

# Integrating Activity-guided Strategy and Fingerprint Analysis to Target Potent Cytotoxic Brefeldin A from a Fungal Library of the Medicinal Mangrove *Acanthus ilicifolius*

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**Figure S1.** Neighbor-joining phylogenetic tree based on ITS sequences of cultured fungi associated with *Acanthus ilicifolius*.

**Figure S2.** Phylogenetic tree of fungi in the order *Hypocreales* based on ITS-rRNA sequence homology.

**Figure S3.** The cytotoxicity relative inhibition rate (%) data of the organic extracts in cancer cell lines.

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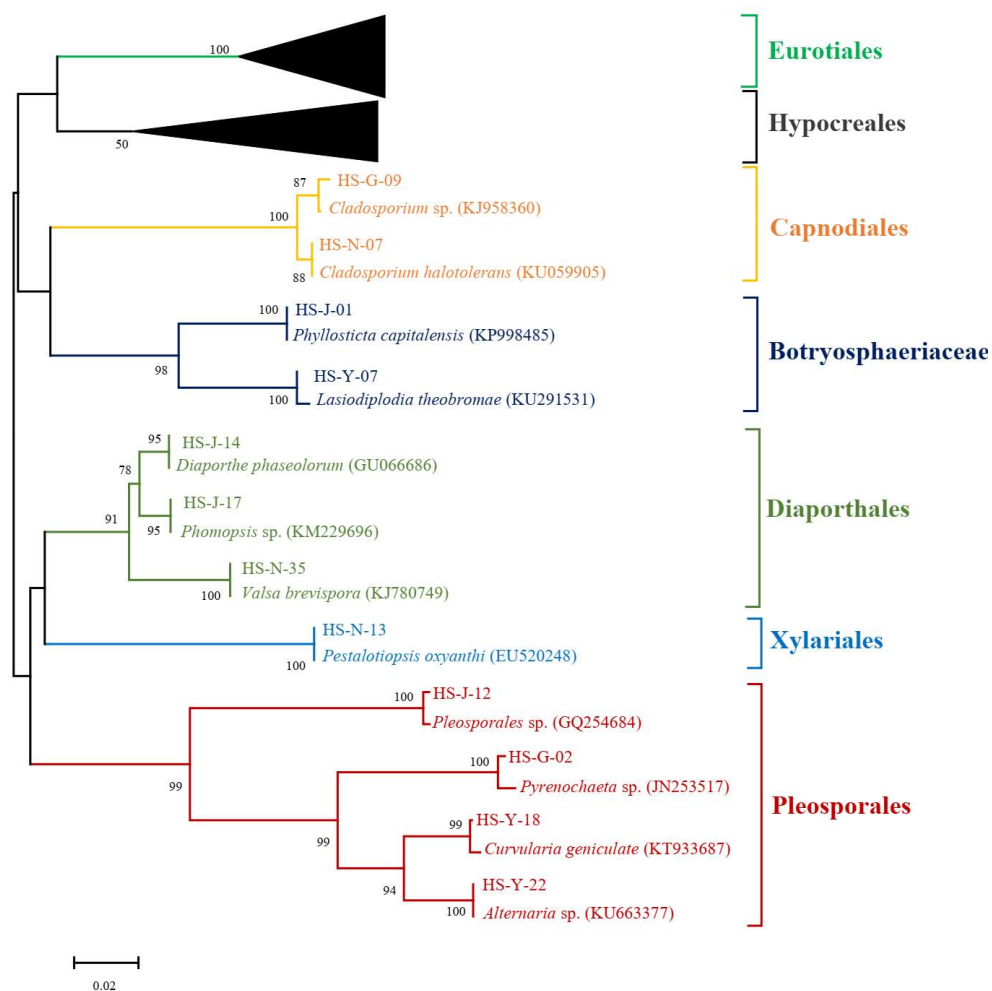
**Figure S5.** The fingerprint analysis of the organic extract of *Penicillium* sp. (HS-N-27).

**Table S1.** Phylogenetic affiliations of cultivable fungi associated with the *Acanthus ilicifolius*

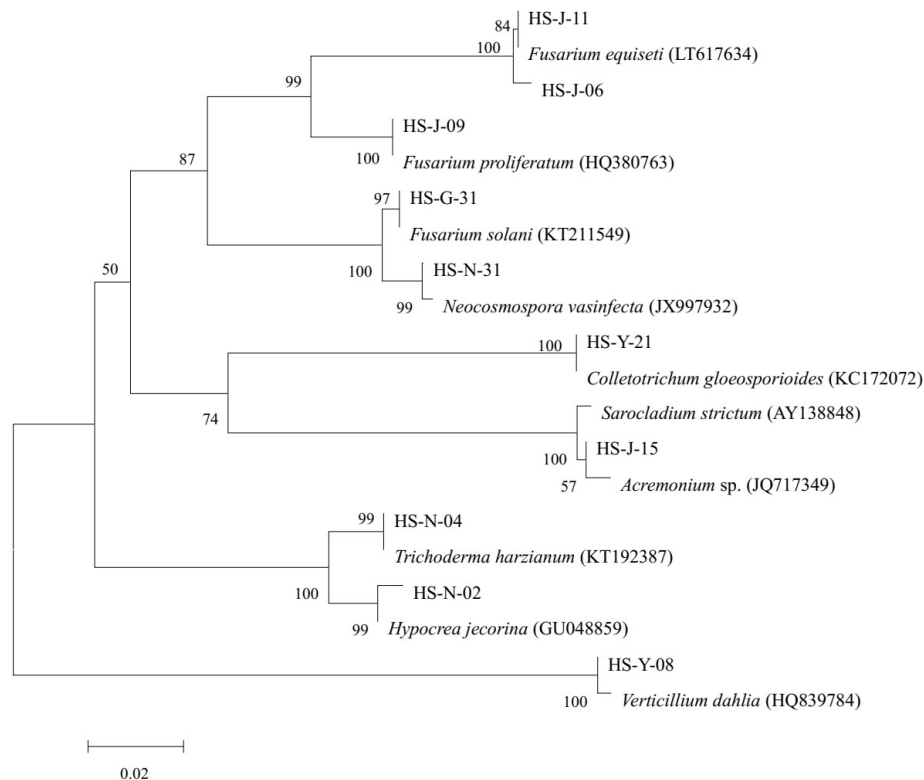
Isolate ID	Order	Genus	Closest identified relative	Identity (%)	Query cover (%)
HS-N-01	Hypocreales	<i>Trichoderma</i>	<i>Trichoderma</i> sp. (KM458790)	99	99
HS-Y-17			<i>Trichoderma reesei</i> (KU377472)	100	100
HS-N-04			<i>Trichoderma harzianum</i> (KT192387)	100	99
HS-N-26			<i>Trichoderma</i> sp. (KM066570)	100	100
HS-N-12		<i>Hypocrea</i>	<i>Hypocrea lixii</i> (HQ229942)	100	99
HS-N-02			<i>Hypocrea jecorina</i> (GU048859)	99	99
HS-J-15		<i>Acremonium</i>	<i>Acremonium</i> sp. (JQ717349)	99	97
HS-J-08			<i>Acremonium</i> sp. (HG936339)	99	98
HS-N-28		<i>Verticillium</i>	<i>Verticillium dahlia</i> (HQ839784)	99	99
HS-N-16			<i>Verticillium dahlia</i> (HQ839784)	99	99
HS-Y-08			<i>Verticillium dahlia</i> (HQ839784)	99	100
HS-N-08		<i>Fusarium</i>	<i>Fusarium solani</i> (KT211526)	100	100
HS-N-09			<i>Fusarium solani</i> (EU719658)	99	99
HS-N-10			<i>Fusarium solani</i> (EU719658)	99	99
HS-G-23			<i>Fusarium solani</i> (AY633744)	99	100
HS-G-15			<i>Fusarium solani</i> (AY633744)	99	99
HS-G-31			<i>Fusarium solani</i> (KT211549)	100	100
HS-Y-09			<i>Fusarium solani</i> (EU719658)	99	100
HS-J-06			<i>Fusarium incarnatum</i> (KU377454)	99	100
HS-J-19			<i>Fusarium</i> sp. (GQ410774)	99	100
HS-Y-20			<i>Fusarium</i> sp. (GQ352485)	99	99
HS-J-27			<i>Fusarium</i> sp. (GQ410774)	99	99
HS-J-09			<i>Fusarium proliferatum</i> (HQ380763)	99	100
HS-J-10			<i>Fusarium incarnatum</i> (KM921663)	99	99
HS-Y-06			<i>Fusarium</i> sp. (GQ352485)	99	100
HS-J-11			<i>Fusarium equiseti</i> (LT617634)	100	100
HS-N-31	Eurotiales	<i>Neocosmospora</i>	<i>Neocosmospora vasinfecta</i> (JX997932)	99	99
HS-N-03		<i>Penicillium</i>	<i>Penicillium janthinellum</i> (KU529846)	100	100
HS-N-22			<i>Penicillium janthinellum</i> (KM461118)	99	98
HS-N-23			<i>Penicillium janthinellum</i> (KM461118)	100	99
HS-G-06			<i>Penicillium chermesinum</i> (KJ767051)	95	100
HS-G-12			<i>Penicillium</i> sp. (KJ935029)	99	100
HS-N-27			<i>Penicillium</i> sp. (KF367521)	99	100
HS-N-29			<i>Penicillium</i> sp. (KU556495)	99	99
HS-G-24			<i>Penicillium</i> sp. (LT558883)	100	99
HS-G-30			<i>Penicillium oxalicum</i> (KM458784)	99	99
HS-N-15			<i>Penicillium chloroleucon</i> (KP016813)	99	98

HS-J-22			<i>Penicillium chrysogenum</i> (KX349473)	100	99
HS-J-26			<i>Penicillium brocae</i> (KJ775608)	100	97
HS-Y-01			<i>Penicillium</i> sp. (LT603036)	100	100
HS-Y-10			<i>Penicillium</i> sp. (FJ647577)	99	100
HS-Y-11			<i>Penicillium rubens</i> (KX011023)	98	99
HS-Y-04			<i>Penicillium sclerotiorum</i> (KC478540)	99	100
HS-G-01			<i>Penicillium</i> sp. (KR093905)	99	97
HS-N-19			<i>Penicillium</i> sp. (KT763472)	100	100
HS-G-20			<i>Penicillium</i> sp. (KT763472)	100	100
HS-N-20		<i>Eupenicillium</i>	<i>Eupenicillium</i> sp. (KC871024)	99	98
HS-N-25			<i>Eupenicillium</i> sp. (KC871024)	99	99
HS-G-29			<i>Eupenicillium</i> sp. (JQ828843)	99	99
HS-N-06		<i>Aspergillus</i>	<i>Aspergillus flavus</i> (KU597196)	99	99
HS-G-05			<i>Aspergillus terreus</i> (KC462061)	100	100
HS-G-07			<i>Aspergillus</i> sp. (JQ717354)	99	100
HS-G-16			<i>Aspergillus</i> sp. (KJ567459)	99	100
HS-Y-12			<i>Aspergillus</i> sp. (KX228408)	100	100
HS-N-30			<i>Aspergillus</i> sp. (JN393254)	100	99
HS-G-04			<i>Aspergillus versicolor</i> (LC105691)	99	99
HS-G-17			<i>Aspergillus</i> sp. (KF888648)	99	99
HS-Y-27			<i>Aspergillus aculeatus</i> (KU310905)	100	100
HS-G-03	Eurotiales	<i>Talaromyces</i>	<i>Talaromyces flavus</i> (KM458831)	99	97
HS-N-13	Xylariales	<i>Pestalotiopsis</i>	<i>Pestalotiopsis oxyanthi</i> (EU520248)	99	100
HS-Y-02			<i>Pestalotiopsis oxyanthi</i> (EU520248)	99	98
HS-J-04			<i>Pestalotiopsis oxyanthi</i> (EU520248)	99	99
HS-J-13	Diaporthales	<i>Diaporthe</i>	<i>Diaporthe phaseolorum</i> (GU066686)	99	100
HS-J-14			<i>Diaporthe phaseolorum</i> (GU066686)	99	100
HS-J-17		<i>Phomopsis</i>	<i>Phomopsis</i> sp. (KM229696)	99	99
HS-J-18			<i>Phomopsis</i> sp. (KM229696)	99	99
HS-J-02			<i>Phomopsis</i> sp. (KM229696)	100	100
HS-J-24			<i>Phomopsis</i> sp. (KM229696)	100	100
HS-J-25			<i>Phomopsis</i> sp. (GU066702)	99	100
HS-N-11		<i>Valsa</i>	<i>Valsa brevispora</i> (KJ780749)	99	99
HS-N-35			<i>Valsa brevispora</i> (KJ780749)	99	99
HS-G-02	Pleosporales	<i>Pyrenochaeta</i>	<i>Pyrenochaeta</i> sp. (JN253517)	97	99
HS-J-12		<i>Pleosporales</i>	<i>Pleosporales</i> sp. (GQ254684)	99	99
HS-Y-18		<i>Curvularia</i>	<i>Curvularia geniculata</i> (KT933687)	100	99
HS-Y-22		<i>Alternaria</i>	<i>Alternaria</i> sp. (KU663377)	100	98
HS-G-09	Capnodiales	<i>Cladosporium</i>	<i>Cladosporium</i> sp. (KJ958360)	100	99
HS-G-08			<i>Cladosporium halotolerans</i> (LN834369)	100	97

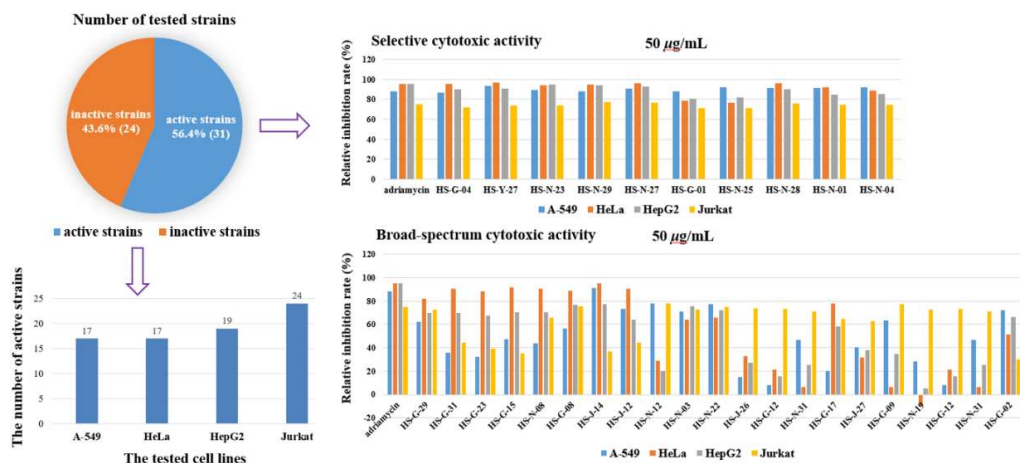
HS-N-07			<i>Cladosporium halotolerans</i> (KU059905)	100	100
HS-J-01	Botryosphaeriaceae	<i>Phyllosticta</i>	<i>Phyllosticta capitalensis</i> (KP998485)	100	100
HS-Y-07		<i>Lasiodiplodia</i>	<i>Lasiodiplodia theobromae</i> (KU291531)	99	96
HS-G-22			<i>Lasiodiplodia theobromae</i> (KT240140)		100
HS-Y-03	Glomerellales	<i>Colletotrichum</i>	<i>Colletotrichum</i> sp. (KT282716)	100	100
HS-Y-05			<i>Colletotrichum gloeosporioides</i> (KC172072)	99	100
HS-Y-21			<i>Colletotrichum gloeosporioides</i> (KC172072)	99	99



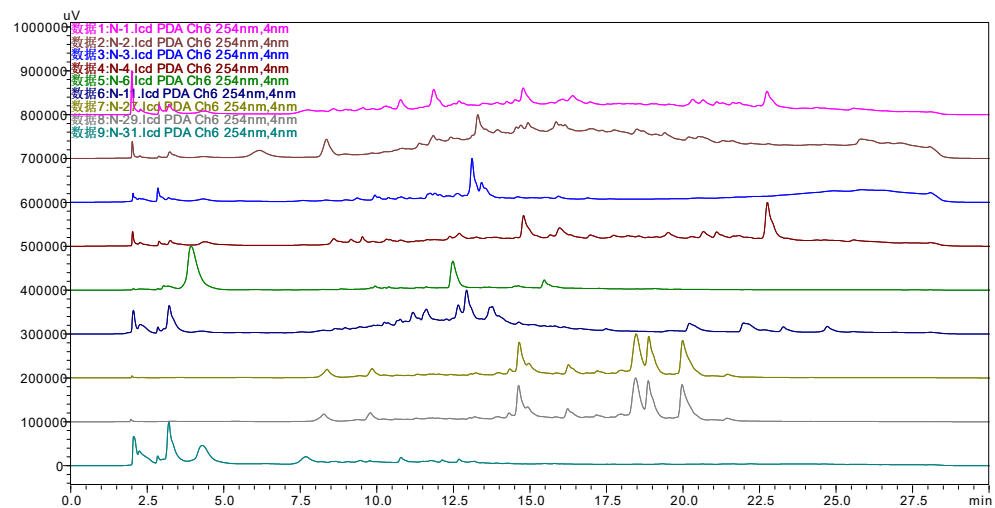
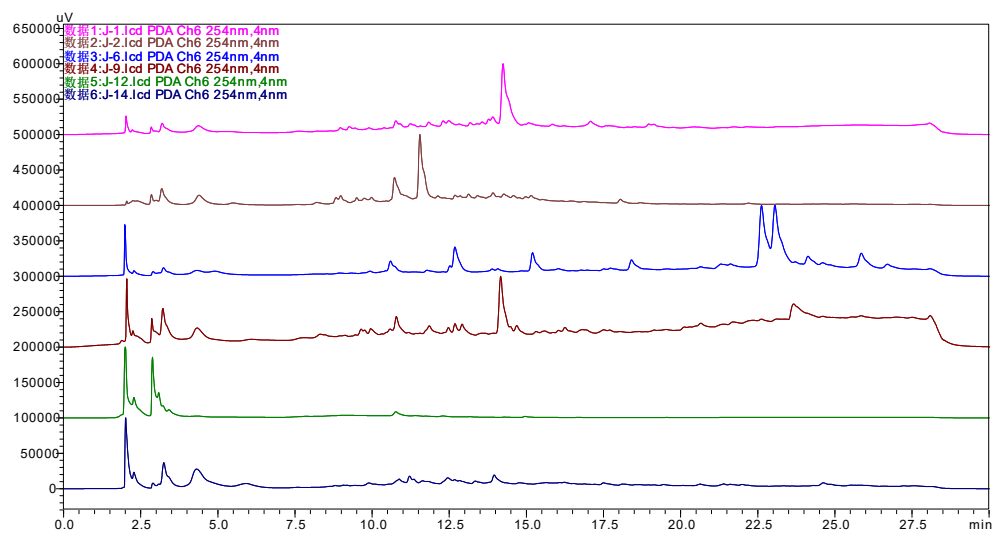
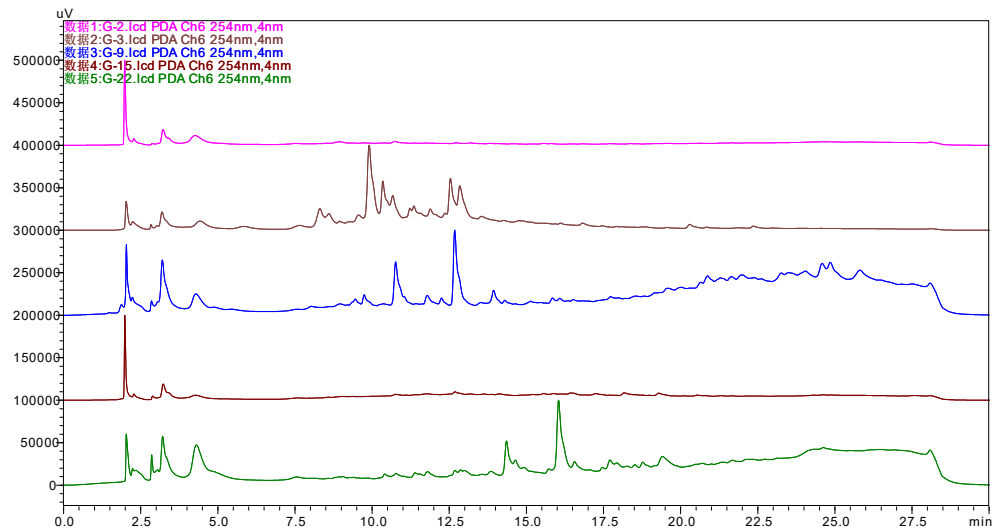
**Figure S1.** Neighbor-joining phylogenetic tree based on ITS sequences of cultured fungi associated with *Acanthus ilicifolius*. The values at each node represent the bootstrap values from 1,000 replicates, and the scale bar=0.02 substitutions per nucleotide.

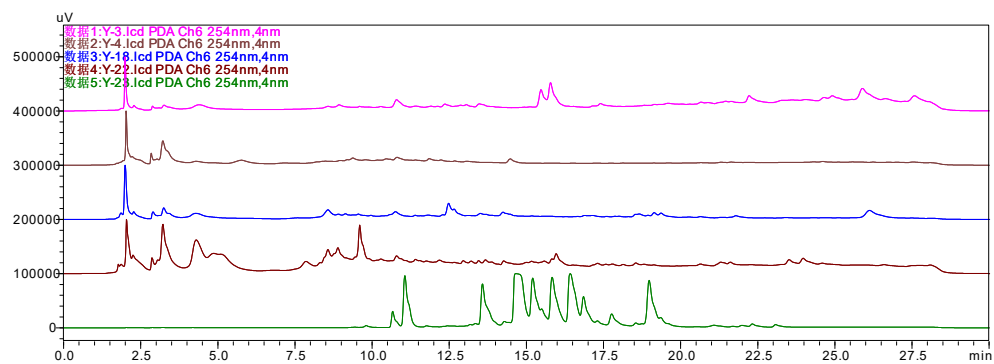


**Figure S2.** Phylogenetic tree of fungi in the order *Hypocreales* based on ITS gene sequence homology. The values at each node represent the bootstrap values from 1,000 replicates, and the scale bar=0.02 substitutions per nucleotide.

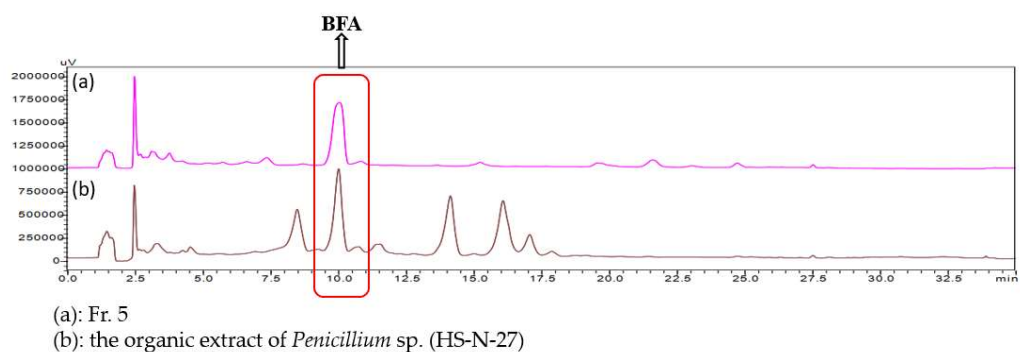


**Figure S3.** The cytotoxicity relative inhibition rate data of the organic extracts in cancer cell lines. To identify active strains for further research as potential cytotoxic strains, the relative inhibition rate of A-549, HeLa and HepG2 cell lines should be greater than 70 %, and the relative inhibition rate of Jurkat cell line should be greater than 60 %.





**Figure S4.** The fingerprint analysis of the organic extract of fungi from different species and genera. the organic extract of fungi (1 mg/mL, 50  $\mu$ L) was analyzed by HPLC (Daojin LC-20AT, C<sub>18</sub> column (250 mm  $\times$  5.0 mm) with a gradient program of MeCN–H<sub>2</sub>O (0.1% formic acid) [0–1 min 10%, 1–20 min 5–100%, 20–23 min 100%, 23–25 min 5%, 25–30 min 5%; 1 mL/min]



**Figure S5.** The fingerprint analysis of the organic extract of *Penicillium* sp. (HS-N-27).