

Supporting Information

A highly efficient extraction of plant essential oils using ball mill-assisted hydrodistillation for antibacterial applications

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Supplementary equation

The prediction equation of TVEO yield is shown in Equation (S1):

$$Y = 0.5128 + 0.043X_1 + 0.0049X_2 + 0.0029X_3 + 0.020X_1X_2 + 0.025X_1X_3 - 0.0002X_2X_3 - 0.0168X_1^2 - 0.0095X_2^2 - 0.0105X_3^2 \quad (6)$$

Supplementary figures

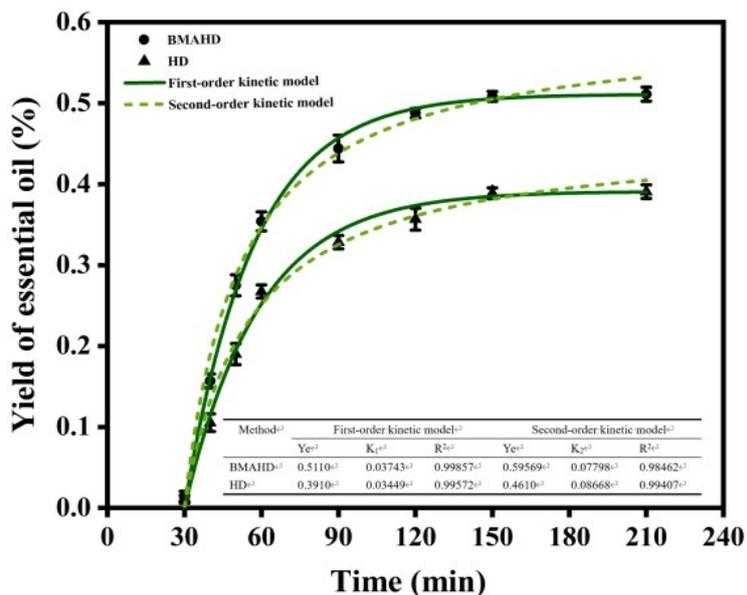


Figure S1 The kinetic curves of TVEO extracted by BMAHD and HD methods.

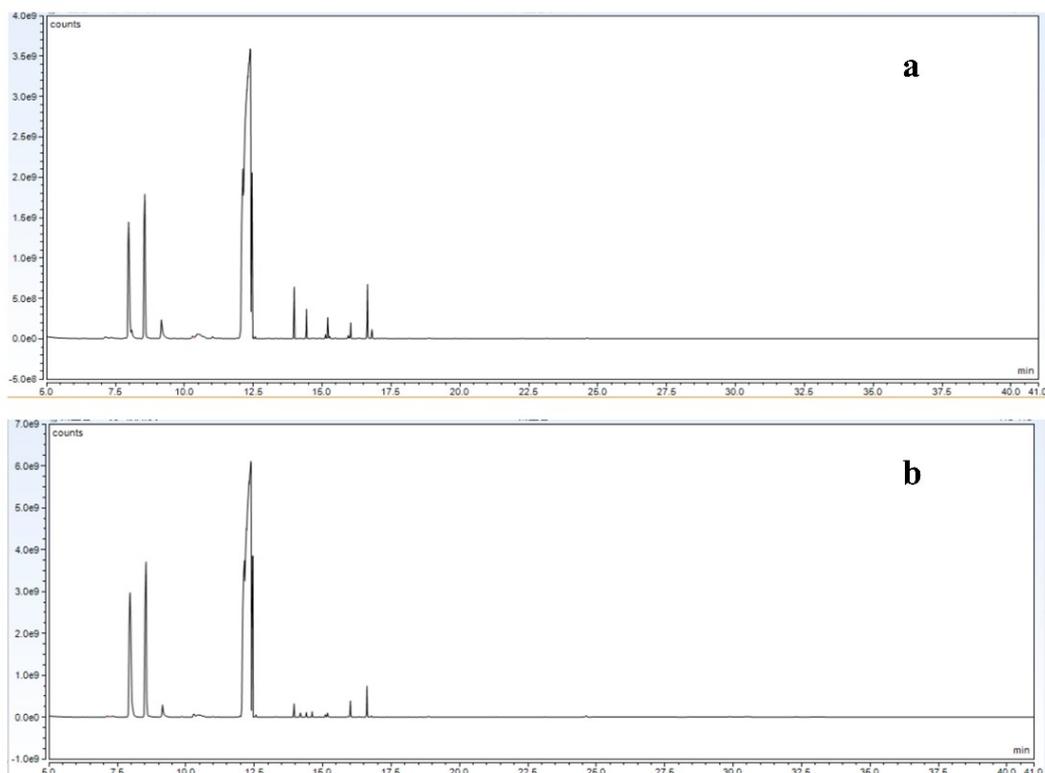


Figure S2 Total ion chromatograms of the essential oil extracted by two different extraction methods. (a) chromatogram for HD sample, (b) chromatogram for BMAHD sample.

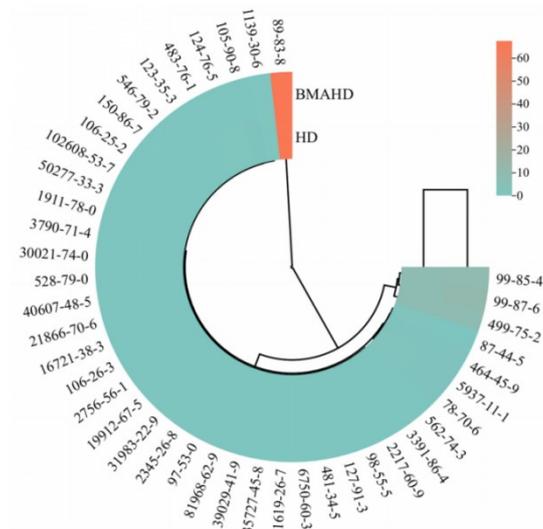


Figure S3 Circular clustering heatmap of TVEO components extracted by different methods. The x-axis represents the extraction methods, and the y-axis represents the essential oil fractions.

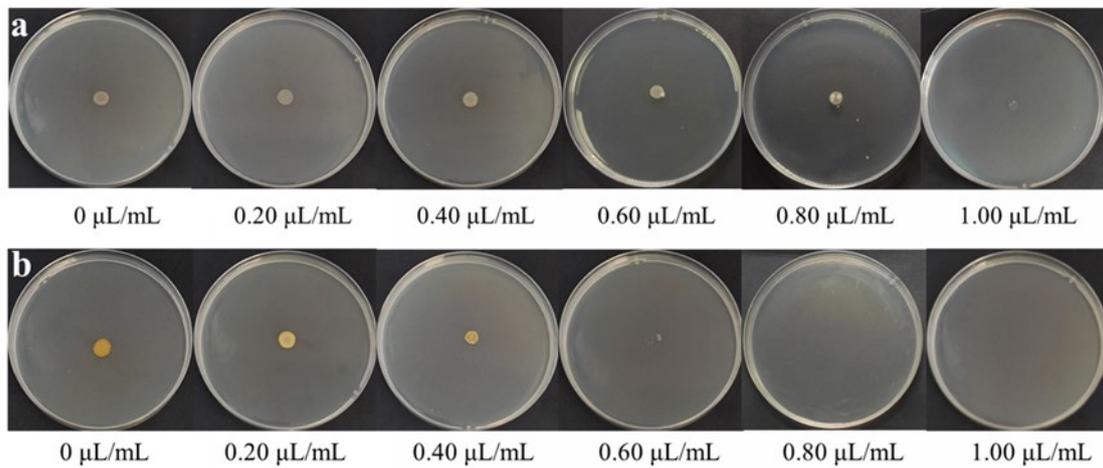


Figure S4 The antibacterial effect of TVEO on two types of bacteria. (a) *Escherichia coli*, (b) *Staphylococcus aureus*.

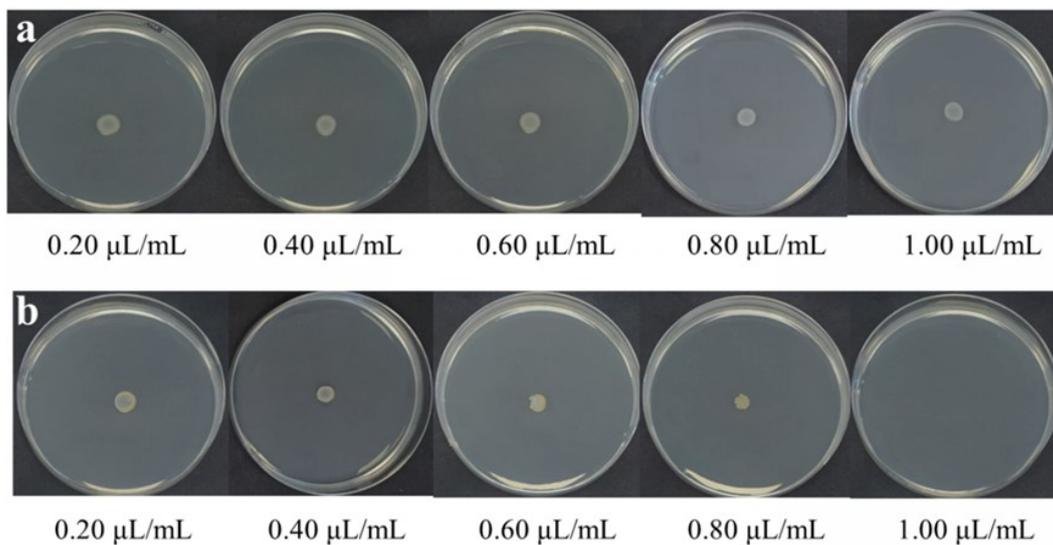


Figure S5 The inhibitory effect of vancomycin on two types of bacteria. (a) *Escherichia coli*, (b) *Staphylococcus aureus*.

Supplementary table

Table S1 Summary of the single factor optimization experiments for BMAHD

	Ball milling time (min)	Rotation speed (r/min)	Liquid-to-solid ratio (mL/g)
1	30	400	20
2	60	400	20
3	90	400	20
4	120	400	20
5	150	400	20
6	90	100	20
7	90	200	20
8	90	300	20
9	90	400	20
10	90	500	20
11	90	400	10
12	90	400	20
13	90	400	30
14	90	400	40
15	90	400	50

Table S2 Compounds with a content below 0.1% as detected by GC-MS.

No. ^a	Components	RI ^b	ID ^c	Molecular formula	CAS number	RA ^f (%) BMAHD	HD
1	Cinnamaldehyde,β-methyl		MS	C ₁₀ H ₁₀ O	21866-70-6	ND	0.01%
2	2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl), cis	1196	RI, MS	C ₁₀ H ₁₈ O	16721-38-3	ND	0.01%
3	2-Octen-1-ol, 3,7-dimethyl	1463	RI, MS	C ₂₀ H ₂₀ O	40607-48-5	ND	0.01%
4	2,6-Octadien-1-ol,3,7-dimethyl-(Z)	1210	RI, MS	C ₁₀ H ₁₈ O	106-25-2	0.05%	0.01%
5	Neral	1214	RI, MS	C ₁₀ H ₁₆ O	106-26-3	0.02%	0.03%
6	(1S,2R,4R,7R)-4-Isopropyl-7-methyl-3,8-dioxatricyclo[5.1.0.02,4] octane	1303	RI, MS	C ₁₀ H ₁₆ O ₂	1619-26-7	0.04%	0.04%
7	Phenol,5-methyl-2-(1-methylethyl)-acetate	1357	RI, MS	C ₁₀ H ₁₆ O ₂	528-79-0	ND	0.01%
8	Eugenol	1368	RI, MS	C ₁₀ H ₁₂ O ₂	97-53-0	ND	0.02%
9	Isobornyl propionate	1384	RI, MS	C ₁₀ H ₂₂ O ₂	2756-56-1	0.02%	0.02%
10	γ-Murolene	1449	RI, MS	C ₁₅ H ₂₄	30021-74-0	ND	0.01%
11	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1α,4α,8α)		MS	C ₁₅ H ₂₄	31983-22-9	ND	0.03%
12	Cyclohexanol,3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-[1R-(1α,2α,3β,6α)]	1930	RI, MS	C ₁₅ H ₂₆ O	35727-45-8	0.01%	0.07%
13	Geranyl isobutyrate	1860	RI, MS	C ₁₄ H ₂₄ O	2345-26-8	ND	0.03%
14	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	1605	RI, MS	C ₁₅ H ₂₄ O	6750-60-3	0.04%	0.06%
15	4a(2H)-Naphthalenol, 1,3,4,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S,4R,4aS,8aR)	1627	RI, MS	C ₁₅ H ₂₆ O	19912-67-5	ND	0.04%
16	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-(Z,E)	1699	RI, MS	C ₁₅ H ₂₆ O	3790-71-4	ND	0.01%
17	14-Hydroxycaryophyllene		MS	C ₁₅ H ₂₄ O	50277-33-3	0.01%	ND
18	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1694.5	RI, MS	C ₁₅ H ₂₄ O	81968-62-9	ND	0.02%
19	1-((1S,3aR,4R,7S,7aS)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl)ethanone	1702	RI, MS	C ₁₅ H ₂₆ O ₂	1911-78-0	ND	0.02%

20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2119.33	RI, MS	C ₂₀ H ₄₀ O	102608-53-7	0.07%	ND
21	Phytol	2122	RI, MS	C ₂₀ H ₄₀ O	150-86-7	0.09%	0.04%

Table S3 The inhibitory effect of TVEO on *Escherichia coli* and *Staphylococcus aureus* was evaluated.

Essential oil concentration (μL/mL)	Inhibition	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
0.20	2.50%	10.34%
0.40	6.52%	32.03%
0.60	20.25%	74.71%
0.80	43.75%	99.99%
1.00	77.15%	99.99%