

Exploring the efficacious subfractions and underlying mechanisms of Herba Siegesbeckiae against myocardial ischemia/reperfusion injury via UCHL5/NLRP3 pathway

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Supplementary Materials

Contents

Figure S1. Primary mass spectrometry of ent-2 β ,15,16-trihydroxypimar-8(14)-en-18-oic acid.....	4
Figure S2. Secondary mass spectrometry of ent-2 β ,15,16-trihydroxypimar-8(14)-en-18-oic acid	4
Figure S3. Primary mass spectrometry of ent-15-oxo-2 β ,16,19-trihydroxypimar-8(14)-ene.....	5
Figure S4. Secondary mass spectrometry of ent-15-oxo-2 β ,16,19-trihydroxypimar-8(14)-ene.....	5
Figure S5. Primary mass spectrometry of ent-15,16-dihydroxypimar-1,8(14)-dien-3-one.....	6
Figure S6. Secondary mass spectrometry of ent-15,16-dihydroxypimar-1,8(14)-dien-3-one.....	6
Figure S7. Primary mass spectrometry of kirenol.....	7
Figure S8. Secondary mass spectrometry of kirenol.....	7
Figure S9. Primary mass spectrometry of siegesbeckic acid.....	8
Figure S10. Secondary mass spectrometry of siegesbeckic acid.....	8
Figure S11. Primary mass spectrometry of eicosapentaenoic acid.....	9
Figure S12. Secondary mass spectrometry of eicosapentaenoic acid.....	9
Figure S13. Comparison of secondary mass spectra of eicosapentaenoic acid standards retrieved by UPLC-HRMS and mzCloud.....	9
Figure S14. Primary mass spectrometry of quercetin.....	10
Figure S15. Secondary mass spectrometry of quercetin.....	10
Figure S16. Comparison of secondary mass spectra of quercetin standards retrieved by UPLC- HRMS and mzCloud.....	10
Figure S17. Primary mass spectrometry of oxydipropyl dibenzoate.....	11
Figure S18. Secondary mass spectrometry of oxydipropyl dibenzoate.....	11
Figure S19. Comparison of secondary mass spectra of oxydipropyl dibenzoate standards retrieved by UPLC-HRMS and mzCloud.....	11
Figure S20. Primary mass spectrometry of dodecyl hydrogen sulphate.....	12
Figure S21. Secondary mass spectrometry of dodecyl hydrogen sulphate.....	12
Figure S22. Comparison of secondary mass spectra of dodecyl hydrogen sulphate standards retrieved by UPLC-HRMS and mzCloud.....	12
Figure S23. Primary mass spectrometry of 4-dodecylbenzenesulfonic acid.....	13
Figure S24. Secondary mass spectrometry of 4-dodecylbenzenesulfonic acid.....	13
Figure S25. Comparison of secondary mass spectra of 4-dodecylbenzenesulfonic acid standards retrieved by UPLC-HRMS and mzCloud.....	13
Figure S26. Primary mass spectrometry of monopalmitin.....	14
Figure S27. Secondary mass spectrometry of monopalmitin.....	14
Figure S28. Secondary mass spectrum of the monopalmitin standard retrieved from PubChem...	14
Figure S29. Standard plot of kirenol.....	15
Table S1. Information on the identification of 11 components of HS-C.....	16
Table S2. Calculation of kirenol content in HS-C.....	17

All sample data collected by UPLC-HRMS were processed using Compound Discoverer software. Each compound was initially identified by comparing the data with the published literature, the online database mzCloud, and the multistage fragment mass spectra containing the compounds in PubChem. Among them, compounds 1-5 and 7 were preliminarily demonstrated in the published literature¹⁻⁵, compounds 6-10 were preliminarily proved by comparing the secondary mass spectrum information in mzCloud database^{6,7}, and compound 11 was preliminarily determined by comparing the secondary mass spectrum information with the PubChem database⁸.

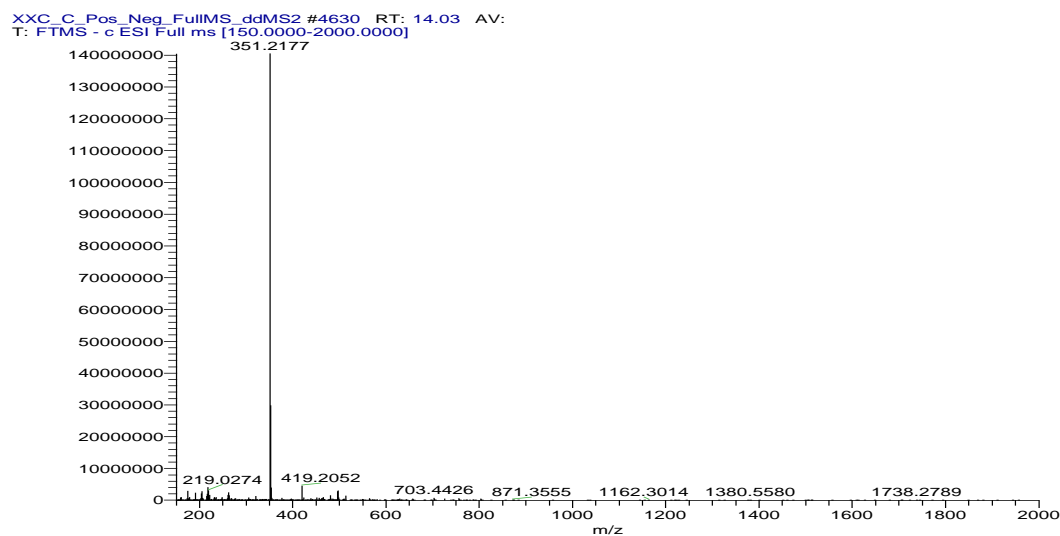


Fig.S1. Primary mass spectrometry of ent-2 β ,15,16-trihydroypimar-8(14)-en-18-oic acid (Compound 1).

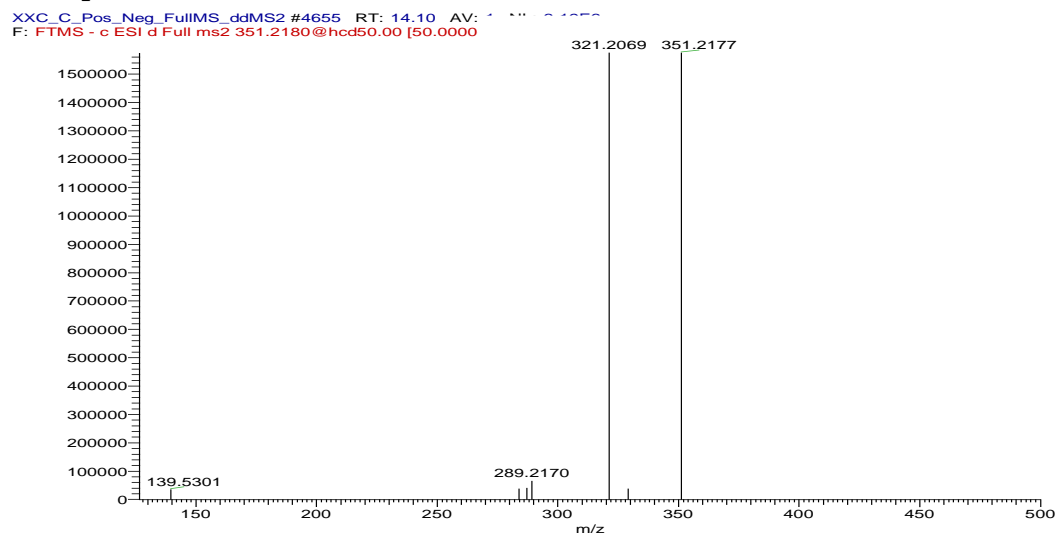


Fig.S2. Secondary mass spectrometry of ent-2 β ,15,16-trihydroypimar-8(14)-en-18-oic acid (Compound 1).

XXC_C_Pos_Neg_FullIMS_ddMS2 #6172 RT: 18.59 AV:
T: FTMS + c ESI Full ms [150.0000-2000.0000]

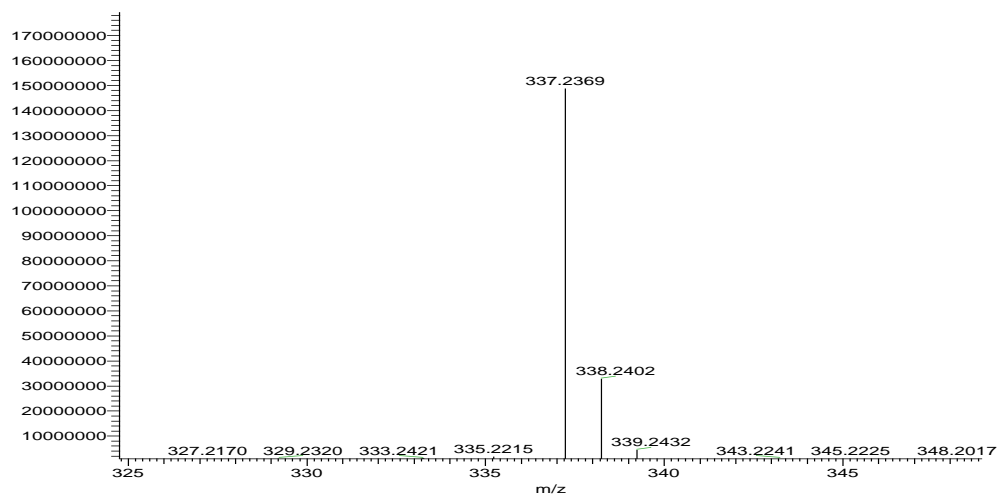


Fig.S3. Primary mass spectrometry of ent-15-oxo-2 β ,16,19-trihydroxypimar-8(14)-ene (Compound 2).

XXC_C_Pos_Neg_FullIMS_ddMS2 #6151 RT: 18.53 AV: 1
F: FTMS + c ESI d Full ms2 337.2382@hcd50.00 [50.0000-2000.0000]

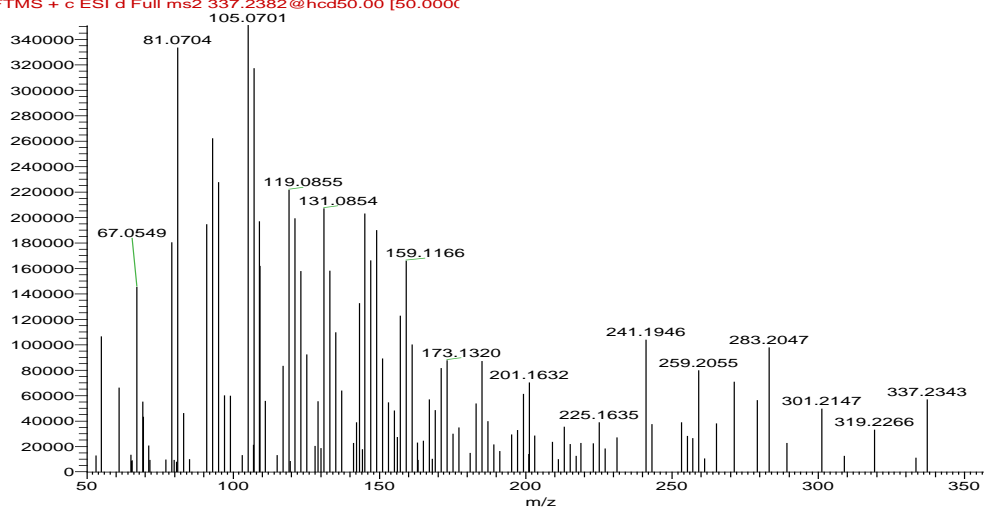


Fig.S4. Secondary mass spectrometry of ent-15-oxo-2 β ,16,19-trihydroxypimar-8(14)-ene (Compound 2).

XXC_C_Pos_Neg_FullMS2 #6184 RT: 18.63 AV:
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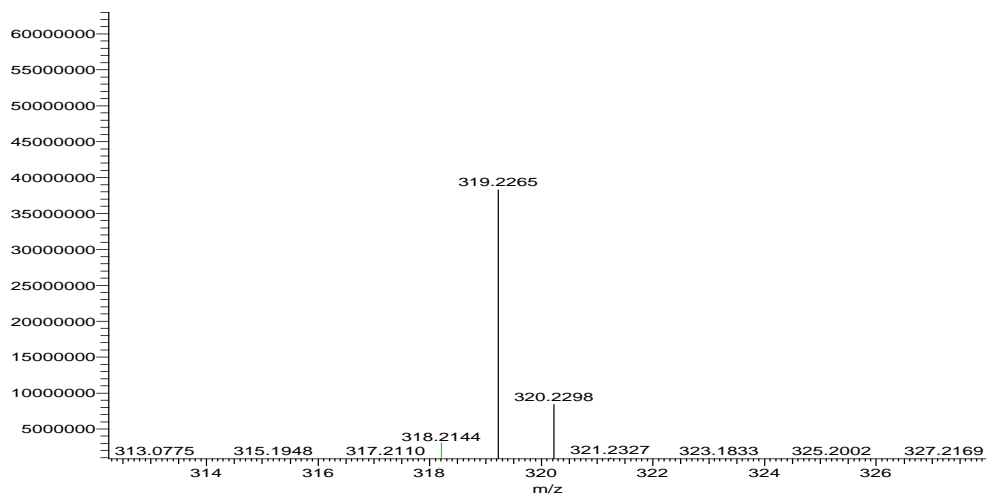


Fig.S5. Primary mass spectrometry of ent-15,16-dihydroxypimar-1,8(14)-dien-3-one (Compound 3).

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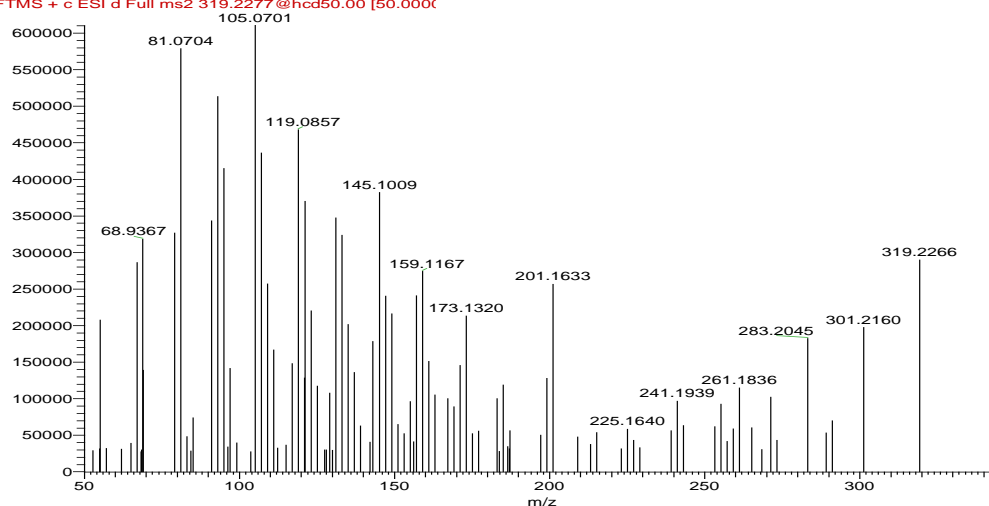


Fig.S6. Secondary mass spectrometry of ent-15,16-dihydroxypimar-1,8(14)-dien-3-one (Compound 3).

XXC_C_Pos_Neg_FullMS_ddMS2 #6292 RT: 18.94 AV:
T: FTMS + c ESI Full ms [150.0000-2000.0000]

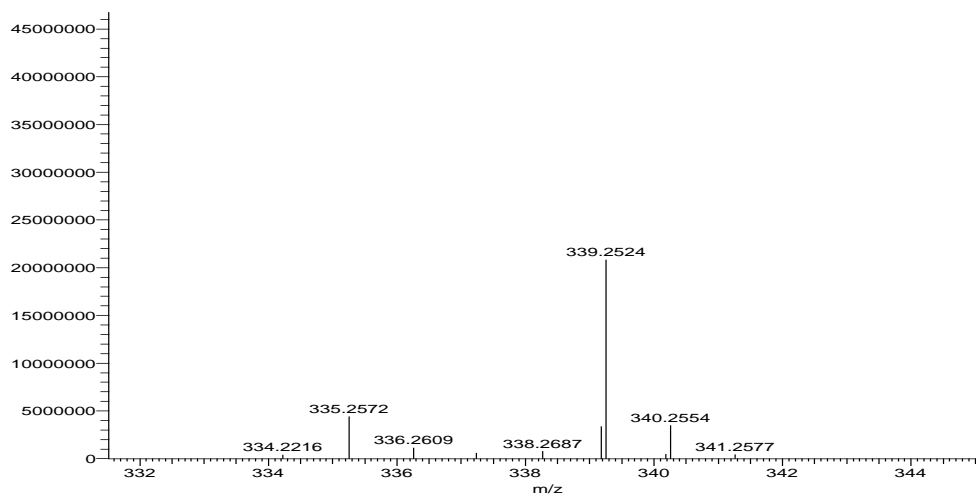


Fig.S7. Primary mass spectrometry of kirenol (Compound 4).

XXC_C_Pos_Neg_FullMS_ddMS2 #6320 RT: 19.02 AV: 1 NI: 5 12F4
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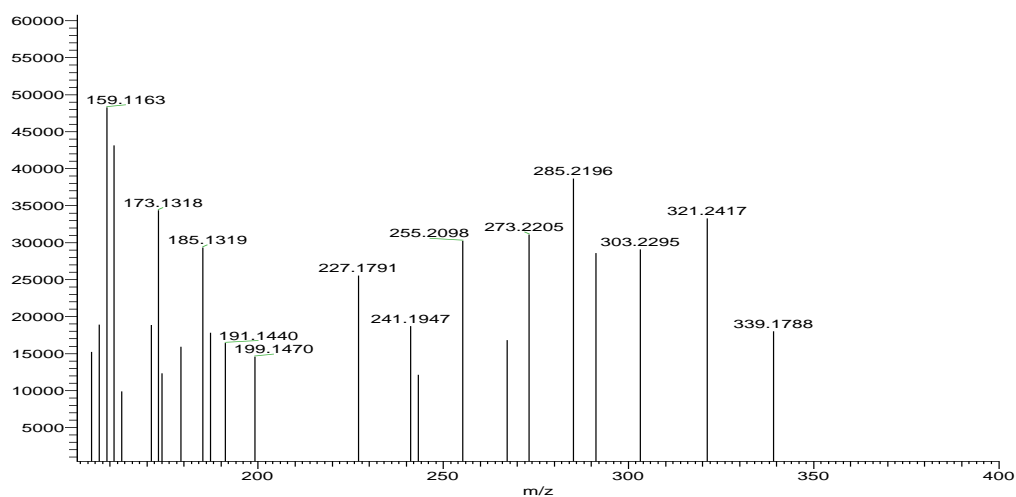


Fig.S8. Secondary mass spectrometry of kirenol (Compound 4).

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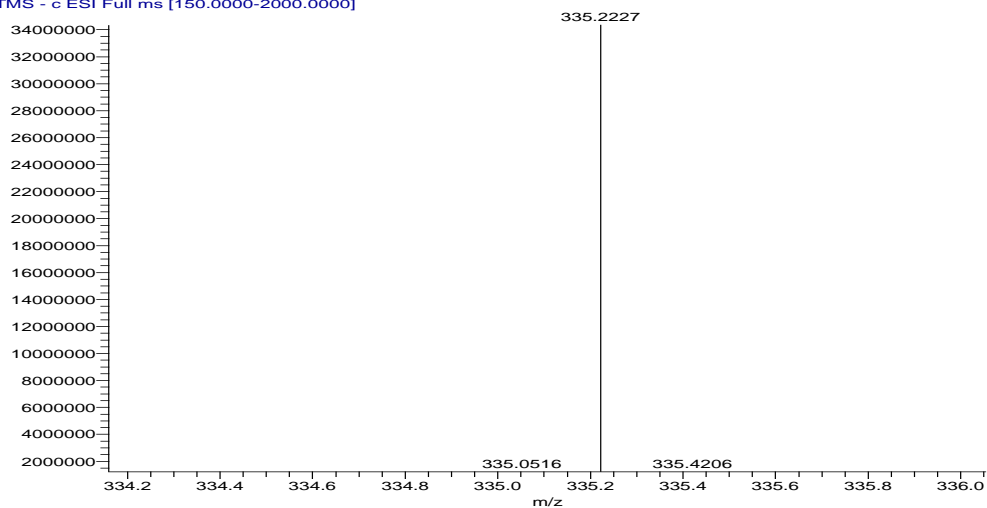


Fig.S9. Primary mass spectrometry of siegesbeckic acid (Compound 5).

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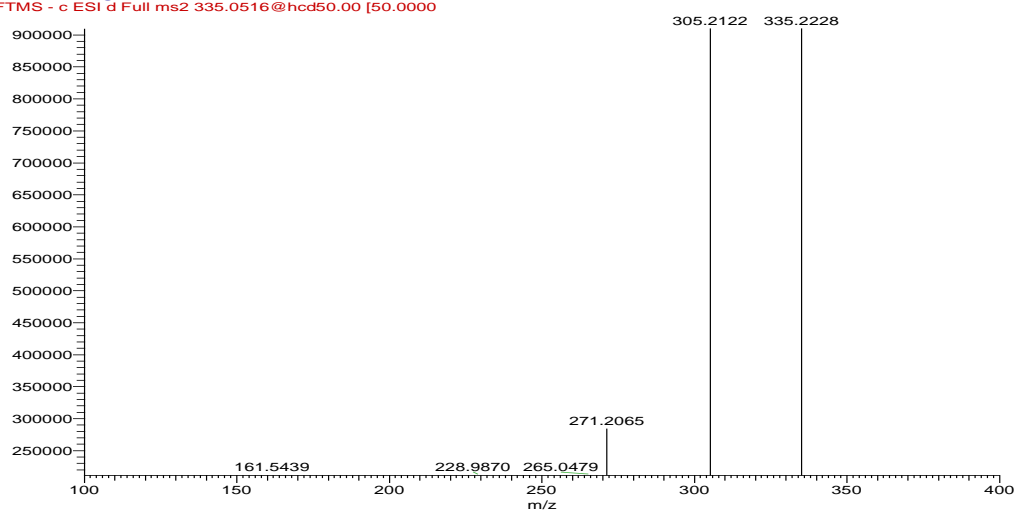


Fig.S10. Secondary mass spectrometry of siegesbeckic acid (Compound 5).

XXC_C_Pos_Neg_FullIMS_ddMS2 #6532 RT: 19.63 AV: 1
 T: FTMS + c ESI Full ms [150.0000-2000.0000]

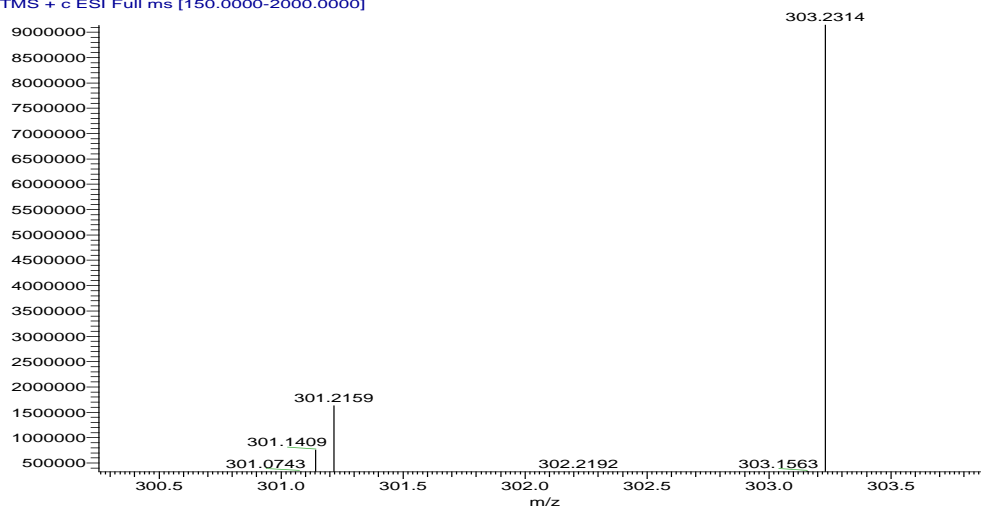


Fig.S11. Primary mass spectrometry of eicosapentaenoic acid (Compound 6).

XXC_C_Pos_Neg_FullIMS_ddMS2 #6546 RT: 19.67 AV: 1 NI: 2 68F5
 F: FTMS + c ESI d Full ms2 303.1962@hcd50.00 [50.0000]

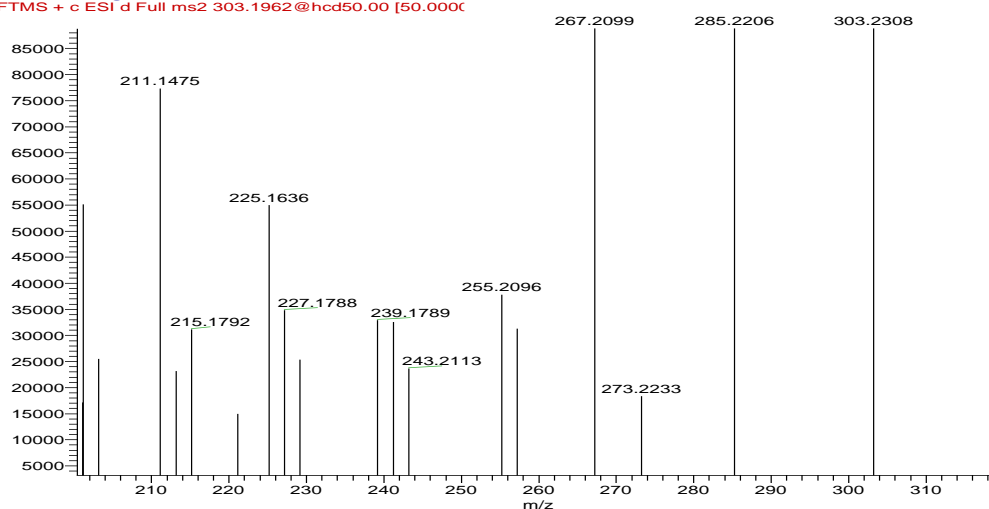


Fig.S12. Secondary mass spectrometry of eicosapentaenoic acid (Compound 6).

RAWFILE(top): XXC_C_Pos_Neg_FullIMS_ddMS2 (F2) #6546, RT=19.667 min, MS2, FTMS (+), (HCD, DDA, 303.2314@(30;50;70), +1)
 REFERENCE(bottom): mzCloud library, Eicosapentaenoic acid, C20 H30 O2, MS2, FTMS, (HCD, 303.2319@(30;50;70))

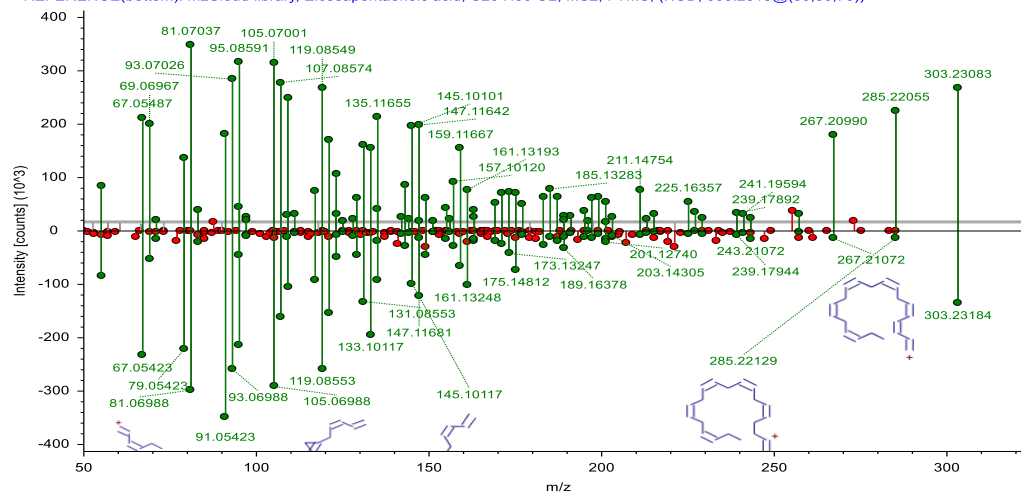


Fig.S13. Comparison of secondary mass spectra of eicosapentaenoic acid (compound 6) standards retrieved by UPLC-HRMS and mzCloud.

XXC_C_Pos_Neg_FullMS_ddMS2 #6580 RT: 19.77 AV: 1 NI: 1.14F4
T: FTMS + c ESI Full ms [150.0000-2000.0000]

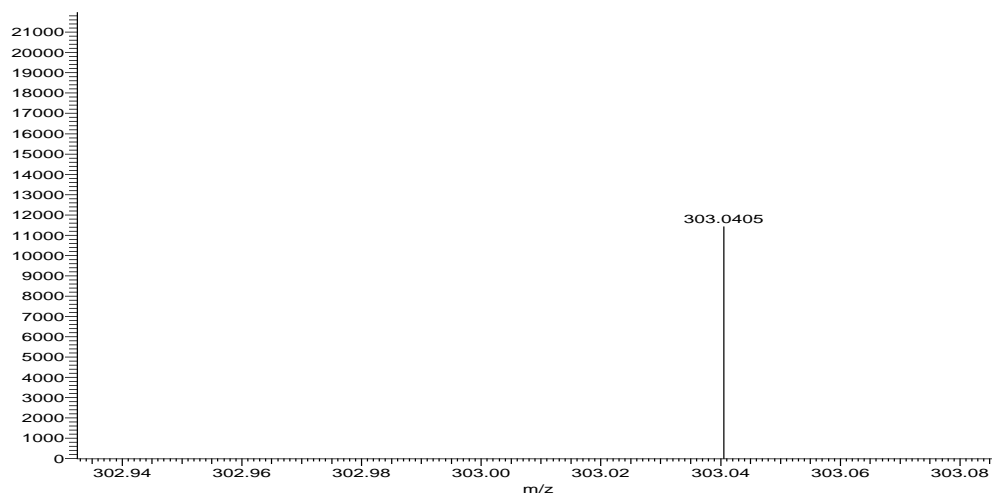


Fig.S14. Primary mass spectrometry of quercetin (Compound 7).

XXC_C_Pos_Neg_FullMS_ddMS2 (F2) #3956 RT: 12.016 min, MS2_FTMS (+), (HCD, DDA, 303.0504@(30;50;70), +1)
F: FTMS + c ESI d Full ms2 303.1962@hcd50.00 [50.0000]

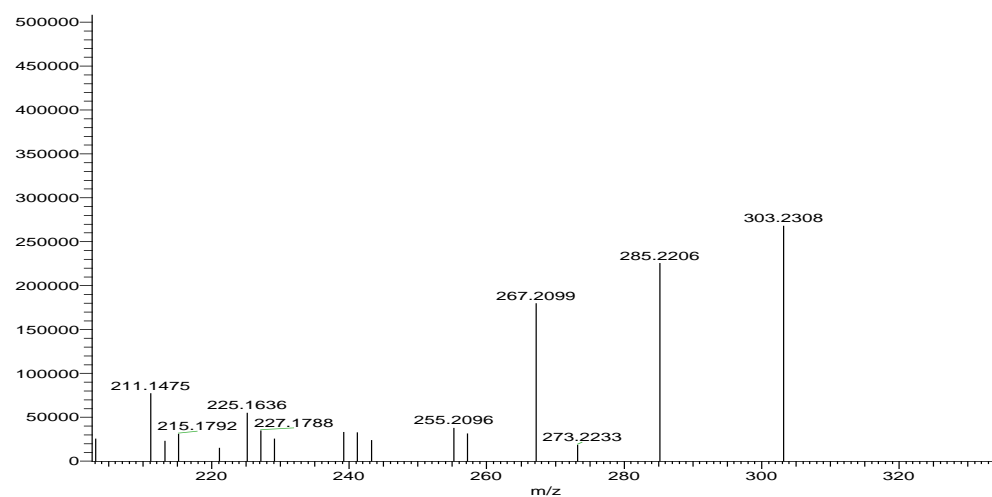


Fig.S15. Secondary mass spectrometry of quercetin (Compound 7).

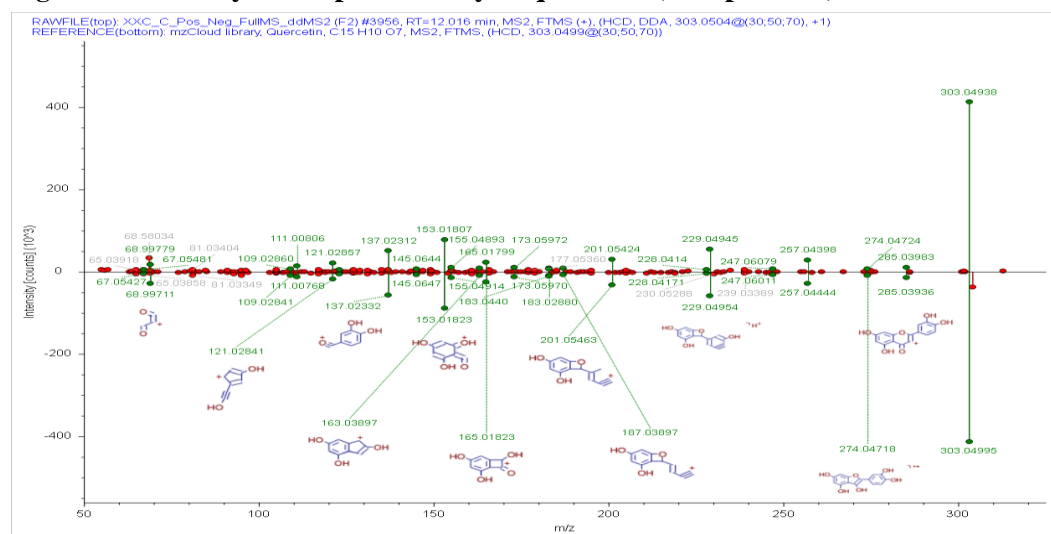


Fig.S16. Comparison of secondary mass spectra of quercetin (compound 7) standards retrieved by UPLC-HRMS and mzCloud.

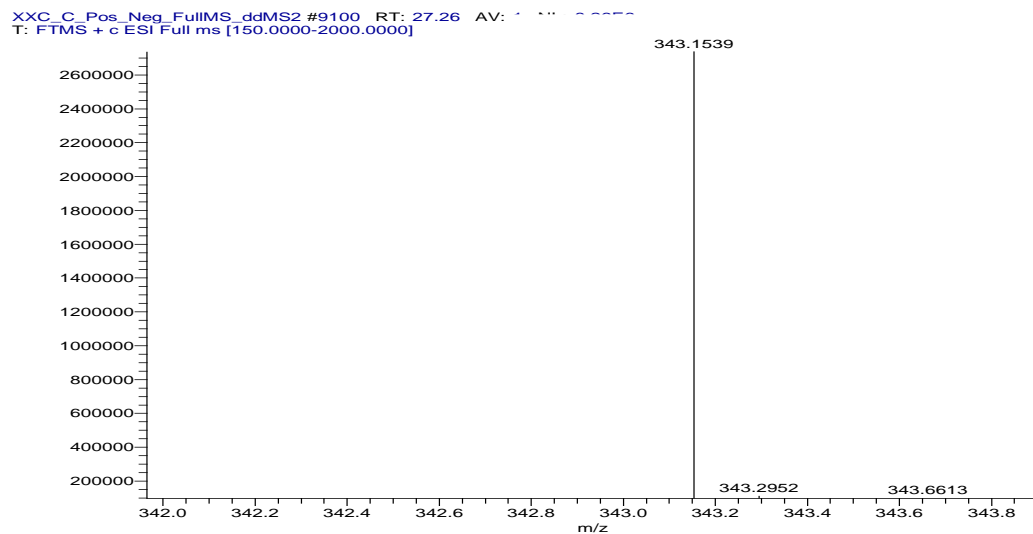


Fig.S17. Primary mass spectrometry of oxydipropyl dibenzoate (Compound 8).

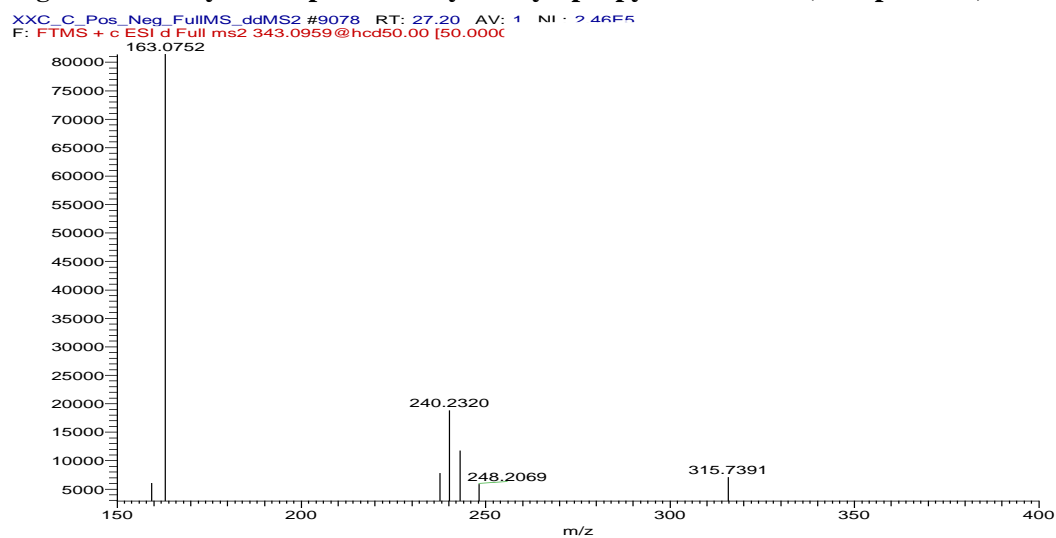


Fig.S18. Secondary mass spectrometry of oxydipropyl dibenzoate (Compound 8).

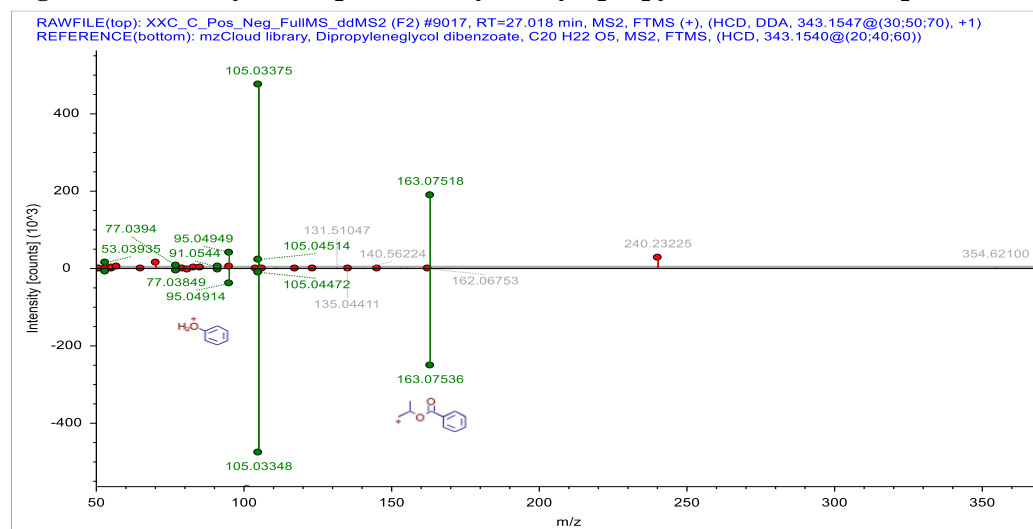


Fig.S19. Comparison of secondary mass spectra of oxydipropyl dibenzoate (Compound 8) standards retrieved by UPLC-HRMS and mzCloud.

XXC_C_Pos_Neg_FullIMS_ddMS2 #10786 RT: 32.27 A\
T: FTMS - c ESI Full ms [150.0000-2000.0000]

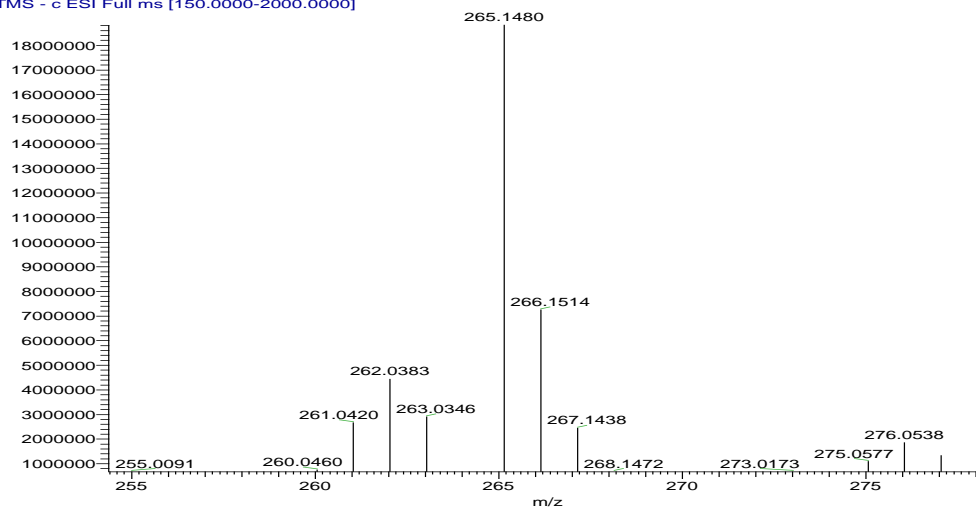


Fig.S20. Primary mass spectrometry of dodecyl hydrogen sulphate (Compound 9).

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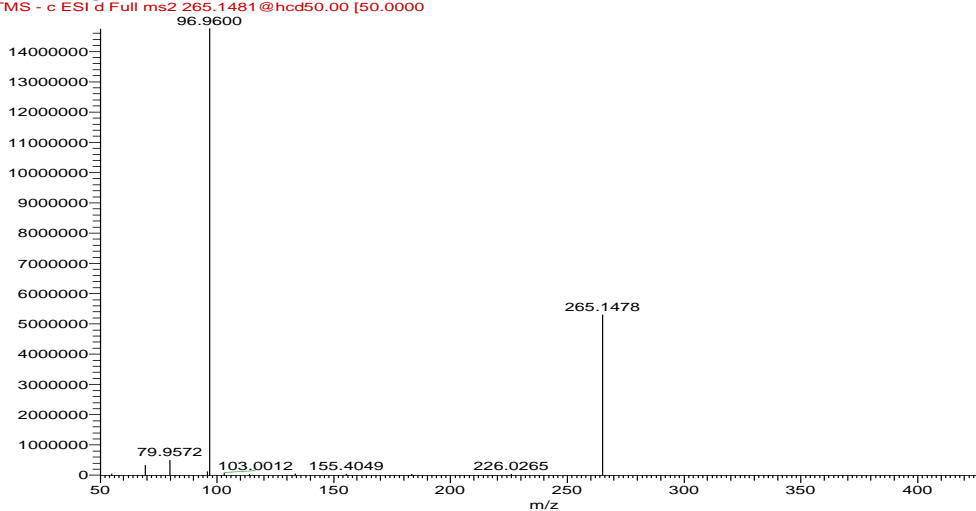


Fig.S21. Secondary mass spectrometry of dodecyl hydrogen sulphate (Compound 9).

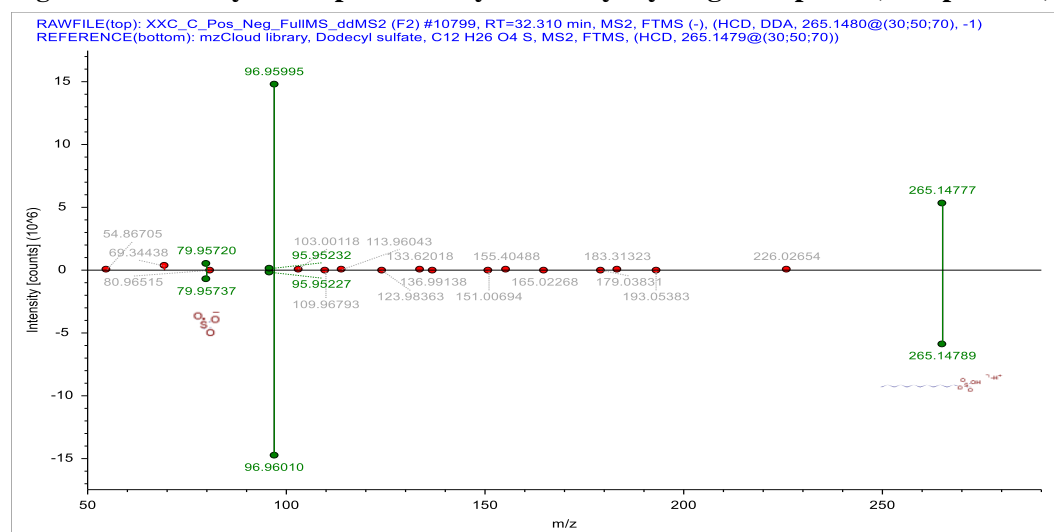


Fig.S22. Comparison of secondary mass spectra of dodecyl hydrogen sulphate (Compound 9) standards retrieved by UPLC-HRMS and mzCloud.

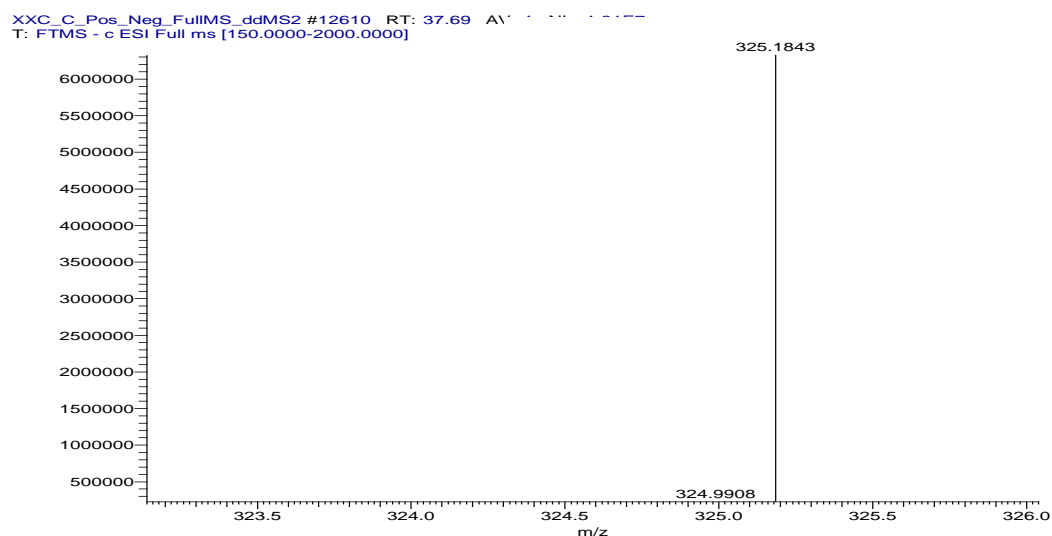


Fig.S23. Primary mass spectrometry of 4-dodecylbenzenesulfonic acid (Compound 10).

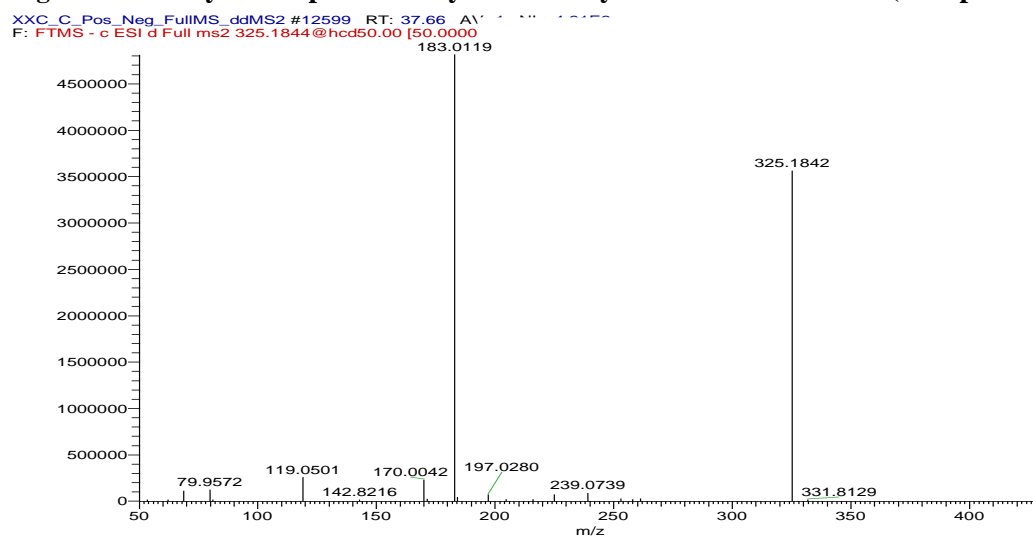


Fig.S24. Secondary mass spectrometry of 4-dodecylbenzenesulfonic acid (Compound 10).

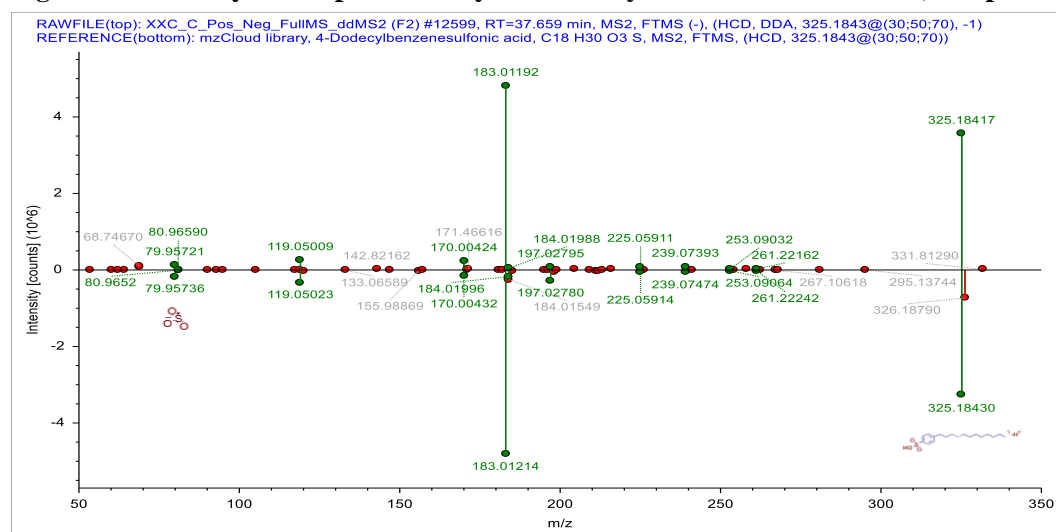


Fig.S25. Comparison of secondary mass spectra of 4-dodecylbenzenesulfonic acid (Compound 10) standards retrieved by UPLC-HRMS and mzCloud.

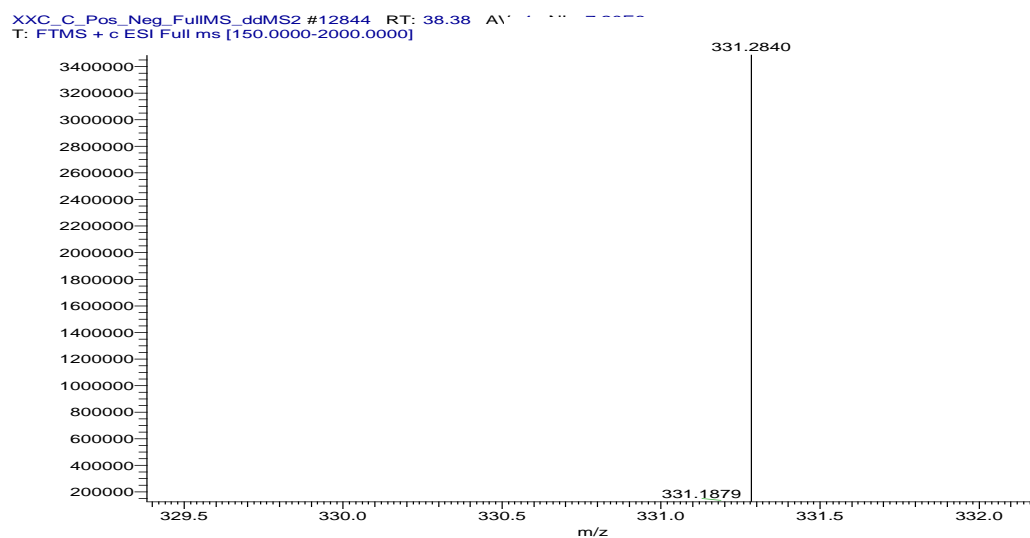


Fig.S26. Primary mass spectrometry of monopalmittin (Compound 11).

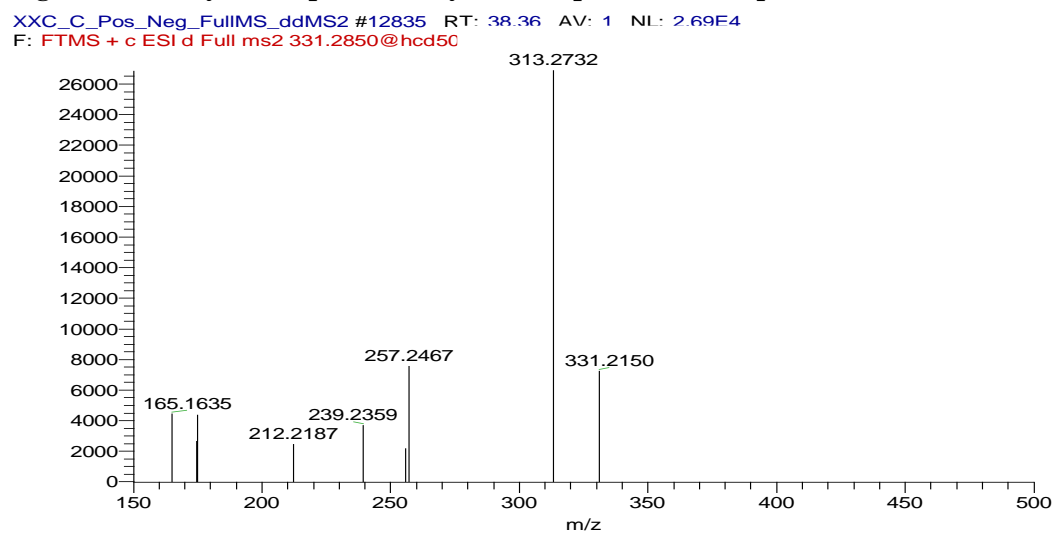


Fig.S27. Secondary mass spectrometry of monopalmittin (Compound 11).

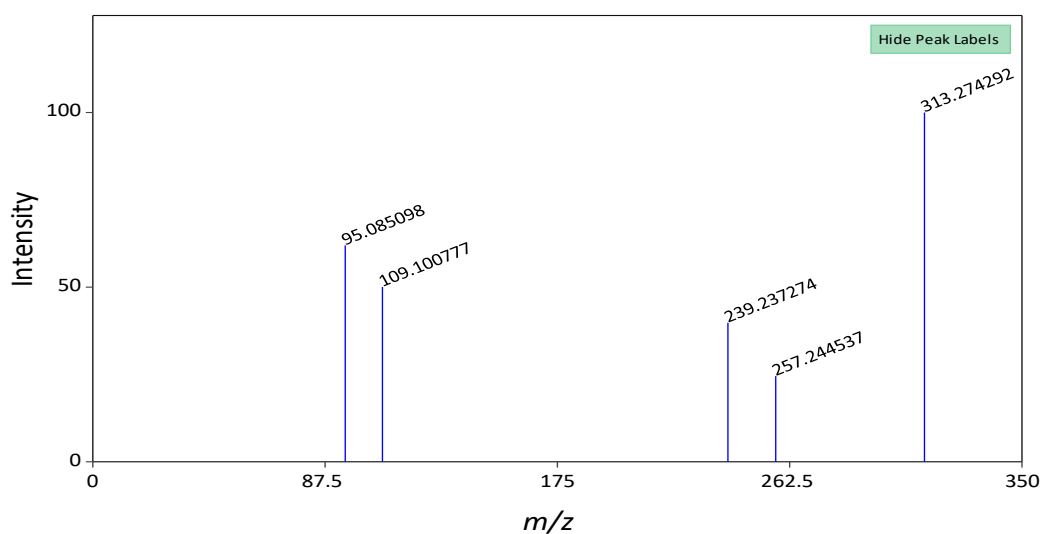


Fig.S28. Secondary mass spectrum of the monopalmittin (Compound 11) standard retrieved from PubChem.

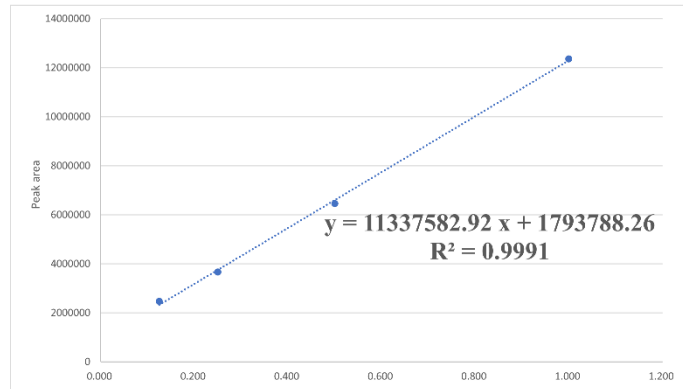
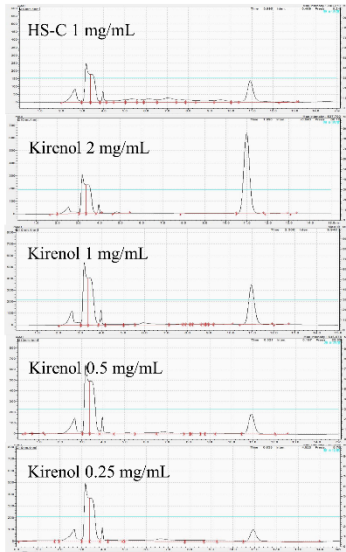


Figure S29. Standard plot of kirenol.

Table S1.

Information on the identification of 11 components of HS-C.

	Name	m/z	RT[min]	$\Delta m/ppm$	Major fragment ion	Reference	mzCloud score (%)
1	Ent-2 β ,15,16-trihydroxypimar-8(14)-en-18-oic acid	[M-H] ⁻ 351.2177	14.03	0.00	[M-H-CH ₂ O] ⁻ 321.2069	¹	-
2	Ent-15-oxo-2 β ,16,19-trihydroxypimar-8(14)-ene	[M+H] ⁺ 337.2369	18.59	-1.19	[M+H-H ₂ O] ⁺ 319.2266 [M+H-2H ₂ O] ⁺ 301.2147 [M+H-3H ₂ O] ⁺ 283.2047	²	-
3	Ent-15,16-dihydroxypimar-1,8(14)-dien-3-one	[M+H] ⁺ 319.2265	18.63	-0.94	[M+H-H ₂ O] ⁺ 301.2160 [M+H-2H ₂ O] ⁺ 283.2045	¹	-
4	Kirenol	[M+H] ⁺ 339.2524	18.97	-1.77	[M+H-H ₂ O] ⁺ 321.2416 [M+H-2H ₂ O] ⁺ 303.2295 [M+H-3H ₂ O] ⁺ 285.2196	³	-
5	Siegesbeckic acid	[M-H] ⁻ 335.2227	19.12	-0.30	[M-H-CH ₂ O] ⁻ 305.2122 [M-H-CH ₂ O-2OH] ⁻ 271.2065	⁴	-
6	Eicosapentaenoic acid	[M+H] ⁺ 303.2314	19.63	-1.32	[M+H-H ₂ O] ⁺ 285.2206 [M+H-2H ₂ O] ⁺ 267.2099	⁶	89

7	Quercetin	[M+H] ⁺ 303.0499	19.87	1.98	[M+H-H ₂ O] ⁺ 285.2206 [M+H-2H ₂ O] ⁺ 267.2099 [M+H-H ₂ O-CO] ⁺ 257.2253 [M+H-2CO-H ₂ O] ⁺ 229.1595 [M+H-2CO-H ₂ O- O] ⁺ 213.1627	⁵	97.6
8	Oxydipropyl dibenzoate	[M+H] ⁺ 343.1539	27.26	-0.29	[M+H-C ₁₀ H ₁₂ O ₃] ⁺ 163.0752	⁶	87.8
9	Dodecyl hydrogen sulphate	[M-H] ⁻ 265.148	32.27	0.38	[M-H-C ₁₂ H ₂₅] ⁻ 96.9600 [M-H-C ₁₂ H ₂₅ -OH] ⁻ 79.9572	⁶	99.8
10	4-dodecylbenzenesulfonic acid	[M-H] ⁻ 325.1843	37.69	0.00	[M-H-C ₁₀ H ₂₂] ⁻ 183.0119	⁶	97.2
11	Monopalmitin	[M+H] ⁺ 31.2848	28.28	1.51	[M+H-H ₂ O] ⁺ 313.2732	⁸	-

Table S2.

Calculation of kirenol content in HS-C.

Name	Concentration (mg/mL)	Peak area
HS-C	1	8879778
Kirenol	2	24606513
Kirenol	1	12862721
Kirenol	0.5	7305618
Kirenol	0.25	4916237

Reference

- 1 Jiang, Z. Studies on active components and quality control method of siegesbeckia pubescens M. *Shenyang Pharmaceutical University* (2011).
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- 4 Ma, Y. H. Investigation on the Chemical Constituents of Diterpenoids in the Isodon Rubescens (Hemsl.) Hara for Quality Control and The Metabolism of Oridonin in Different Liver Microsomes. *Hebei medical university* (2017).
- 5 Guo, S. H. *et al.* Analysis of Quercetin in Sedum Aizoon L. by Using HPLC-MS/MS and HPLC Determination of Quercetin in the Sedum Aizoon L. and in Yano Xin Cao Capsule. *Chinese Archives of Traditional Chinese*. **3**, 460-462 (2006). <https://doi.org/10.3969/j.issn.1673-7717.2006.03.037>.
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- 7 Xiao, Y. *et al.* Components analysis of San-Bai decoction, and its pharmacodynamics and mechanism on preventing and treating melasma. *J Ethnopharmacol*, **5**, 118388 (2024). <https://doi.org/10.1016/j.jep.2024.118388>.
- 8 Kim, S. *et al.* PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Res*, **49**, D1388-D1395 (2021). <https://doi.org/10.1093/nar/gkaa971>. Available online: <https://pubchem.ncbi.nlm.nih.gov> (accessed 15 May 2024)