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Metabolomic reveal the protective effect of Farfarae Flos against asthma using an OVA-induced rat model

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The identification of the compounds by LC-MS

Compound **2**, gave $[M+H]^+$ ion at *m/z* 431.2794 and $[M+Na]^+$ ion at *m/z* 453.2617 in positive ion mode, and was identified as 7β-(3'-Ethyl-cis-crotonoyloxy)-1α-(2'-methylbutyryloxy)-3(14)-dehydro-*Z*-notonipetranone ($C_{26}H_{38}O_5$). It produced fragment ions at *m/z* 351.1933 $[M+Na\text{-}MebuO]^+$, *m/z* 339.1933 $[M+Na\text{-}MesenO]^+$, *m/z* 255.1352 $[M+Na\text{-}MesenO\text{-}Mebu]^+$, *m/z* 237.1252 $[M+Na\text{-}MesenO\text{-}MebuO]^+$, *m/z* 215.1433 $[M+H\text{-}MesenO\text{-}MebuO]^+$, *m/z* 173.0963 in MS^2 spectra, and its possible fragment pathway was shown in Fig. S9A. The identification of the compound **2** was further confirmed by comparison with the authentic standard. Compounds **5** showed the same adduct ion at m/z at 431.2794 $[M+H]^+$ and 453.2617 $[M+Na]^+$, suggesting the same molecular formula of $C_{26}H_{38}O_5$. Thus, it was an double bond isomer of compound **5**, and identified as 7β-(3'-Ethyl-cis-crotonoyloxy)-1α-(2'-methylbutyryloxy)-3(14)-dehydro-*E*-notonipetranone. Compound **3** showed $[M+H]^+$ ion at *m/z* 391.2478, and was identified as tussilagone by comparing with the standard compound. Compound **6** exhibited $[M+H]^+$ ion at *m/z* 491.3003 and $[M+Na]^+$ ion at *m/z* 513.2819, which was 100 Da than that of tussilagone. As C-1 was usually substituted by a MebuO group, thus, compound **6** was tentatively identified as 14-acetoxy-7β-(3'-ethyl cis-crotonoyloxy) -lα-(2'-methyl butyryloxy)-notonipetranone. Compound **8** gave $[M+H]^+$ ion at *m/z* 507.2949 and $[M+Na]^+$ ion at *m/z* 529.2769 and produced predominant fragment ions at *m/z* 447.2741 $[M+H\text{-}OAC]^+$, *m/z* 431.2792 $[M+H\text{-}OAC\text{-}O]^+$, *m/z* 347.2215 $[M+H\text{-}OAC\text{-}O\text{-}Mebu]^+$, *m/z* 317.2110 $[M+H\text{-}OAC\text{-}O\text{-}MesenO]^+$, *m/z* 233.1535 $[M+H\text{-}OAC\text{-}O\text{-}MesenO\text{-}Mebu]^+$ in MS^2 spectra, and was identified as tussilagolactone. The possible fragment pathway for compound **8** was shown in Fig. S9B. Compound **9** showed protonated molecule $[M+H]^+$ at *m/z* 345.2061 in positive MS spectra, suggesting a possible molecular formula of $C_{21}H_{28}O_4$. It was 162 Da smaller than compound **8**, which was in agreement with a MebuO at C-1 and an AcO group at C-3, thus, it was tentatively identified as 7β-(3'-ethylcis-crotonoyloyx)-5,6-dehydro-3,14-dehydro-Z-notonipetralactone.

For the standard available compound **1**, it was identified by comparing retention time and accurate mass and identified as 2,2-dimethyl-6-acetylchromanone (Fig. S9C).

Table S1 ^{1}H NMR assignments of major metabolites from the petroleum ether extract of FF.

No.	Metabolite	Assignment	δ 1H multiplicity
1	2,2-dimethyl-6-acetylchromanone	CH3	1.50 (s)
		-COCH3	2.60 (s)
		2-H	2.78 (s)
		8-H	7.00 (d, 8.8 Hz)
		7-H	8.14 (dd, 2.4, 8.0 Hz)
		5-H	8.44 (d, 2.4 Hz)
2	7β-(3-Ethyl-ciscrotonoyloxy)-1α-(2-methylbutyryloxy)-3(14)-dehydro-Z-notonipetranone	CH3-4"	0.88 (t, 7.4 Hz)
		CH3-5'	1.07 (t, 7.5 Hz)
		CH3-5"	1.13 (d, 6.6 Hz)
		CH3-6'	2.15 (s)
		CH-10a	4.80 (s)
		CH-10b	5.17 (s)
		CH-14	6.38 (q, 6.6 Hz)
		CH3-13	0.78 (d, 6.6 Hz)
		CH3-12	0.98 (d, 6.6 Hz)
3	Tussilagone	CH3-5'	1.08 (t, 7.3 Hz)
		CH3-15	1.22 (d, 6.7 Hz)
		OAc	2.11 (s)
		CH3-6'	2.15 (s)
		CH-10a	4.79 (s)
		CH-10b	5.14 (s)
		CH-7	5.58 (brt, 2.3 Hz)
		CH-2'	5.63 (s)
		CH-1	5.86 (d, 12.5)
4	1β,8-bisangeloyloxy -3β,4β-epoxybisabola-7(14),10-diene	CH2-14	6.08 (S)
		CH3-15	1.46 (S)
		CH2-3'	6.06 (qq, 1.5,7.0)
		CH2-3"	6.06 (qq, 1.5,7.0)
		CH3-5'	1.89 (qq, 1.5,1.5)
		CH3-5"	1.91 (qq, 1.5,1.5)
5	7β-(3-Ethyl-ciscrotonoyloxy)-1α-(2-methylbutyryloxy)-3(14)-dehydro-E-notonipetranone	CH3-4"	0.88 (t, 7.4 Hz)
		CH3-5'	1.07 (t, 7.5 Hz)
		CH3-5"	1.13 (d, 6.6 Hz)
		CH3-6'	2.15 (s)
		CH-10a	4.80 (s)
		CH-10b	5.17 (s)
		CH-14	6.70 (q, 6.6 Hz)

6	14-acetoxy-7β-(3'-Ethyl-ciscrotonoyloxy)-1α-(2'-methylbutyryloxy)-notonipetranone	CH-1	5.45 (d, 3.0)
		CH3-12	1.00 (d, 6.6 Hz)
		CH3-13	0.82 (d, 6.6 Hz)
		CH2-14	5.16 (m)
		CH3-15	1.23 (d, 6.0)
		CH3-5'	1.07 (t, 7.8)
		CH3-6'	2.10 (S)
		CH3-4"	0.89 (t, 7.8)
		CH3-5"	1.15 (t, 7.8)
7	14-acetoxy-7β-angeloyloxy-notonipetranone	CH-7	5.57 (t, 3.0)
		CH3-12	0.98 (d, 6.9 Hz)
		CH3-13	0.78 (d, 6.9 Hz)
		CH3-15	1.23 (d, 6.0)
		CH3-4'	0.89 (d, 1.1)
		CH3-5'	2.15 (d, 1.1)

TableS2 Chemical constituents identified in extracts from the petroleum ether extract of FF.

No.	t_R	Molecular Formula	Selected ion	Experimental	Theoretical	Error (ppm)	MS/MS fragmentaion	Identification
1	1.211	C13H14O3	$[M+H]^+$	219.10179	219.10157	1	177.0910 163.0389	2,2-dimethyl-6-acetylchromanone
2	8.930	C26H38O5	$[M+H]^+$ $[M+Na]^+$	431.27942 453.26178	431.27920 453.26137	0.508 1.400	351.1933 339.1933 255.1352 237.1252 215.1433 173.0963 137.0578	7β-(3'-Ethyl-cis-crotonoyloxy)-1α-(2'-methylbutyryloxy)-3(14)-dehydro-*Z*-notonipetranone
3	7.94	C23H34O5	$[M+H]^+$	391.24789	391.24790	-0.027	331.2270 218.1301 217.1590 175.1482 133.1014	Tussilagone
5	10.413	C26H38O5	$[M+H]^+$ $[M+Na]^+$	431.27942 453.26178	431.27920 453.26137	0.508 1.400	351.1931 339.1933 255.1351 237.1250 215.1433 173.0963 137.0578	7β-(3'-Ethyl-cis-crotonoyloxy)-1α-(2'-methylbutyryloxy)-3(14)-dehydro-*E*-notonipetranone
6	6.071	C28H42O7	$[M+H]^+$	491.30033	491.30033	1.099	453.2612	14-acetoxy-7β-

			$[M+Na]^+$	513.28198	513.30087	2.290	351.1926 339.1928 255.1349 237.1246 215.1432 173.0958 137.0573 125.0573	(3'-ethyl cis-crotonoyloxy) -lα-(2'-methyl butyryloxy)-notonipetranone
8	4.962	$C_{28}H_{42}O_8$	$[M+H]^+$	507.29498	507.29587	1.233	447.2741 431.2792 347.2215 317.2110 233.1535	tussilagolactone
9	2.936	$C_{21}H_{28}O_4$	$[M+H]^+$	345.20618	345.20604	0.4	217.1587	7β-(3'-ethylcis-crotonoyloyx)-5,6-dehydro-3,14-dehydro-Z-notonipetralactone

Table S3 [1]H NMR assignments of major metabolites from rat lung.

No.	Metabolites	[1]H chemical shift (multiplicity)	No.	Metabolites	[1]H chemical shift (multiplicity)
1	Isoleucine	0.95(d)[a], 1.01(d)	22	Choline	3.2(s)
2	Leucine	0.97(t),	23	GPC[b]	3.22(s), 3.63(m)
3	Valine	0.99(d), 1.05(d)	24	PC[b]	3.23(s), 3.61(t)
4	3-HB[b]	1.20(d), 2.31(dd) ,2.41(dd)	25	PE[b]	3.23(t), 3.99(m)
5	Lactate	1.33(d), 4.12(q)	26	Taurine	3.27(t), 3.43(t)
6	lysine	1.49(m), 1.72(m), 1.92(m)	27	Betaine	3.27(s), 3.90(s)
7	Alanine	1.49(d)	28	*scyllo*-inositol	3.36(s)
8	Arginine	1.70(m), 1.92(m)	29	Methyl phosphate	3.47(d)
9	Ornithine	1.75(m), 1.93(m)	30	Adenosine	6.10(d), 8.24(s), 8.35(s)
10	Acetate	1.93(s)	31	β-glucose	4.65(d)
11	Glutamate	2.05(m), 2.34(m), 3.75(m)	32	Glycine	3.56(s)
12	Methionine	2.14(s), 2.14(m), 2.64(t), 3.85(m)	33	α-glucose	5.24(d)
13	Glutamine	2.15(m), 2.44(m), 3.77(m)	34	Cytidine	5.91(d), 6.07(d), 7.85(d)
14	GSSG[b]	2.17(m), 2.54(m), 2.95(m), 3.25(m)	35	Uracil	5.81(d), 7.55(d)
15	Ethanolamine	3.15(t), 3.84(t)	36	Fumarate	6.53(s)
16	Succinate	2.40(s)	37	Tyrosine	6.91(d), 7.20(d)
17	Pyruvate	2.37(s)	38	Phenylalanine	7.33(d), 7.43(t)
18	Aspartate	2.67(dd), 2.82(dd)	39	Niacinamide	7.60(dd), 8.26(d), 8.72(d), 8.94(s)
19	DMA[b]	2.72(s)	40	Xanthine	7.91(s)
20	TMA[b]	2.88(s)	41	Hypoxanthine	8.20(s), 8.22(s)
21	Creatine	3.04(s), 3.94(s)	42	Formate	8.46(s)

[a]Multiplicity for [1]H resonances: s: singlet, d: doublet, t: triplet, m: multiplet, dd: doublet of doublet.

[b]Keys: GSSG, glutathione disulfide; 3-HB, 3-hydroxybutyrat; DMA, dimethylamine; TMA, trimethylamine; GPC, glycerophosphocholine; PC, phosphocholine; PE, phosphoethanolamine;,

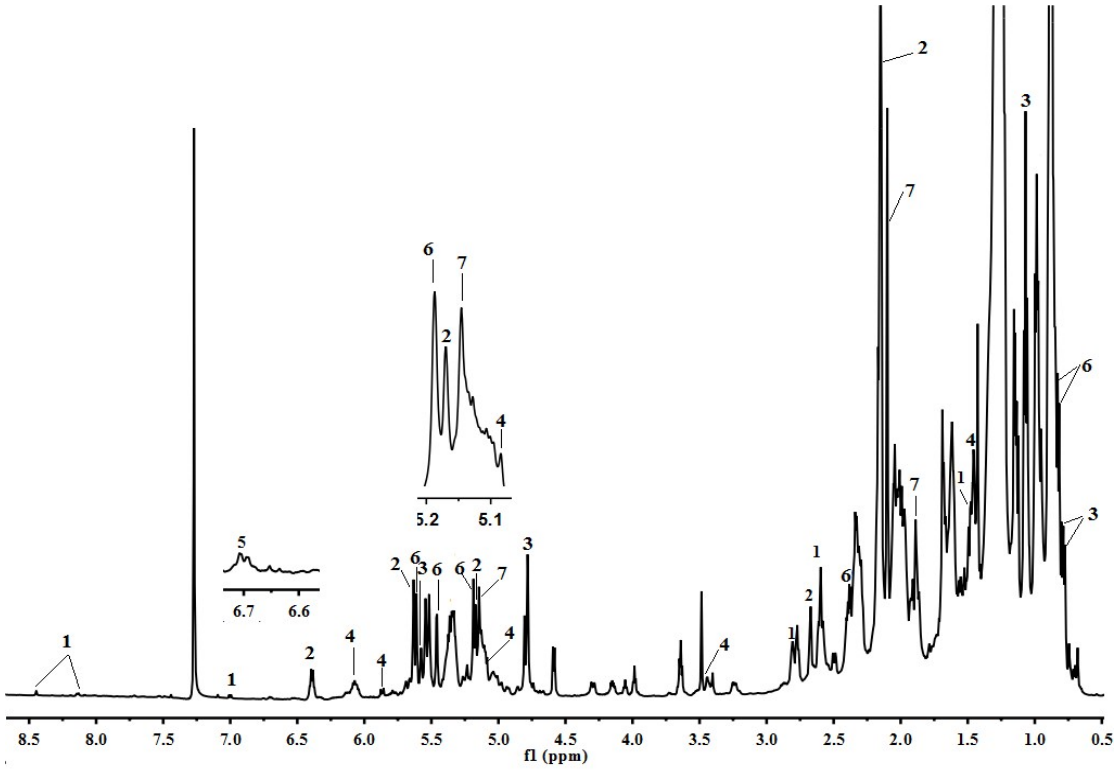


Figure S1 Typical ^{1}H NMR spectra of PEFF of FF.

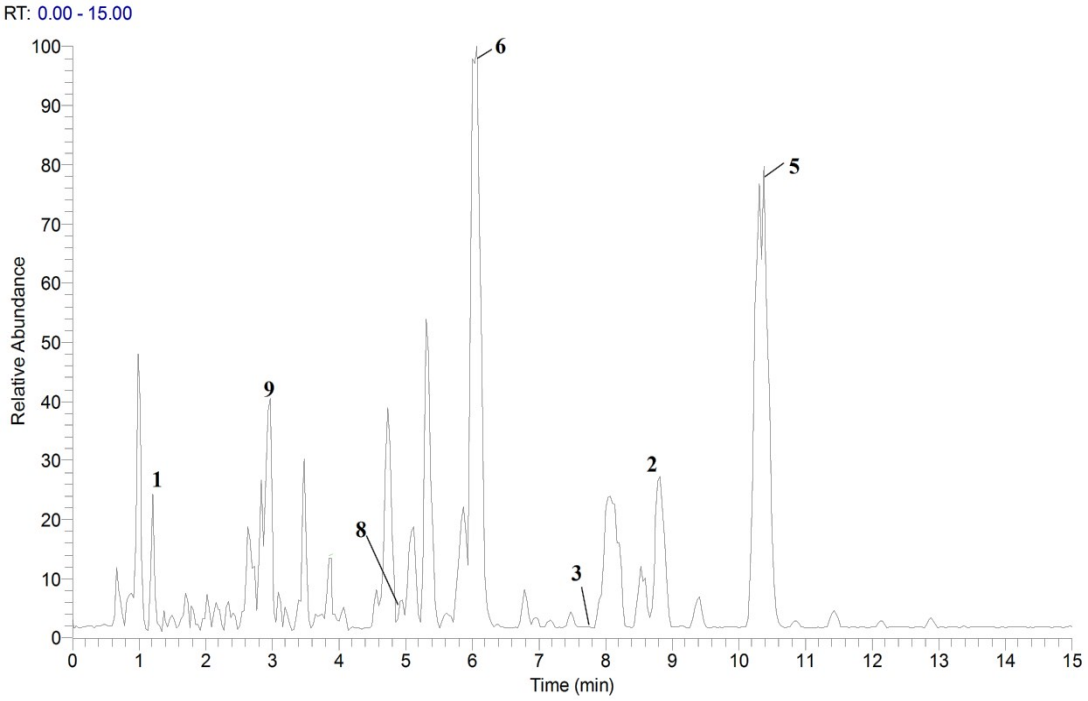


Figure S2 LC-MS chromatograms of PEFF of FF.

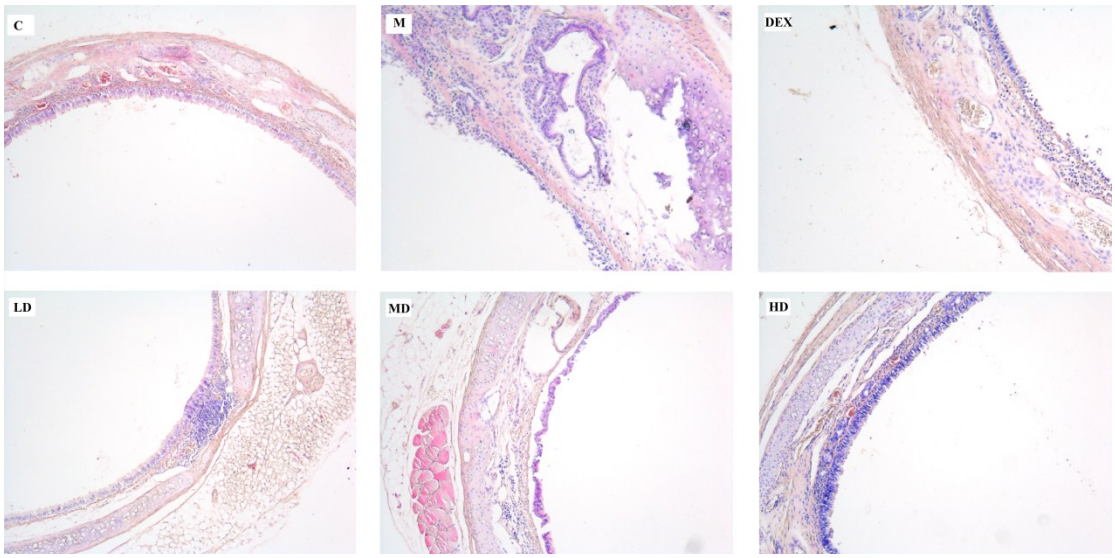


Figure S3 Histopathological photomicrographs of trache, and C mean control group, M mean model group, DEX mean dexamethasone group, LD mean low dose group, MD mean middle dose group, and HD mean high dose group.

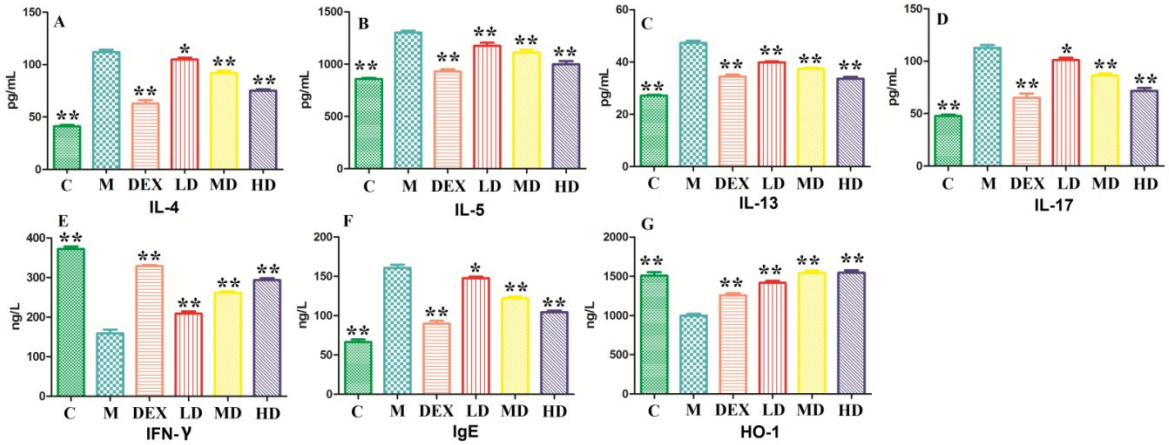


Figure S4 Quantification of inflammatory cytokines in serum as determined by ELISA. The statistical significance of differences between model group and other groups (n=6) are indicated as $p<0.05$ (*) and $p<0.01$ (**).

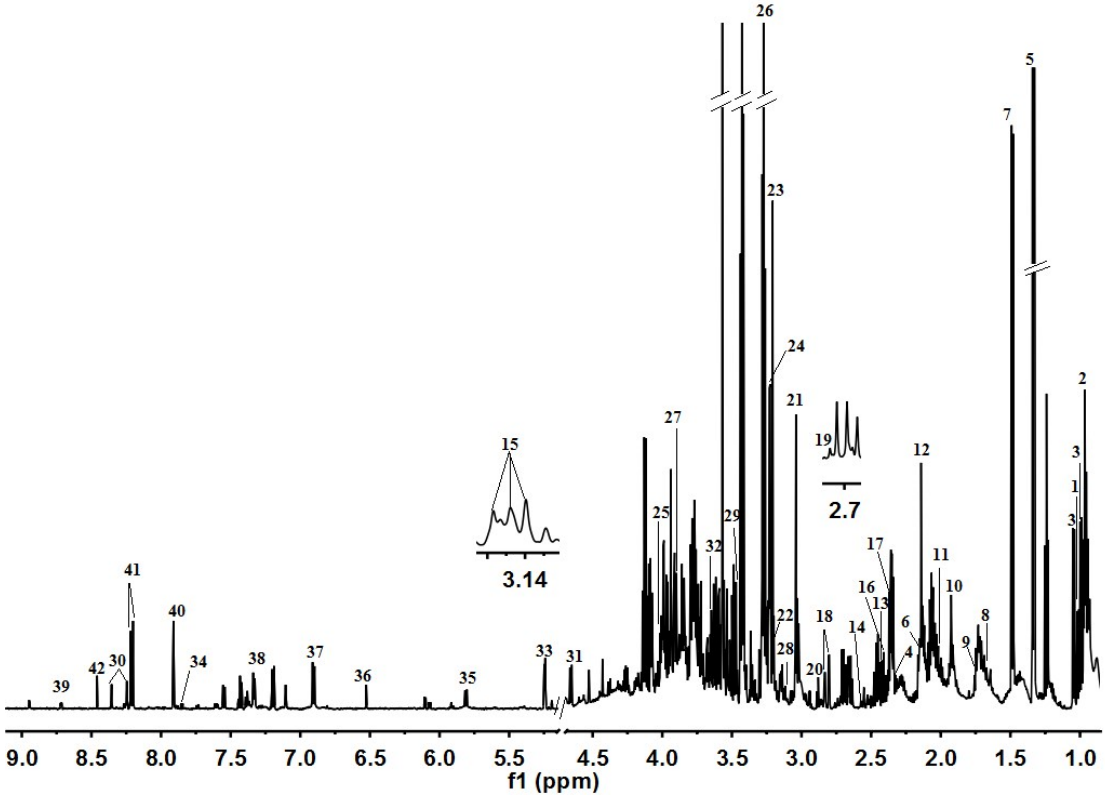


Figure S5 Representative ^{1}H NMR spectra of lung samples obtained from control group of rats.

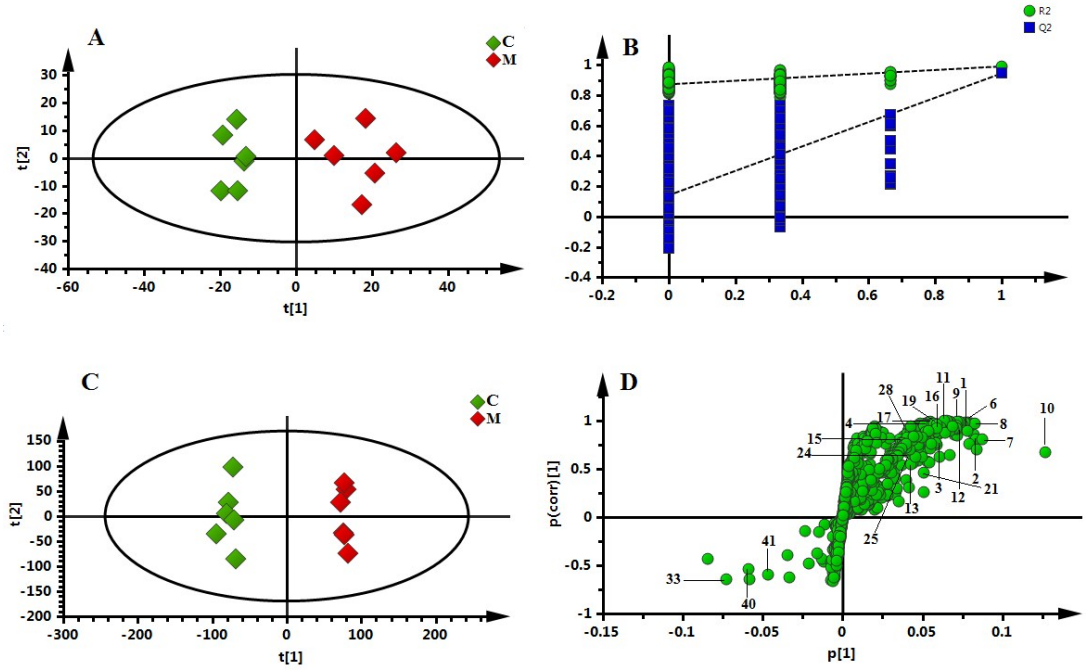


Figure S6 PCA (A) score plots, permutation test model validation plots (B), OPLS-DA (C) score plots, , and S-plot (D) based on ^{1}H-NMR data of lung homogenates from the control

and model groups (n=6).

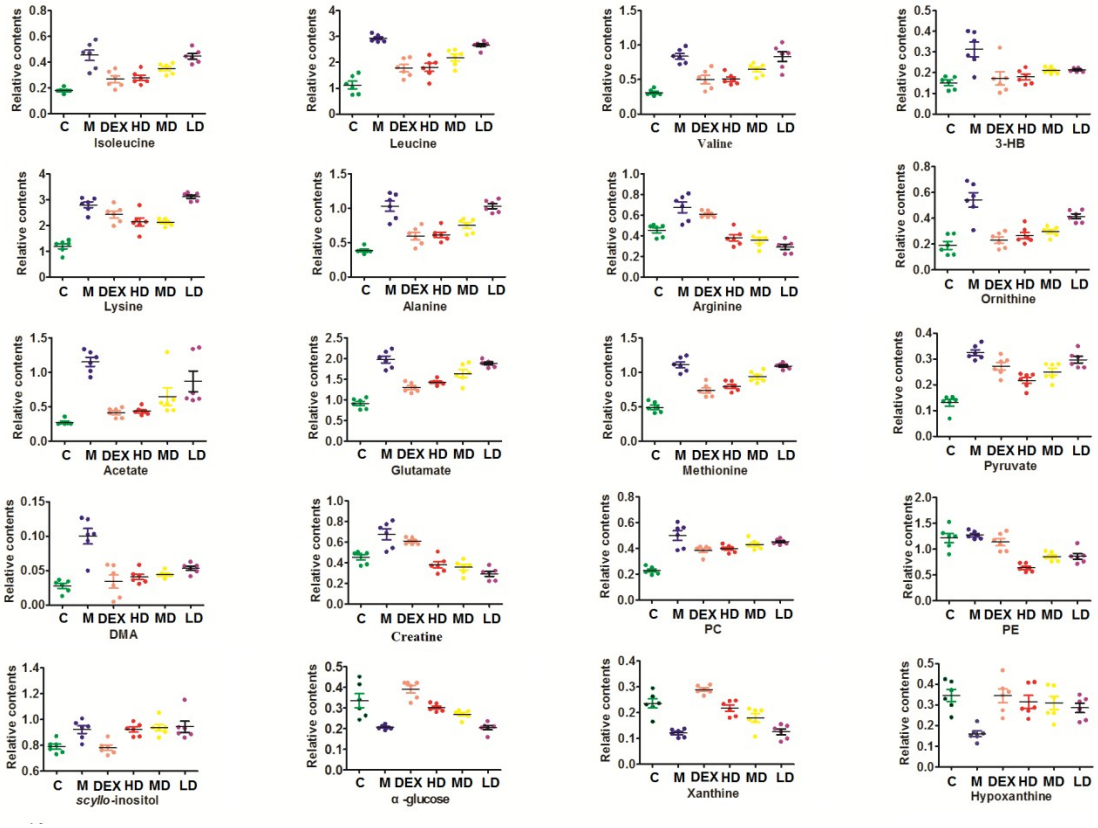


Figure S7 Quantification of metabolites indentified of lung homogenates of PEFF (n=6).

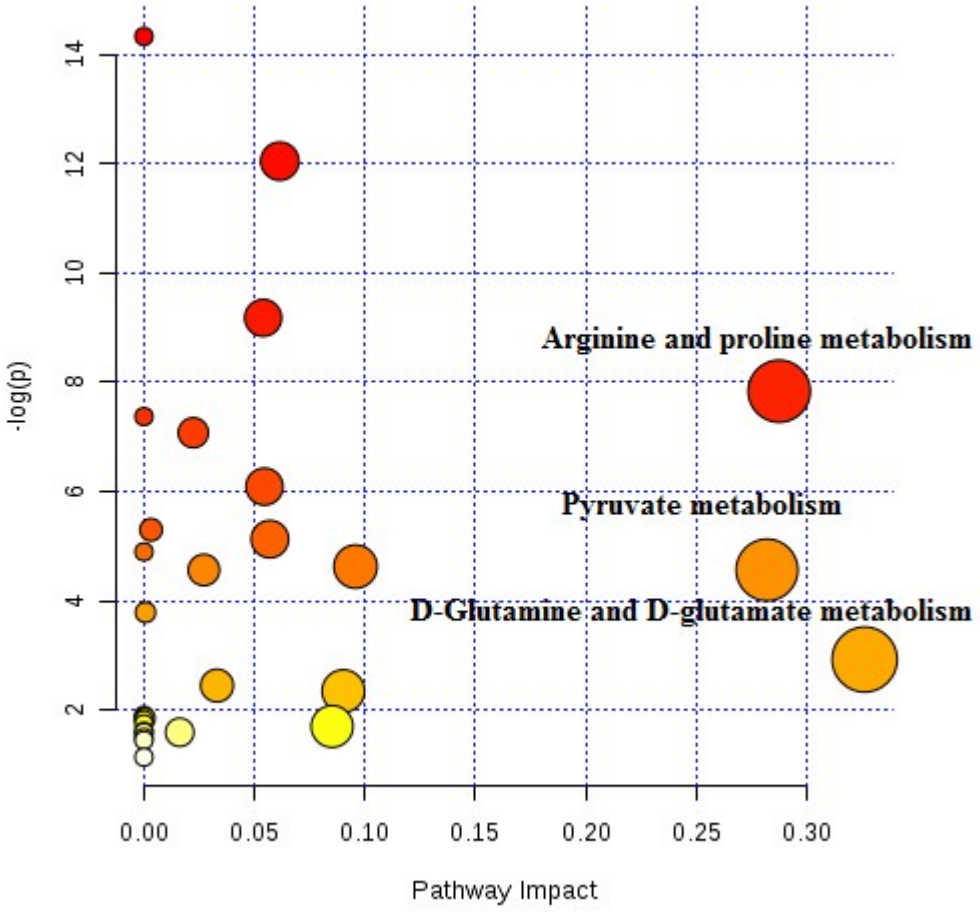
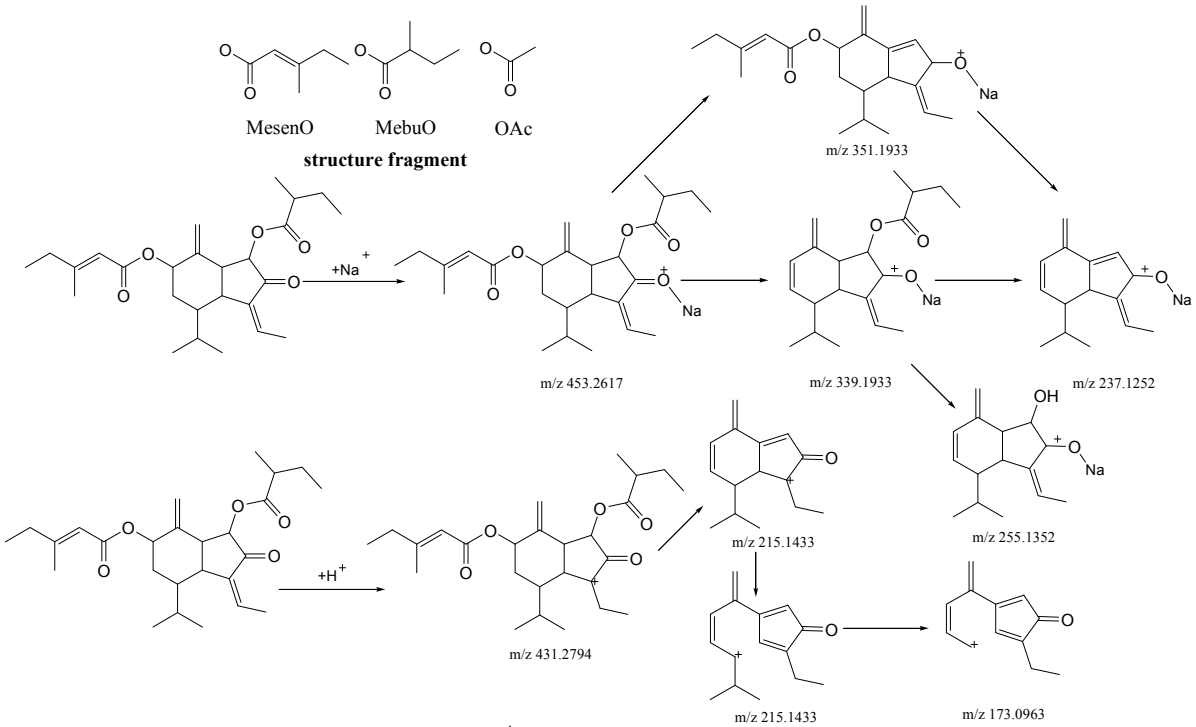


Figure S8 Summary of pathway analysis with MetPA.



A

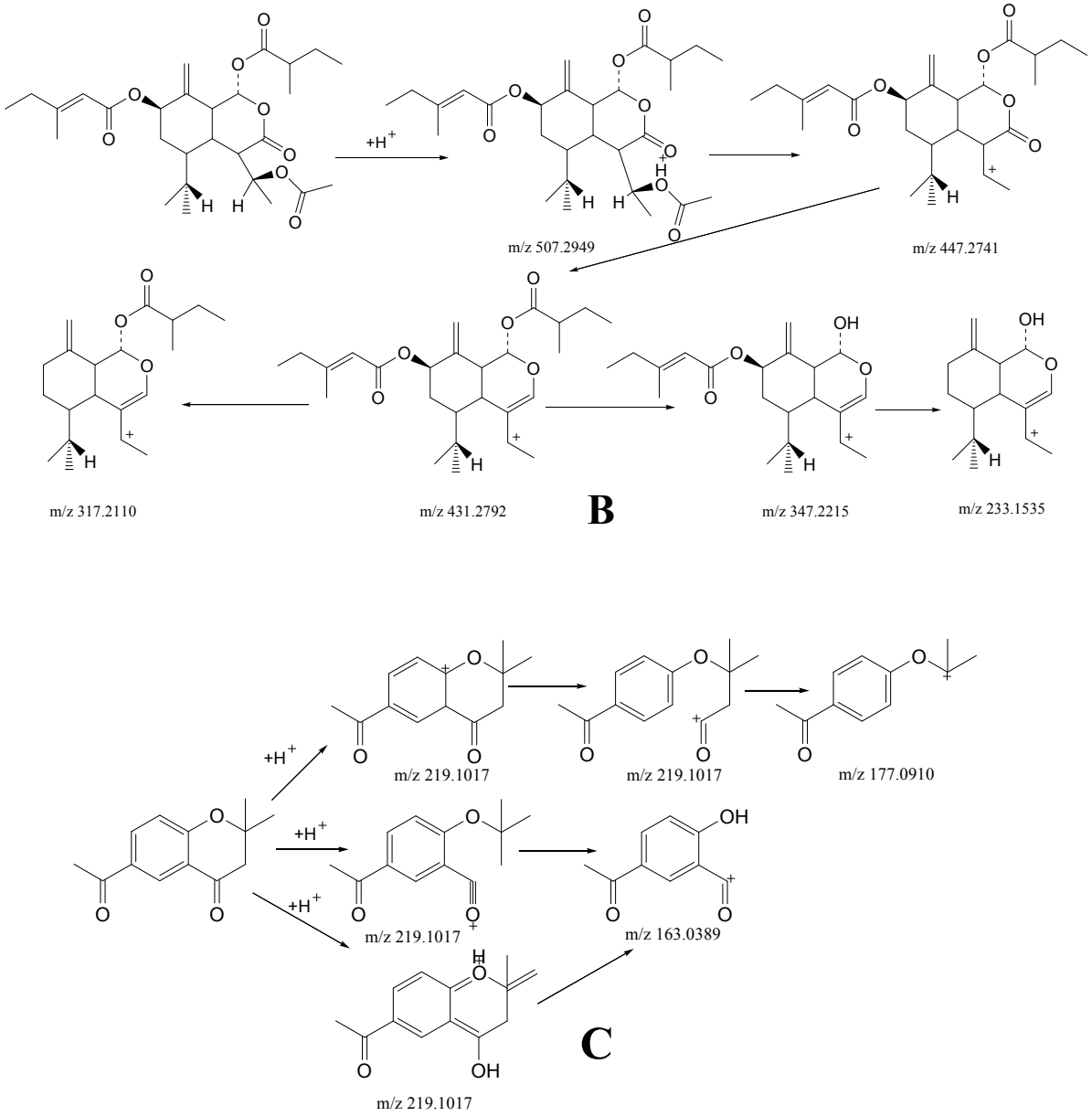


Figure S9 The possible fragment pathways of 7β-(3'-Ethyl-cis-crotonoyloxy)-1α-(2'-methylbutyryloxy)-3(14)-dehydro-*Z*-notonipetranone (A), tussilagolactone (B), 2,2-dimethyl-6-acetylchromanone(C).