

**Ebeiedinone and peimisine inhibit cigarette smoke extract-induced  
oxidative stress injury and apoptosis in BEAS-2B cells**

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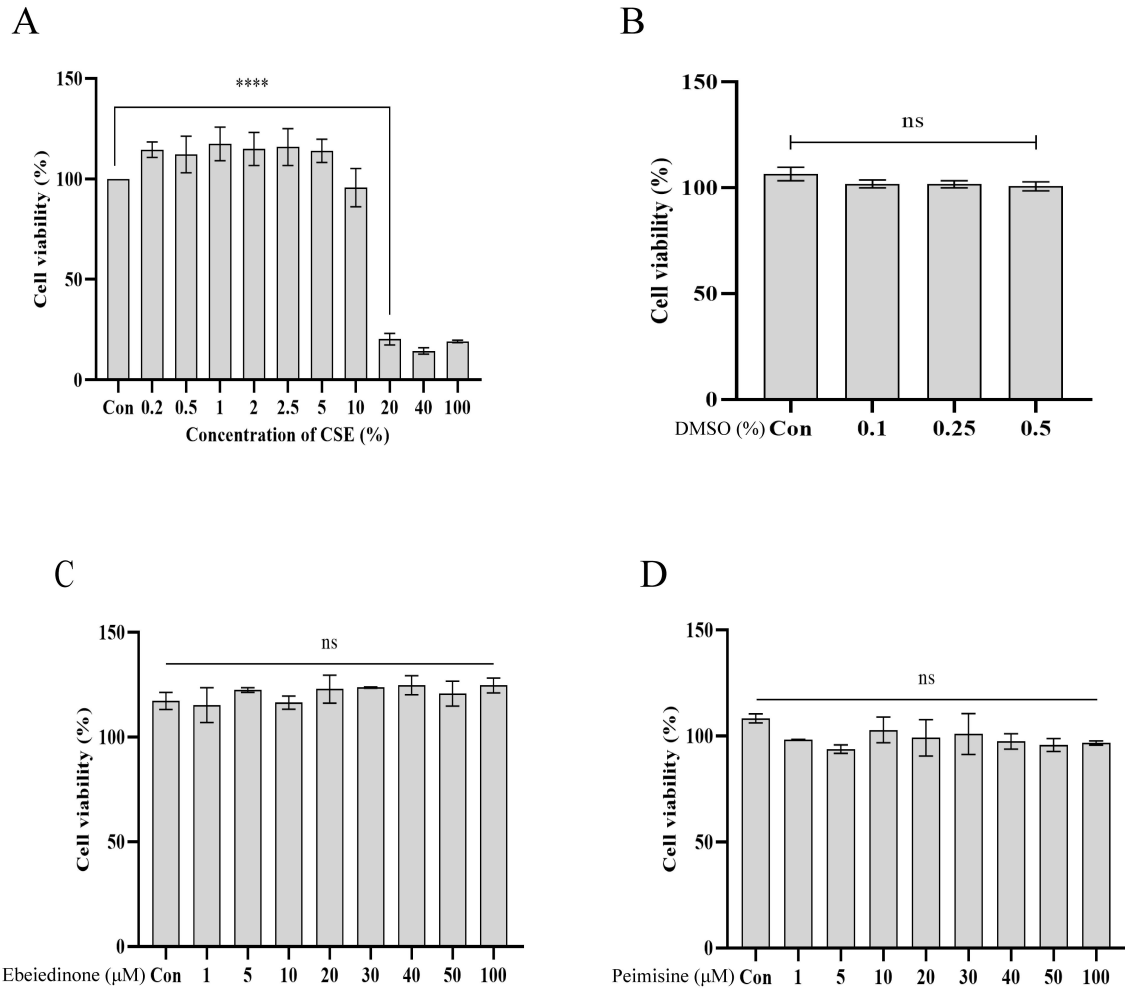
Chuanlan Liu and Xiaomu Zhu contributed equally to this work.

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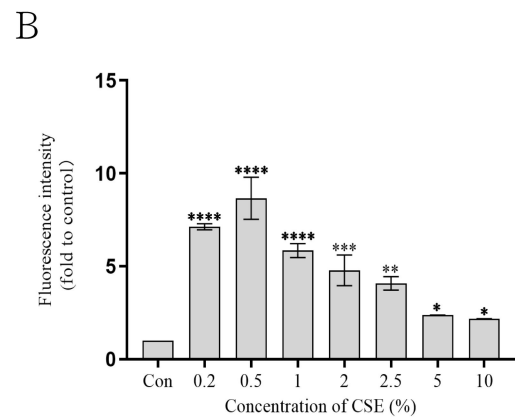
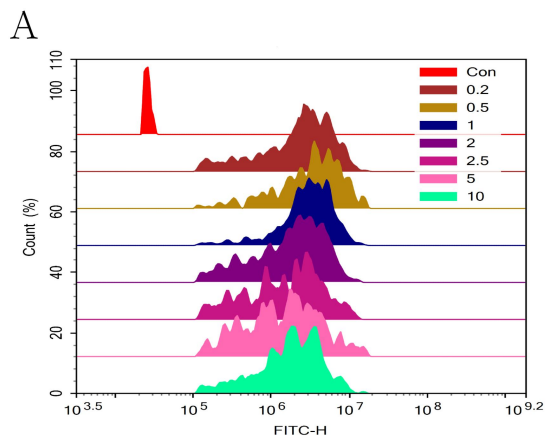
**Email:** benguiye513@163.com

Supplementary dates consist of Supplementary Figures 1–4 and Tables 1–3.

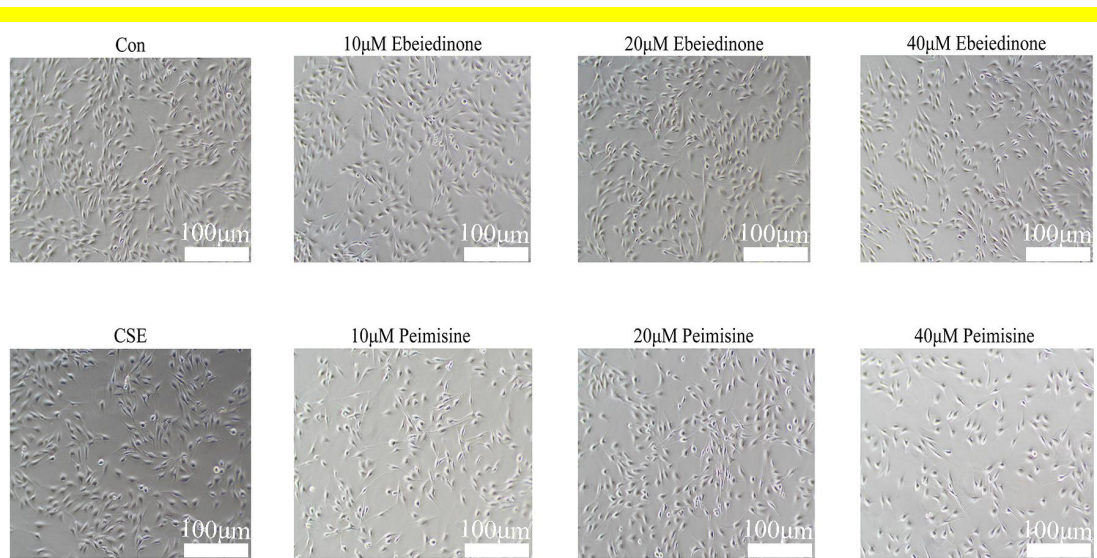
### Supplementary Figures



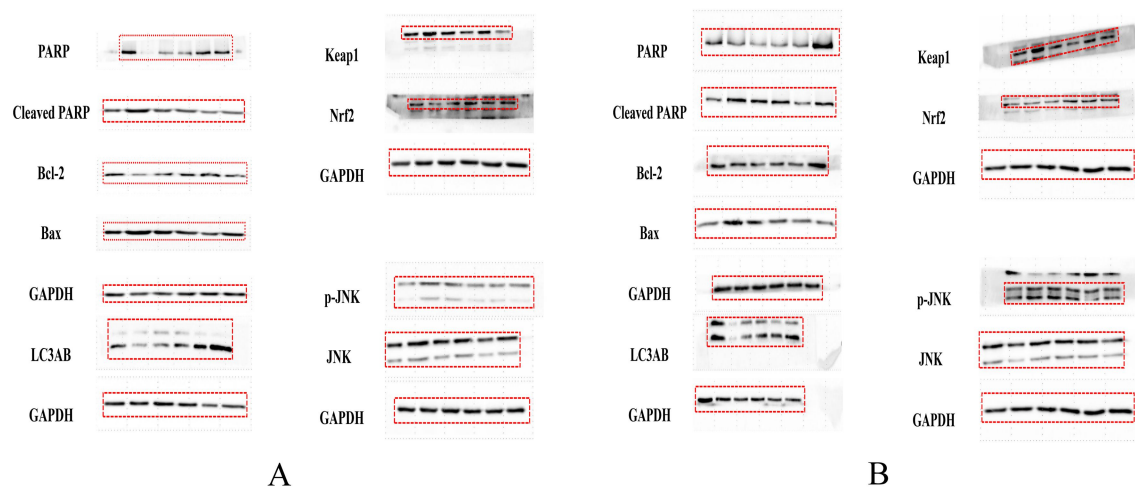
**Supplementary Fig. 1. The cytotoxicity was caused by CSE, DMSO, ebeiedinone, and peimisine.** (A) Effect of CSE on cell viability in BEAS-2B cells. (B) Effect of DMSO on cell viability in BEAS-2B cells. (C and D) Effects of ebeiedinone and peimisine on cell viability in BEAS-2B cells. The data are represented as the mean  $\pm$  SD of three independent experiments. In (A)–(D), the data are mean  $\pm$  SD ( $n = 3$ ). \*\*\*\* $P < 0.0001$  compared with the control group; *ns*=not significant.



**Supplementary Fig. 2. Effects of CSE exposure on ROS production in BEAS-2B cells.** The cells were treated with 0-10% CSE for 24 h. Cells were washed, trypsinized, centrifuged, incubated with 10 $\mu$ M DCFDA fluorescent probe for 25 min, and analyzed by FACS. The data are represented as the mean  $\pm$  SD of three independent experiments. ### $P$ <0.0001 compared with the control group. \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001, and \*\*\*\* $P$ <0.0001 compared with the control group.



**Supplementary Fig. 3. Effects of ebeiedinone and peimisine on the cell morphology of BEAS-2B cells damaged by CSE.** BEAS-2B cells were cultured with CSE (0.5%), ebeiedinone, and peimisine (10, 20, and 40 μM) for 24 h, and then the images were obtained (10×magnification).



**Supplementary Fig. 4. Original image for checking. (A)**The gel blot images of ebeiedinone. **(B)**The gel blot images of peimisine.

## Supplementary Tables

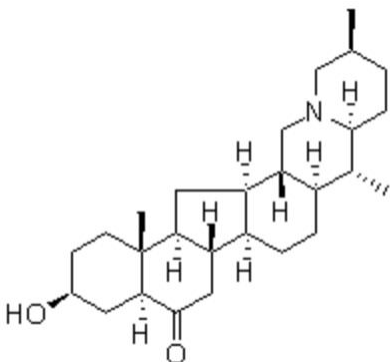
### Supplementary Tables 1. Antibodies used for western blot analysis.

Antibody	Dilution	Catalog no	Company
Bax	1:1000	ET1603-34	Huaan Biotechnology, Hangzhou,China
Bcl-2	1:1000	ET1603-11	Huaan Biotechnology, Hangzhou,China
PARP	1:1000	9531	Cell Signaling Technology, MA, USA
Cleaved-PARP	1:1000	5625	Cell Signaling Technology, MA, USA
LC3A/B	1:1000	12741	Cell Signaling Technology, MA, USA
Keap1	1:1000	AF5266	Affinity Biosciences, Jiangsu, China
Nrf2	1:1000	AF0639	Affinity Biosciences, Jiangsu, China
JNK	1:1000	9252	Cell Signaling Technology, MA, USA
P-JNK	1:1000	AP1337	ABclonal, Wuhan, China

**Supplementary Tables 2. The analysis and NMR spectrum of ebeiedinone.**

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<b>Description</b>	<b>Ebeiedinone</b>
<b>CAS No.</b>	<b>25650-68-4</b>
<b>MF</b>	<b>C<sub>27</sub>H<sub>43</sub>NO<sub>2</sub></b>
<b>MW</b>	<b>413.64</b>
<b>Lot No.</b>	<b>MUST-22031506</b>
<b>Report Date</b>	<b>2021-03-15</b>



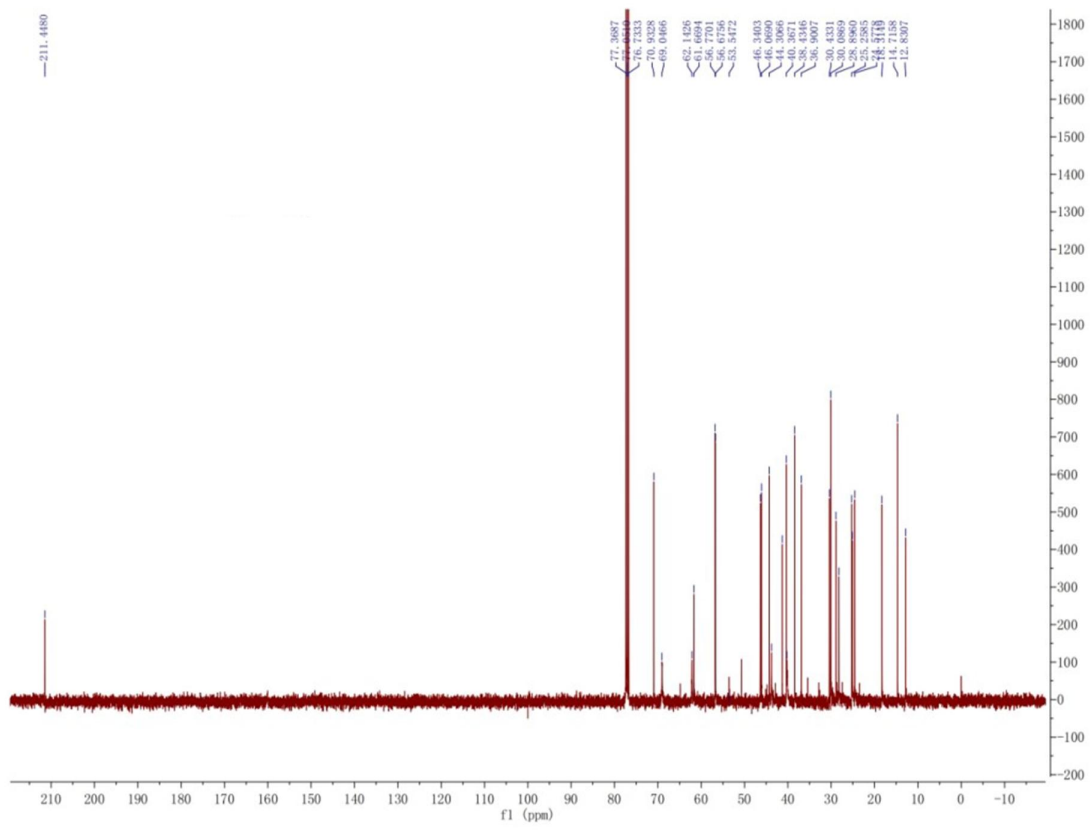
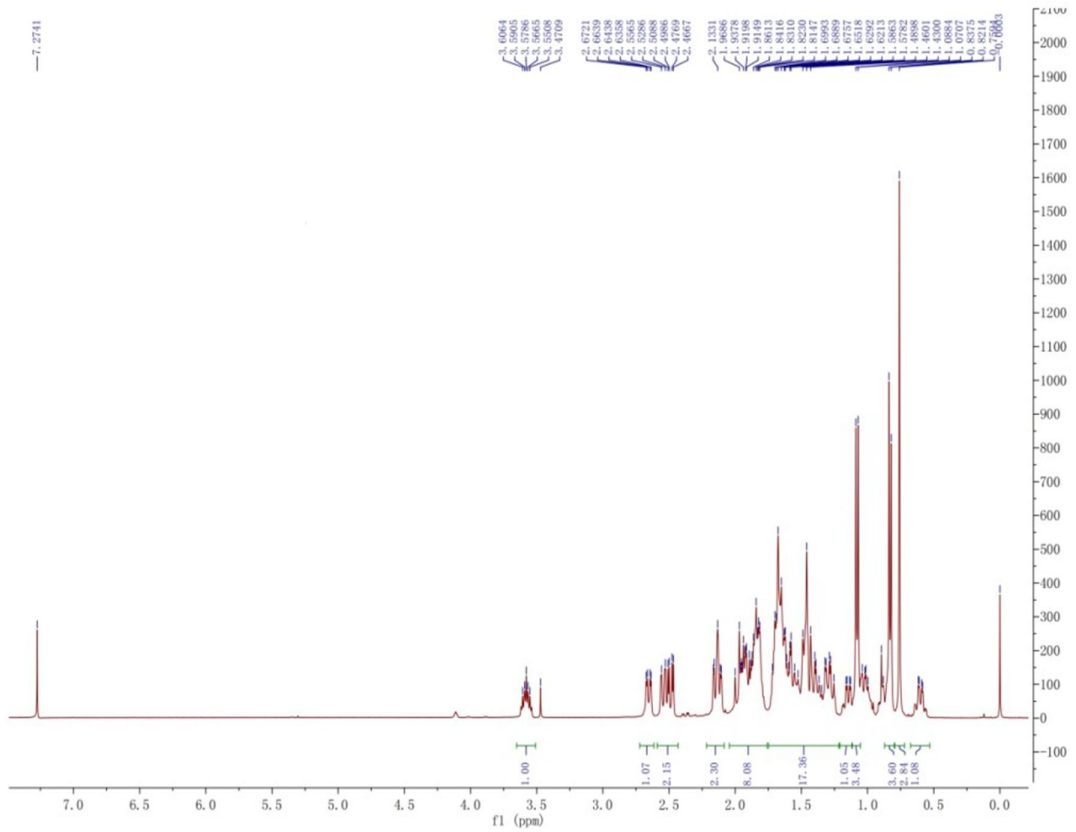
**TEST ITEM AND RESULT**

<b>Item</b>	<b>Standard</b>	<b>Result</b>	<b>REMARKS</b>
<b>Appearance</b>	<b>White Powder</b>	<b>Conforms</b>	
<b>Loss on drying</b>	<b>≤ 2.0%</b>	<b>Conforms</b>	
<b>Assay by HPLC</b>	<b>≥ 98%</b>	<b>99.63%</b>	

**ATTENTION**

<b>Storage</b>	<b>Keep tightly sealed and store under dry and dark conditions.</b> <b>Recommended storage temperature: below 4°C, special varieties below -20°C.</b>
<b>Warranty</b>	<b>Two years</b>
<b>Usage</b>	<b>Because some compounds may change at room temperature after dissolved in solvents, please use the dissolved sample early. Chromatographic pure reagents are recommended to dissolve samples for HPLC analysis.</b>
<b>Note</b>	<b>In case of quality problem, please contact us within 15 days after receiving the products.</b>

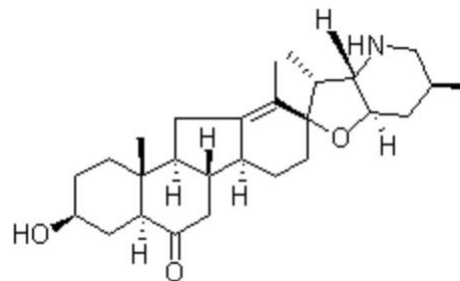
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### Supplementary Tables 3. The analysis and NMR spectrum of peimisine.

Description	Peimisine
CAS No.	19773-24-1
MF	C <sub>27</sub> H <sub>41</sub> NO <sub>3</sub>
MW	427.31
Lot No.	MUST-22032810
Report Date	2022-03-28



#### TEST ITEM AND RESULT

Item	Standard	Result	REMARKS
Appearance	White Powder	Conforms	
Loss on drying	≤ 2.0%	Conforms	
Assay by HPLC	≥ 98%	99.60%	

#### ATTENTION

Storage	Keep tightly sealed and store under dry and dark conditions. Recommended storage temperature: below 4°C, special varieties below -20°C.
Warranty	Two years
Usage	Because some compounds may change at room temperature after dissolved in solvents, please use the dissolved sample early. Chromatographic pure reagents are recommended to dissolve samples for HPLC analysis.
Note	In case of quality problem, please contact us within 15 days after receiving the products.

