**Supporting Information**

**Table S1. Ion pair informations**

| Component | Q1 Mass（Da） | Q3 Mass （Da） | DP（V） | CE(V) |
|---|---|---|---|---|
| Dendrobine | 264.2 | 105.2 | 140 | 50 |

# Metabolites from the *Dendrobium* Endophyte Pseudomonas protegens CM-YJ44 Alleviate Insulin Resistance in HepG2 Cells via the IRS1/PI3K/Akt/GSK3β/GLUT4 Pathway

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## 1. UPLC-MS/MS Analysis of CM-YJ44-3

## 2. RNA isolation and RT-PCR

**Table S2. Target genes and primers for RT - PCR**

| Gene | Forward primer (5'- 3') | Reverse primer (5'- 3') |
|---|---|---|
| *β-actin* | TGTCCACCTTCCAGCAGATGT | AGCTCAGTAACAGTCCGCCTAGA |
| *TNF-α* | AGGACCAGCTAAGAGGGAGA | TTCAGTGCTCATGGTGTCCT |
| *IL-8* | TGGCAGCCTTCCTGATTTCT | AATTTCTGTGTTGGCGCAGT |
| *IL-10* | AGGGCACCCAGTCTGAGAACA | AGGGCACCCAGTCTGAGAACA |

## 3. Validation of the molecular docking methodology

To validate the molecular docking methodology used in this study, GLUT4 protein was selected as a representative example. The co-crystallized ligand Cytochalasin B (PubChem CID: 5311281) was extracted from the GLUT4 structure using PyMOL. The ligand was then re-docked into the binding site of GLUT4

using the same docking protocol applied in this study. The root mean square deviation (RMSD) between the re-docked pose and the original co-crystallized pose was calculated. An RMSD value of less than 2 Å indicates that the docking method is reliable. The results showed that the re-docked pose of Cytochalasin B closely overlapped with its original position and orientation in the crystal structure, with an RMSD of 0.011 Å, confirming the validity of the docking methodology.

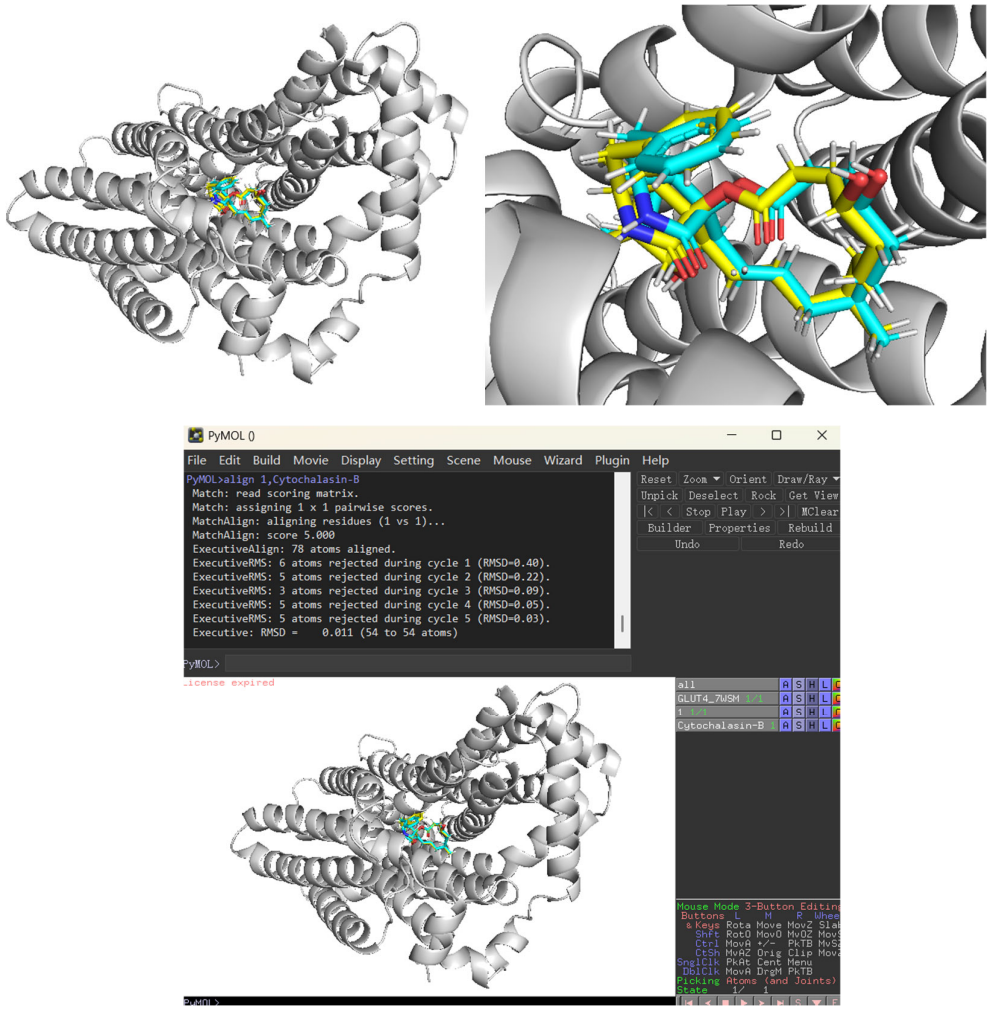



**Figure S1** Results of validation

**4. Components Identification of CM-YJ44-3 Using Q Exactive Focus LC-MS System**

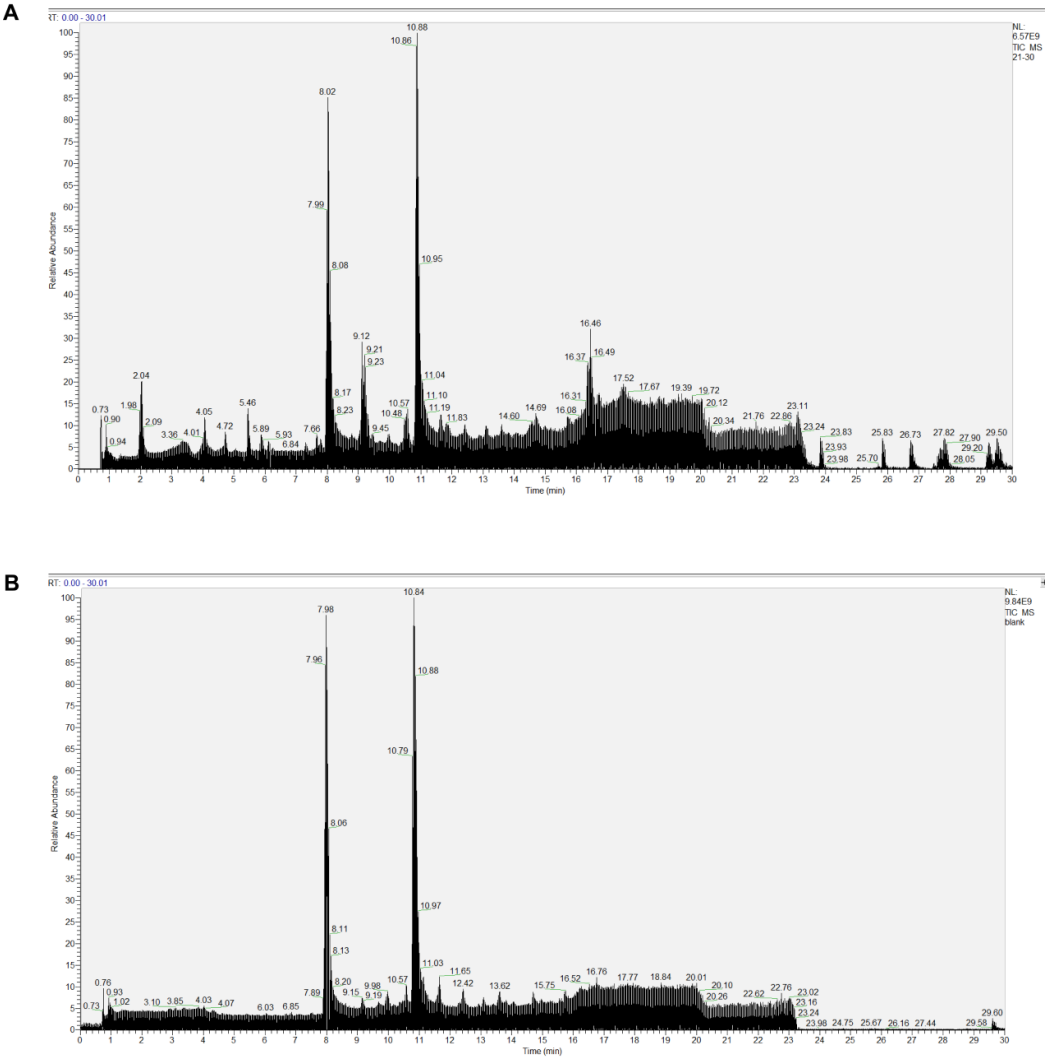



**Figure S2.** Total ion chromatogram. (A) CM-YJ44-3. (B) Blank control

## 5. UPLC-MS/MS analysis dendrobine of CM-YJ44-3

**Table S3. Calibration curve and CM-YJ44-3 concentration table for dendrobine quantification**

| Sample Name | Sample Type | Area (cps) | RT (min) | Target [Conc]. (ng/ml) | Calculated Conc. (ng/ml) |
|---|---|---|---|---|---|
| Rstd_0.1ng | Standard | 7.150e+02 | 3.60 | 0.100 | 0.150 |
| Rstd_0.2ng | Standard | 2.190e+03 | 3.60 | 0.200 | 0.375 |
| Rstd_0.5ng | Standard | 2.916e+03 | 3.61 | 0.500 | 0.486 |
| Rstd_1ng | Standard | 6.433e+03 | 3.61 | 1.000 | 1.023 |
| Rstd_2ng | Standard | 1.373e+04 | 3.61 | 2.000 | 2.135 |
| Rstd_5ng | Standard | 3.211e+04 | 3.61 | 5.000 | 4.941 |
| Rstd_10ng | Standard | 6.612e+04 | 3.61 | 10.000 | 10.130 |

| | | | | | |
|---|---|---|---|---|---|
| Rstd_20ng | Standard | 1.343e+05 | 3.60 | 20.000 | 20.531 |
| Rstd_50ng | Standard | 3.309e+05 | 3.61 | 50.000 | 50.534 |
| Rstd_100ng | Standard | 6.146e+05 | 3.61 | 100.000 | 93.822 |
| Rstd_200ng | Standard | 1.259e+06 | 3.61 | 200.000 | 192.200 |
| CM-YJ44-3-1 | Unknown | 5.182e+05 | 3.60 | N/A | 79.117 |
| CM-YJ44-3-2 | Unknown | 4.864e+05 | 3.60 | N/A | 74.263 |
| CM-YJ44-3-3 | Unknown | 5.424e+05 | 3.60 | N/A | 82.810 |

y = 6553.47949 x -268.84194 ($R^2$ = 0.99800)

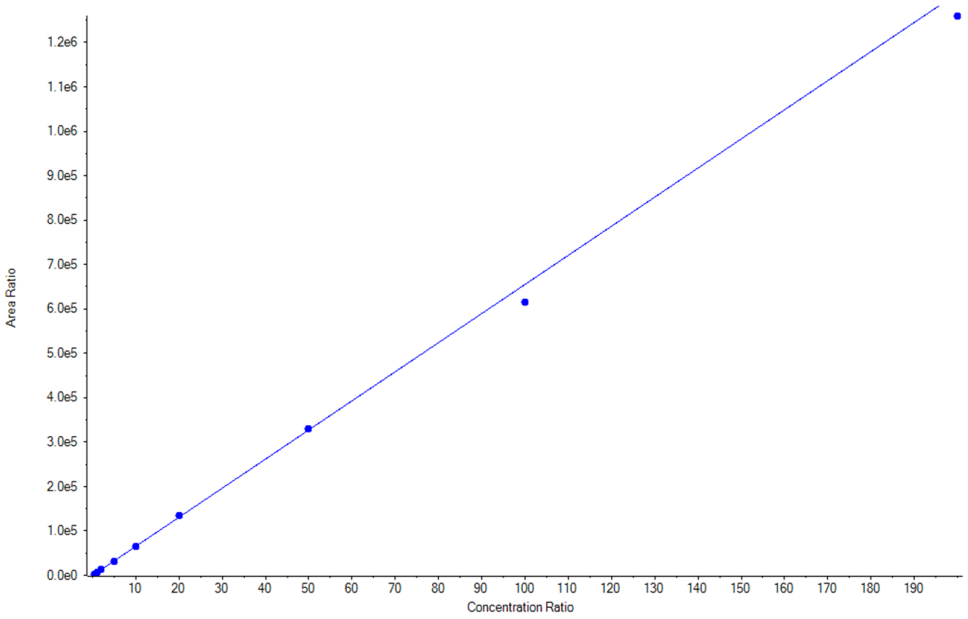
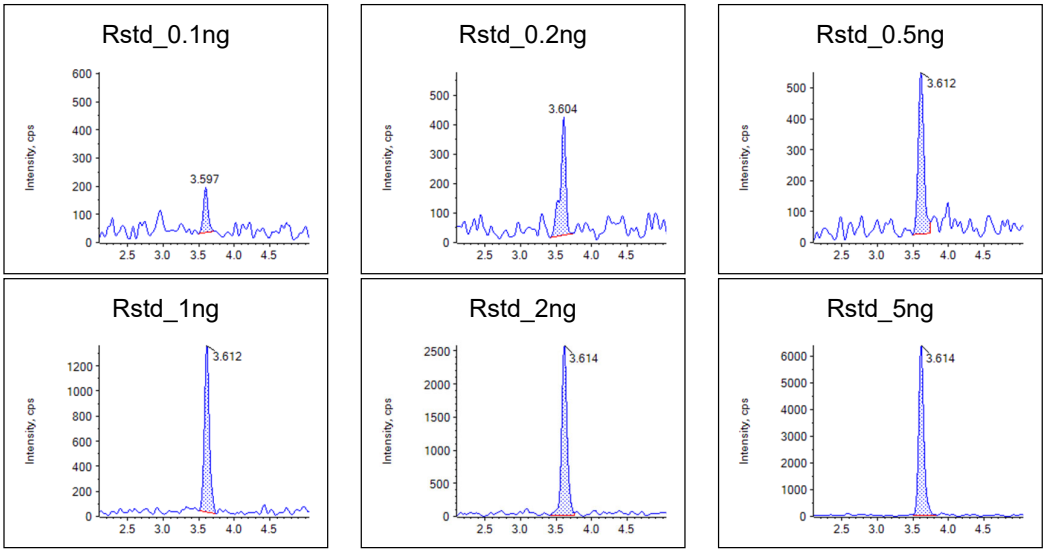



**Figure S3.** Standard curve of dendrobine.



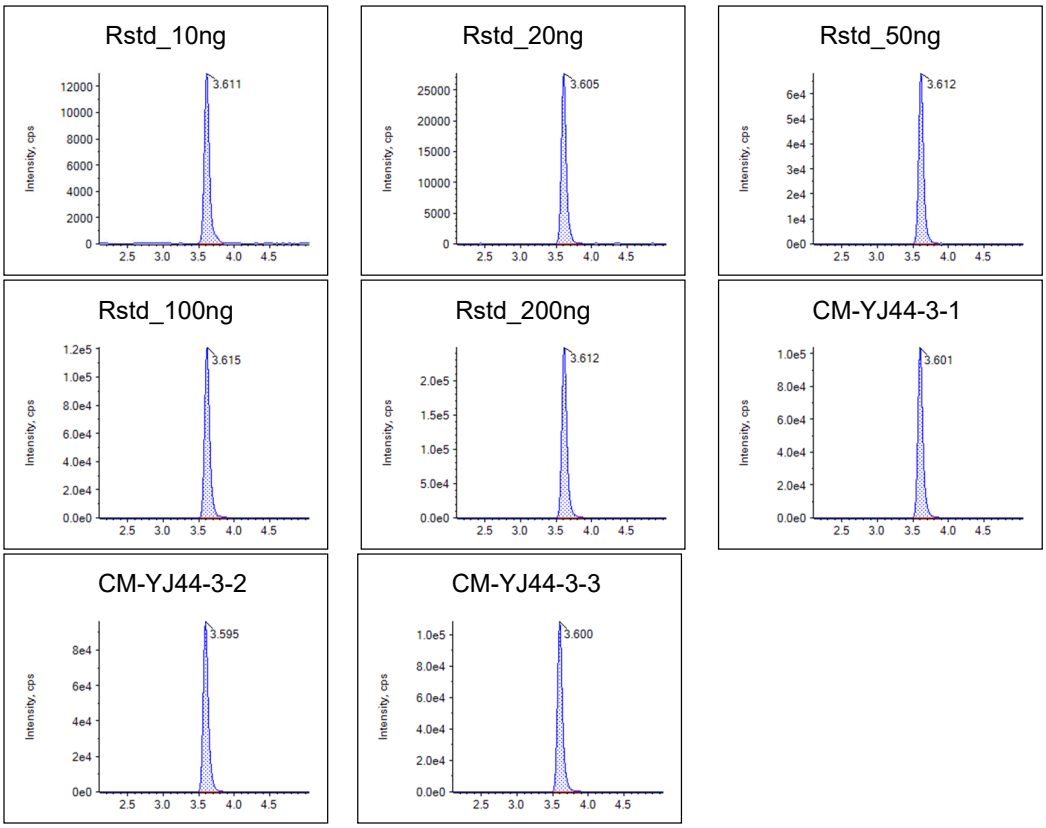



**Figure S4.** Calibration curve and chromatogram of CM-YJ44-3 in dendrobine quantitative analysis.