

Supplementary Material

The Natural Product, Echinatin, Protects Mice from Methicillin-resistant *Staphylococcus aureus* Pneumonia by Inhibition of Alpha-hemolysin Expression

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Figure .1 Quality test report of echinatin.



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刺甘草查尔酮

Retrochalcone/Echinatin

检测信息

(Detection
Information)

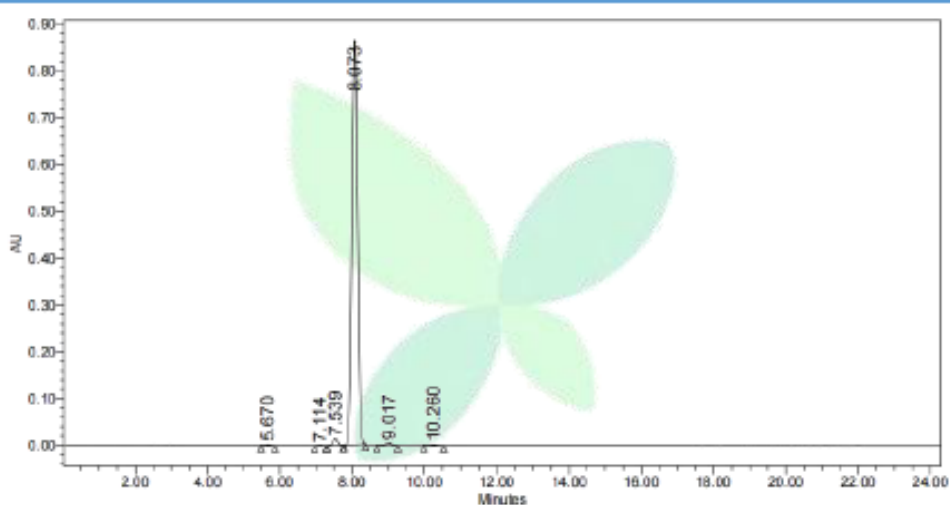
色谱柱 (Column): Kromasil 100-5-C18 4.6*250mm

流动相 (Mobile phase): A Acetonitrile B 0.1% phosphoric acid solution

0min38%A, 15min38%A, 25min80%A

检测波长 (Detection mode): 360nm

柱温 (Column temperature): 30°C



	RT	Area	Height	% Area
1	5.670	15903	1988	0.17
2	7.114	5169	587	0.05
3	7.539	135520	13597	1.44
4	8.073	9184182	864721	97.43
5	9.017	65004	5358	0.69
6	10.260	20410	1658	0.22

Figure. 2 Analysis of pulmonary immune cell proportions in mice with *S. aureus* pneumonia by flow cytometry.

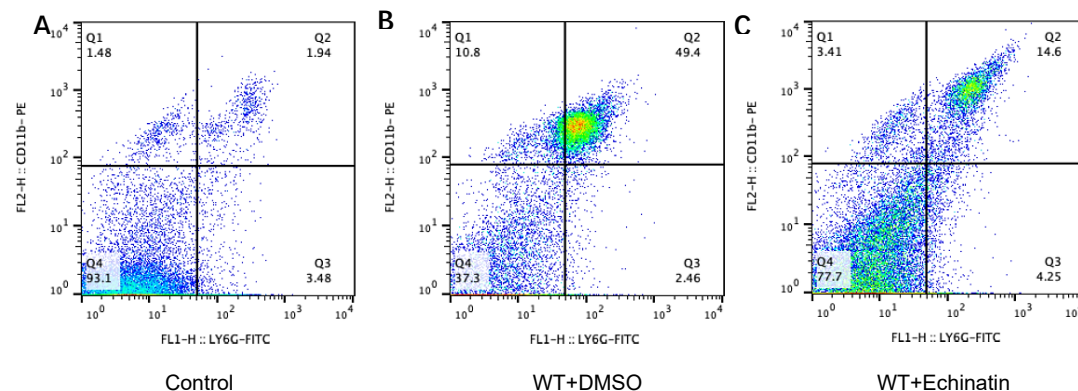


Figure. 2 Analysis of pulmonary immune cell proportions in mice with *S. aureus* USA300 pneumonia by flow cytometry. (A) Proportions of neutrophils and macrophages in the lungs of untreated mice. After 24 hours of infection with *S. aureus* USA300, mice were euthanized, and lung tissue cells were collected to detect the proportions of neutrophils and macrophages in the lungs of the (B) infected group and (C) echinatin-treated group.

Table. 1 Screening of effective inhibitors from small molecule libraries by hemolysis assay

No.	Compounds	CAS	Categories	Inhibition effect
1	Bilobeti	521-32-4	Biflavones	—
2	Irigenin	548-76-5	Isoflavones	—
3	Baohuoside V	118544-18-6	Flavonols	—
4	Baohuoside I	113558-15-9	Flavonols	—
5	Baohuoside VII	119730-89-1	Flavonols	—
6	Procyanidin C1	37064-30-5	Biflavones	—
7	Apiin	26544-34-3	Flavones	—
8	Alpha-Naphthoflavone	604-59-1	Flavones	—
9	<i>Sinensetin</i>	2306-27-6	Flavones	—
10	Schaftoside	51938-32-0	Flavones	—
11	Galangin 3-methyl ethe	6665-74-3	Flavonols	—
12	Flavokawain B	1775-97-9	Chalcones	—
13	7,4'-Dihydroxyflavone	2196-14-7	Flavones	—
14	Taxifolin	111003-33-9	Flavanonols	—
15	Prunetin	552-59-0	Isoflavones	—
16	Leucoside	27661-51-4	Flavonols	—
17	Engeletin	572-31-6	Flavanonols	—
18	Mirificin	103654-50-8	Isoflavones	—
19	Artemitin	479-90-3	Flavonols	—
20	Complanatuside	116183-66-5	Flavonols	—
21	Complanatoside A	146501-37-3	Flavonols	—
22	Tectoridin	611-40-5	Isoflavones	—
23	Trifoside	154-36-9	Isoflavones	—
24	Typhaneoside	104472-68-6	Flavonols	—
25	Narirutin	14259-46-2	Flavonones	—
26	Isomangiferin	24699-16-9	Polyphenols	—
27	Ieariline	489-32-7	Flavonols	—
28	Orientin	28608-75-5	Flavones	—
29	Homoorientin	4261-42-1	Flavones	—

30	Trihydroxyethylrutin	7085-55-4	Flavones	—
31	Isoschaftoside	52012-29-0	Flavones	—
32	Trifolirhizin	6807-83-6	Flavonoids	+
33	Desmethyglycitein	17817-31-1	Isoflavones	—
34	Luteolin-3-O-beta-D-glucuronide	53527-42-7	Flavones	—
35	Ginkgetin	481-46-9	Biflavones	—
36	Tilianin	4291-60-5	Flavones	—
37	Kuwanon G	75629-19-5	Flavones	—
38	Echinatin	34221-41-5	Chalcones	+++
39	Spinosin	72063-39-9	Flavones	—
40	Vicenin 2	23666-13-9	Flavones	—
41	Pectolinarin	28978-02-1	Flavones	—
42	Oroxin B	114482-86-9	Flavones	—
43	Oroxin A	57396-78-8	Flavones	—
44	Isobacachalcone	20784-50-3	Chalcones	+++
45	4',7-Dimethoxy-5-Hydroxyflavone	5128-44-9	Flavones	—
46	7,4'-Dihydroxyflavone	2196-14-7	Flavones	—
47	4',5-Dihydroxyflavone	6665-67-4	Flavones	—
48	Neoisoliquiritin	59122-93-9	Chalcones	—
49	Neeriocitrin	13241-32-2	Flavonones	—
50	Hesperetin 7-rutinoside	520-26-3	Flavonones	—
51	Pedalitin permethyl ether	2306-27-6	Flavones	—
52	Vincetoxicoside B	22007-72-3	Flavonols	—
53	Eriocitrin	13463-28-0	Flavonones	—
54	Wogonoside methyl ester	82475-01-2	Flavones	—
55	Wogonoside	51059-44-0	Flavones	—

Triton and USA300 were used as negative controls for hemolytic activity, and PBS and myricetin (Wang et al., 2020)

were used as negative controls. “—” represents no hemolysis inhibition effect; “++” represents significant inhibition of hemolysis; “+++” stands for extremely significant inhibition of hemolysis.

References

- Wang, T., Zhang, P., Lv, H., Deng, X., and Wang, J. (2020). A Natural Dietary Flavone Myricetin as an α -Hemolysin Inhibitor for Controlling *Staphylococcus aureus* Infection. *Front Cell Infect Microbiol* 10, 330. doi: 10.3389/fcimb.2020.00330.