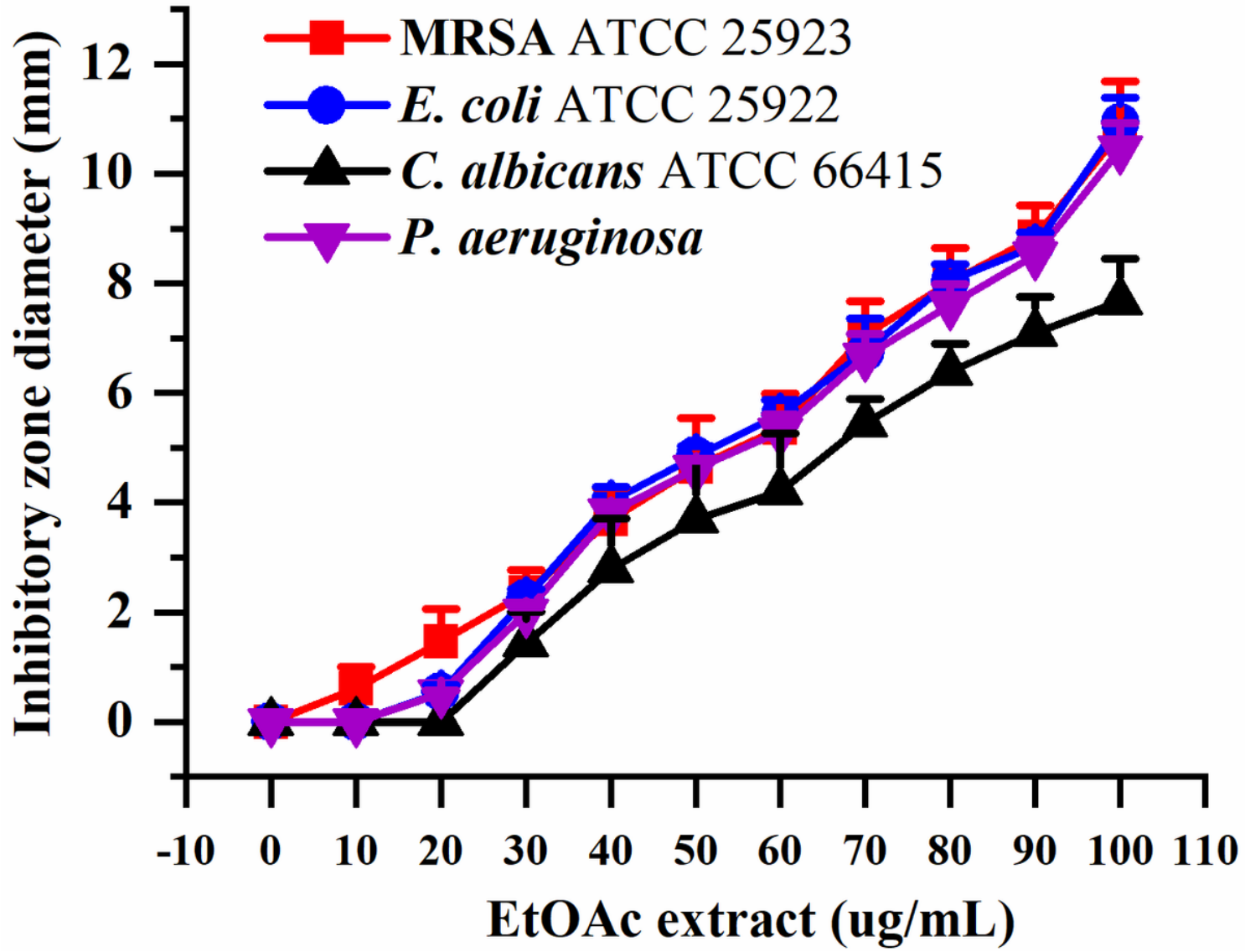


Figure 6

Effects of the EtOAc extract on various microbial pathogens



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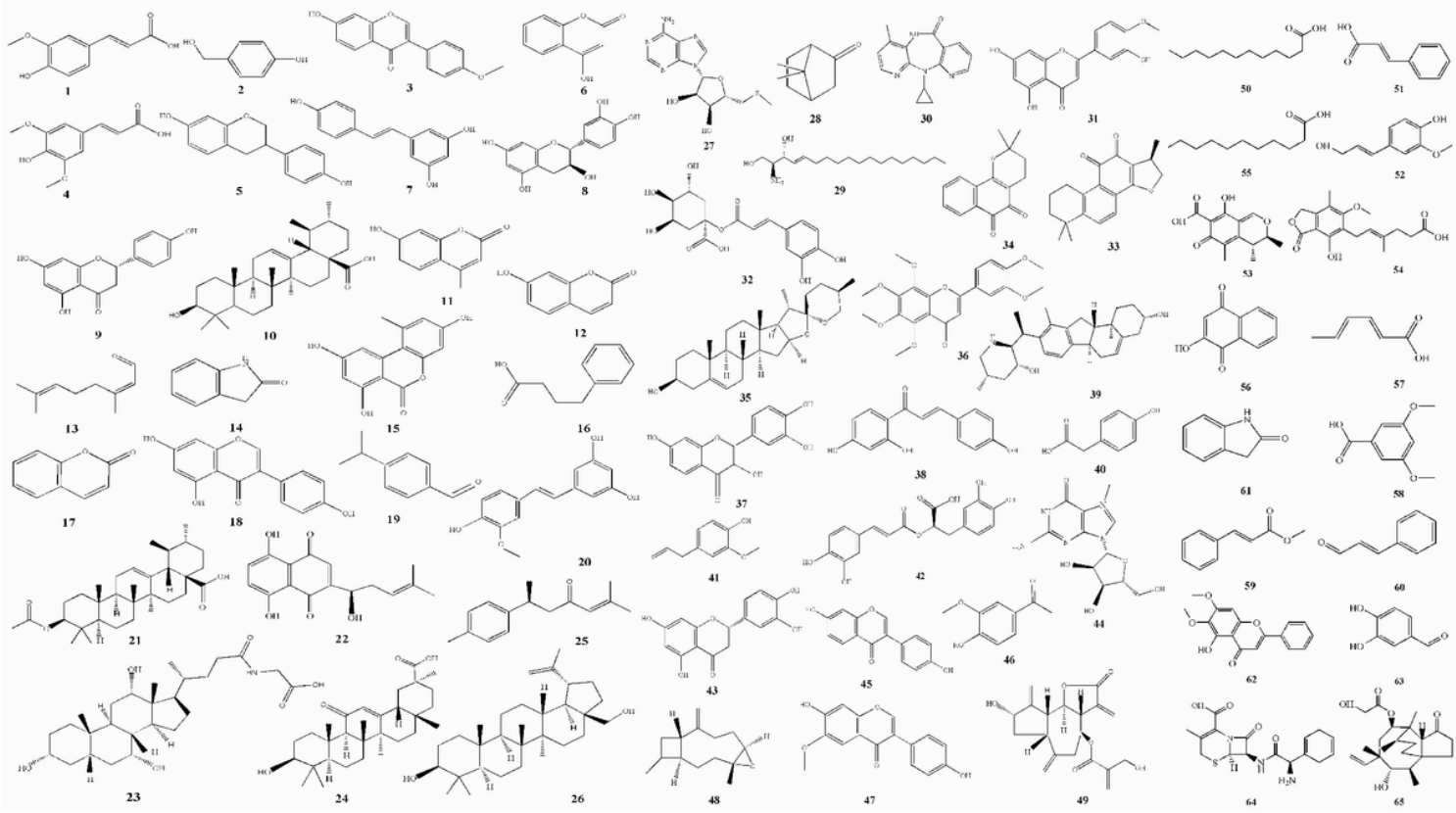
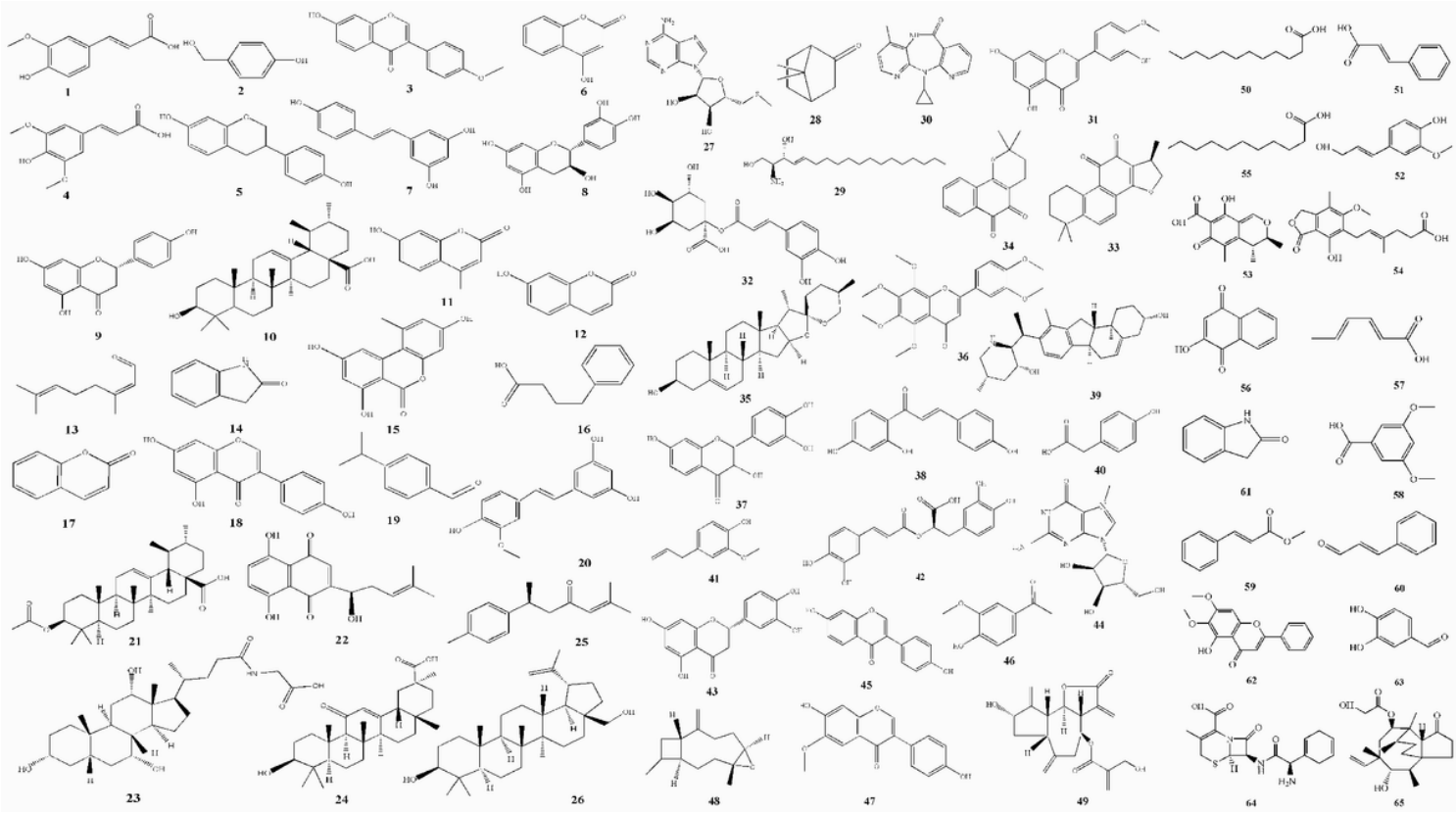


Figure 7

Chemical structures of the antitumor (1 ~ 39), potential antitumor (40 ~ 49) and antimicrobial (50 ~ 65) compounds in the EtOAc extract.



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