

*Supplemental online material*

**Metabolite profiling of Huaiyang *Medicago polymorpha* with different mowing crops**

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## 1. Experimental

### 1.1 Chemicals, solvents and herbal materials

Apigenin, Apigenin-7-O- $\beta$ -D-glucopyranoside, Luteolin, Isoliquiritigenin, Daidzein, Formononetin, Genkwanin, Soyasaponin Bb,  $\beta$ -Sitosterol were purchased from Chengdu must bio-technology co., Ltd. (Chengdu, China)  $\alpha$ -Hederin, Tricin, Cynaroside, Chryseriol were purchased from Saipuruisi (beijing) co. (Beijing, China) The purity of the standard was no less than 98%. Oleic acid was purchased from the National Institute for Control of Pharmaceutical and Biological Products. Cultivated *Medicago polymorpha* (Yang Cao) in six greenhouses in November at Yangzhou (YC-201801, Figure S5), Jiangsu Province, one of the indigenous cultivating regions in China, were mowed twice with every 30 days. After vacuum dried at low temperature then grinded into superfine powder, and approximately 1 kg. HPLC grade methanol was purchased from DIKMA (America) and MS grade formic acid from Fisher Scientific. All other chemicals and solvents were of an analytical grade. Ultra-pure water (18.2M $\Omega$ ) was prepared with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

### 1.2 Standard solutions and sample preparation

The mentioned standard were prepared in methanol. Samples of the fruits of *L. lucidum* Ait from different collections were pulverized, and then the powder was sieved through 40 mesh sieves. The pulverized samples were accurately weighed (approximately 0.2 g), and ultrasonically-extracted with 1 ml methanol for 20 min. The resulting solutions were centrifuged at 4000 r/min. The extraction procedure was repeated twice. After cooling, the solutions were combined. Prior to use, all samples were filtered through a 0.22  $\mu$ m membrane filter.

In the sample preparation procedure, the extraction solvent, extraction procedure, and extraction time were also optimized. Samples of 0.2 g were accurately weighed and extracted using different volumes and percentages of methanol. It was found that the sample extracted by 1 mL methanol showed the greatest

number of detectable components in *Medicago polymorpha*. And moreover, ultrasonic extraction was simple, reproducible and effective. The weighed powdered samples were extracted with 1 mL methanol for 15 min, 30 min, 60 min, respectively. It was found that the samples with 30, 60 extraction time gave a very similar amount of compounds, and all showed a higher level of compounds than 15 min extraction time. So a 30 min extraction method repeated twice was used throughout this study.

### ***1.3 RRLC-TOFMS conditions***

For qualitative analysis of metabolite profiling of *Medicago polymorpha*, an Agilent 1200 series rapid resolution liquid chromatography system was coupled to an Agilent 6520 quadrupole time-of-flight mass spectrometer. An Agilent 6520 quadrupole time-of-flight mass spectrometer (Agilent Technologies, California, USA) which was equipped with an electrospray ionization (ESI) source and operated in positive ion mode and negative mode. The separation was performed on Waters ACQUITY BEH C<sub>18</sub> column (100 mm×2.1 mm, 1.7 μm). The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile containing 0.1% formic acid. The linear gradient conditions were as follows: 0-5 min, 10-50% B; 5-25 min, 50-100%; 25-30 min, 100%. The flow rate was 0.3 ml/min. The conditions of MS analysis were as follows: the nebulization gas was set to 45 psig, the drying gas was set at 10 L/min, the gas temperature of 350 °C. The capillary voltage was set to 3000 V. All MS data was acquired using reference masses to ensure mass accuracy and reproducibility. The [M+H]<sup>+</sup> ion of purine at m/z 121.0510 and the [M+H]<sup>+</sup> ion of HP-0921 at m/z 922.0098 were used as the lock mass in positive ESI mode. The [M-H]<sup>-</sup> ion of purine at m/z 119.0363 and the [M-H]<sup>-</sup> ion of HP-0921 at m/z 966.0007 were used as the lock mass in negative ESI mode. Data was acquired for each sample from 100 to 1500 Da. The RRLC-QTOFMS data of all determined samples were analyzed by Masshunter qualitative analysis version B. 03. 01 (Agilent, USA) to identify the potential variables for *Medicago polymorpha* collected. Different kinds of mobile phases, such as acetonitrile and methanol 0.05%, 0.1% and 0.2% aqueous formic acid, were tested. The best peak shape and resolution was obtained from a mixture of methanol and aqueous 0.1% formic acid solution. By an optimized gradient elution in the column, the main components were separately eluted within 30 min. The nebulization gas was set to 45 psi, the drying gas was set at 10

L/min, the gas temperature of 350 °C. The capillary voltage was set to 3000 V, so as to remove redundant solvent resulting from a flow rate of 0.3 mL/min for mass spectrometer. The MS/MS analysis of components in this study was performed at different collision energies ranging from 25 to 110 V.

#### ***1.4 Data processing and statistical analysis***

The peak finding, peak alignment, and peak filtering of the ESI+ and ESI- raw data were carried out using Mass Profiler Professional version B.12.05 (Agilent). For data collection, the method parameters were set as follows: retention time range 0-30 min, mass range 100-1500 Da, retention time tolerance 0.01 min, mass tolerance 0.02 Da. For peak integration, the peak intensity threshold was set to 5000. No specific mass or adduct was excluded. The resulting 3-D matrix containing arbitrarily assigned peak index, retention time, and normalized peak area were further exported to a web-based analytical pipeline Metaboanalyst for multivariate statistical analysis (Xia et al. 2015). Mathematical methods, mean-centered and divided by the standard deviation of each variable, were applied to pretreat the data sets resulting from the above samples. Principal Component Analysis (PCA) was to visualize general clustering, trends and outliers among the observations. In order to select potential biomarkers worthy of preferential study in the next step, these differential metabolites were validated using Independent-Samples T-test (SPSS 17.0). *P*-values were generated for all metabolites.

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**Table S1.** Components identified using ESI + from the Medicago Polymorpha Linnin the first mowing crops.

t <sub>R</sub>	Assigned identity	Molecular formula	[M+H] <sup>+</sup> m/z			[M+Na] <sup>+</sup> m/z			Product ion	References
			Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)	Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)		
2.75	Kaempferol-3-robinobioside*	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.1664	595.1657	0.05	-	617.1477	-	578, 449, 287	He et al. (2006); Lang (2008)
2.86	Riboflavin*	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	377.1459	377.1456	-0.9	-	399.1275	-	243, 172	Peng et al. (2010); Han et al. (2012)
2.99	Apigenin-7-O- $[\beta$ -D-GluA-O- $\beta$ -D-GluA]*	C <sub>27</sub> H <sub>26</sub> O <sub>17</sub>	623.1253	623.1243	-1.65	-	645.1062	-	607, 447, 271	He et al. (2006); Lang (2008); Yan et al. (2012); Liu et al. (2016)
3.59	Cynaroside*#	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	449.1068	449.1078	2.4	-	471.0898	-	287	Wang et al. (2009); Zhao HF and Zhao Q (2013); Yuan (2016)
4.03	Apigenin-7-O- $\beta$ -D-glucopyranoside*#	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	433.1133	433.1129	-0.87	-	455.0949	-	271	Yin and Qin (2008); Yan et al. (2012)
4.69	Daidzein*#	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	255.0651	255.0652	0.34	-	277.0471	-	181, 137	Lang (2008); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014)
4.87	Luteolin*#	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.0551	287.0550	-0.3	-	309.0370	-	153	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Zheng et al. (2015) Liu et al. (2016); Yuan (2016)
5.43	Apigenin*#	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	271.0599	271.0601	0.74	-	293.0420	-	153	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Zheng et al. (2015) Liu et al. (2016); Yuan (2016)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S1.** Components identified using ESI + from the Medicago Polymorpha Linnin the first mowing crops (continued).

t <sub>R</sub>	Assigned identity	Molecular formula	[M+H] <sup>+</sup> m/z			[M+Na] <sup>+</sup> m/z			Product ion	References
			Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)	Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)		
5.60	Chryseriol <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	301.0704	301.0707	0.88	-	323.0526	-	155	Lang (2008); Wang et al. (2009); Zhu et al. (2009); Chen et al. (2012); Liu et al. (2016); Yin and Qin (2008); Wang et al. (2012); Yan et al. (2012); Chen et al. (2014)
5.96	Isoliquiritigenin <sup>#</sup>	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	257.0809	257.0808	-0.25	-	279.0628	-	240	Lang (2008); Zhu et al. (2009); Chen et al. (2012); Zhao HF and Zhao Q (2013); Chu et al. (2015); Yang et al. (2016)
6.25	Formononetin <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	269.0805	269.0808	1.25	-	291.0628	-	197,252, 238,221	He et al. (2005); Jin (2007); Shi (2007); Lang (2008); Tava et al. (2011)
6.89	Soyasaponin Bb <sup>★#</sup>	C <sub>48</sub> H <sub>78</sub> O <sub>18</sub>	943.5269	943.5261	-0.86	965.512	965.5080	-4.2	798, 636, 599, 520, 441	He et al. (2006); Xu et al. (2014); Zheng et al. (2015)
7.05	Genkwanin <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	285.0776	285.0757	-6.51	-	307.0577	-	254, 251, 189	Yang (2004); Wang et al. (2009)
18.94	Palmitic acid <sup>★</sup>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	257.2471	257.2475	1.59	279.2299	279.2295	-1.75	240	Yang (2004)
19.55	Oleic acid <sup>★</sup>	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	283.2631	283.2632	0.2	305.2441	305.2451	3.55	266	Yang (2004)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S2.** Components identified using ESI - from the Medicago Polymorpha Linnin the first mowing crops.

t <sub>R</sub>	Assigned identity	Molecular formula	[M-H] <sup>-</sup> m/z			Product ion	References
			Mean measured mass(Da)	Theoretical extract mass(Da)	Mass Accuracy (ppm)		
2.75	Kaempferol-3-robinobioside <sup>★</sup>	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	593.1500	593.1512	2.01	285	He et al. (2006); Lang (2008)
2.86	Riboflavin <sup>★</sup>	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	375.1299	375.1310	2.95	358	Peng et al. (2010); Han et al. (2012)
2.99	Apigenin-7-O- [β-D-GluA-O-β-D-GluA] <sup>★</sup>	C <sub>27</sub> H <sub>26</sub> O <sub>17</sub>	621.1070	621.1097	0.04	445,552	He et al. (2006); Lang (2008); Yan et al. (2012); Liu et al. (2016)
3.59	Cynaroside <sup>★#</sup>	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0925	447.0933	1.75	285, 431	Wang et al. (2009); Zhao HF and Zhao Q (2013); Yuan (2016)
4.03	Apigenin-7-O- β-D-glucopyranoside <sup>★#</sup>	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	431.0972	431.0984	2.71	269, 225, 201, 183	
4.69	Daidzein <sup>★#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	253.0495	253.0506	4.46	209, 185, 167	Lang (2008); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014)
4.87	Luteolin <sup>★#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	285.0405	285.0405	-0.13	241, 199, 217,175,133	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Liu (2010); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014); Liu et al. (2016)
5.43	Apigenin <sup>★#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	269.0458	269.0455	-0.94	117,225, 201, 183	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Zheng et al. (2015); Liu et al. (2016); Yuan (2016)
5.60	Chryseriol <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	299.0542	299.0561	6.37	268, 225, 201, 183	Lang (2008); Wang et al. (2009); Zhu et al. (2009); Chen et al. (2012); Liu et al. (2016)
5.96	Isoliquiritigenin <sup>#</sup>	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	255.0664	255.0663	-0.46	135,119	Yin and Qin (2008); Wang et al. (2012); Yan et al. (2012); Chen et al. (2014)
6.25	Formononetin <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	267.0659	267.0663	1.43	252	Lang (2008); Zhu et al. (2009); Chen et al. (2012); Zhao HF and Zhao Q (2013); Chu et al. (2015); Yang et al. (2016)
6.89	Soyasaponin Bb <sup>★#</sup>	C <sub>48</sub> H <sub>78</sub> O <sub>18</sub>	941.5156	941.5115	-4.31	880,796,734,616,598	He et al. (2005); Jin (2007); Shi (2007); Lang (2008); Tava et al. (2011)
7.05	Genkwanin <sup>★#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	283.0585	283.0612	9.49	116, 268	He et al. (2006); Xu et al. (2014); Zhang et al. (2015)
18.94	Palmitic acid <sup>★</sup>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	255.2327	255.2330	0.99	238	Yang (2004); Wang et al. (2009)
19.55	Oleic acid <sup>★</sup>	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	281.2476	281.2486	3.56	264	Yang (2004)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S3.** Components identified using ESI+ from the *Medicago Polymorpha* Linn in the second mowing crops.

t <sub>R</sub>	Assigned identity	Molecular formula	[M+H] <sup>+</sup> m/z			[M+Na] <sup>+</sup> m/z			Product ion	References
			Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)	Mean measured mass(Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)		
2.75	Kaempferol-3-robinobioside <sup>*</sup>	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.1661	595.1657	-0.59	-	617.1477	-	578, 449, 287	He et al. (2006); Lang (2008); Liu et al. (2016)
2.85	Riboflavin <sup>*</sup>	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	377.1459	377.1456	-0.9	-	399.1275	-	243, 172	Peng et al. (2010); Han et al. (2012)
2.98	Apigenin-7 <sup>*</sup> -O-[β-D-GluA-O-β-D-GluA] <sup>*</sup>	C <sub>27</sub> H <sub>26</sub> O <sub>17</sub>	623.1242	623.1243	0.12	-	645.1062	-	607, 447, 271	He et al. (2006); Lang (2008); Yan et al. (2012); Liu et al. (2016)
4.01	Apigenin-7-O-β-D-glucopyranoside <sup>*#</sup>	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	433.1123	433.1129	1.44	-	455.0949	-	271	Yin and Qin (2008); Yan et al. (2012)
4.69	Daidzein <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	255.0653	255.0652	-0.45	-	277.0471	-	181, 137	Lang (2008); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014)
4.87	Luteolin <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.0551	287.055	-0.3	-	309.037	-	153	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Liu (2010); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014); Liu et al. (2016);
5.32	Hederacoside I <sup>*</sup>	C <sub>35</sub> H <sub>86</sub> O <sub>22</sub>	1075.5695	1075.5684	-1.07	1097.549	1097.5503	1.21	1058.5	Tava et al. (2011); Meng et al. (2013)
5.44	Apigenin <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	271.0615	271.0601	-5.18	-	293.042	-	153	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Zheng et al. (2015) Liu et al. (2016); Yuan (2016)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S3.** Components identified using ESI+ from the *Medicago Polymorpha* Linn in the second mowing crops (continued).

t <sub>R</sub>	Assigned identity	Molecular formula	[M+H] <sup>+</sup> m/z			[M+Na] <sup>+</sup> m/z			Product ion	References
			Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)	Mean measured mass(Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)		
5.60	Chryseriol <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	301.0706	301.0707	0.22	-	323.0526	-	155	Lang (2008); Wang, et al. (2009); Zhu et al. (2009); Chen et al. (2012); Liu et al. (2016)
5.97	Isoliquiritigenin <sup>#</sup>	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	257.081	257.0808	-0.64	-	279.0628	-	240	Yin and Qin (2008); Wang et al. (2012); Yan et al. (2012); Chen et al. (2014)
6.25	Formononetin <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	269.081	269.0808	-0.61	-	291.0628	-	197,252, 238,221	Lang (2008); Zhu et al. (2009); Chen et al. (2012); Chu et al. (2015); Yang et al. (2016); Zhao HF and Zhao Q (2013)
6.90	Soyasaponin Bb <sup>*</sup>	C <sub>48</sub> H <sub>78</sub> O <sub>18</sub>	943.5261	943.5261	-0.01	965.508	965.508	0.04	798, 636, 599, 520, 441	He et al. (2005); Jin (2007); Shi (2007); Lang (2008); Tave et al. (2011)
7.09	Genkwanin <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	285.075	285.0757	2.64	-	307.0577	-	254, 251, 189	He et al. (2006); Xu et al. (2014); Zheng et al. (2015)
7.97	α-Hederin <sup>*#</sup>	C <sub>41</sub> H <sub>66</sub> O <sub>12</sub>	751.4625	751.4627	0.27	773.4452	773.4446	-0.73	734.4	Tava et al. (2011); Meng et al. (2013); Liu et al. (2016)
15.58	Oleanolic acid <sup>*#</sup>	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	457.364	457.3676	7.96	479.3443	479.3496	11.54	439, 411, 393	Kinjo et al. (1994); Chen (2012); Yu et al. (2016); Xiong et al. (2017)
19.01	Palmitic acid <sup>*</sup>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	257.2476	257.2475	-0.36	279.2302	279.2295	1.13	240	Yang (2004); Wang et al. (2009)
19.51	Oleic acid <sup>*</sup>	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	283.2629	283.2632	0.91	305.2445	305.2451	2.13	266	Yang (2004)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S4.** Components identified using ESI- from the Medicago Polymorpha Linn in the second mowing crops.

t <sub>R</sub>	Assigned identity	Molecular formula	[M-H] <sup>-</sup> m/z			Product ion	References
			Mean measured mass (Da)	Theoretical extract mass (Da)	Mass Accuracy (ppm)		
2.75	Kaempferol-3-robinobioside <sup>*</sup>	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.1661	595.1657	-0.59	285	He et al. (2006); Lang (2008); Peng et al. (2010); Han et al. (2012)
2.85	Riboflavin <sup>*</sup>	C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub>	377.1459	377.1456	-0.9	358	Peng et al. (2010); Han et al. (2012)
2.98	Apigenin-7 <sup>*</sup> -O-[β-D-GluA-O-β-D-GluA] <sup>*</sup>	C <sub>27</sub> H <sub>26</sub> O <sub>17</sub>	623.1242	623.1243	0.12	445,552	He et al. (2006); Lang (2008); Yan et al. (2012); Liu et al. (2016)
4.01	Apigenin-7-O-β-D-glucopyranoside <sup>*#</sup>	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	433.1123	433.1129	1.44	269, 225, 201, 183	Yin and Qin (2008); Yan et al. (2012)
4.69	Daidzein <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	255.0653	255.0652	-0.45	209, 185, 167	Lang (2008); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014)
4.87	Luteolin <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.0551	287.055	-0.3	241, 199, 217,175,133	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Liu (2010); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Fan (2014); Liu et al. (2016)
5.32	Hederacoside I <sup>*</sup>	C <sub>35</sub> H <sub>86</sub> O <sub>22</sub>	1075.5695	1075.5684	-1.07	927,765,603,471	Tava et al. (2011); Meng et al. (2013)
5.44	Apigenin <sup>*#</sup>	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	271.0615	271.0601	-5.18	117,225, 201, 183	Lang (2008); Yin and Qin (2008); Zhu et al. (2009); Chen et al. (2012); Yan et al. (2012); Zhao HF and Zhao Q (2013); Zheng et al. (2015); Liu et al. (2016); Yuan (2016)
5.60	Chryseriol <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	301.0706	301.0707	0.22	268, 225, 201, 183	Lang (2008); Wang et al. (2009); Zhu et al. (2009); Chen et al. (2012); Liu et al. (2016)
5.97	Isoliquiritigenin <sup>#</sup>	C <sub>15</sub> H <sub>12</sub> O <sub>4</sub>	257.081	257.0808	-0.64	135,119	Yin and Qin (2008); Wang et al. (2012); Yan et al. (2012); Chen et al. (2014)
6.25	Formononetin <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	269.081	269.0808	-0.61	252	Lang (2008); Zhu et al. (2009); Chen et al. (2012); Zhao HF and Zhao Q (2013); Chu et al. (2015); Yang et al. (2016)
6.90	Soyasaponin Bb <sup>*</sup>	C <sub>48</sub> H <sub>78</sub> O <sub>18</sub>	943.5261	943.5261	-0.01	880,796,734,616,598	He et al. (2005); Jin (2007); Shi (2007); Lang (2008); Tava et al. (2011)
7.09	Genkwanin <sup>*#</sup>	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	285.075	285.0757	2.64	116, 268	He et al. (2006); Xu et al. (2014); Zheng et al. (2015)

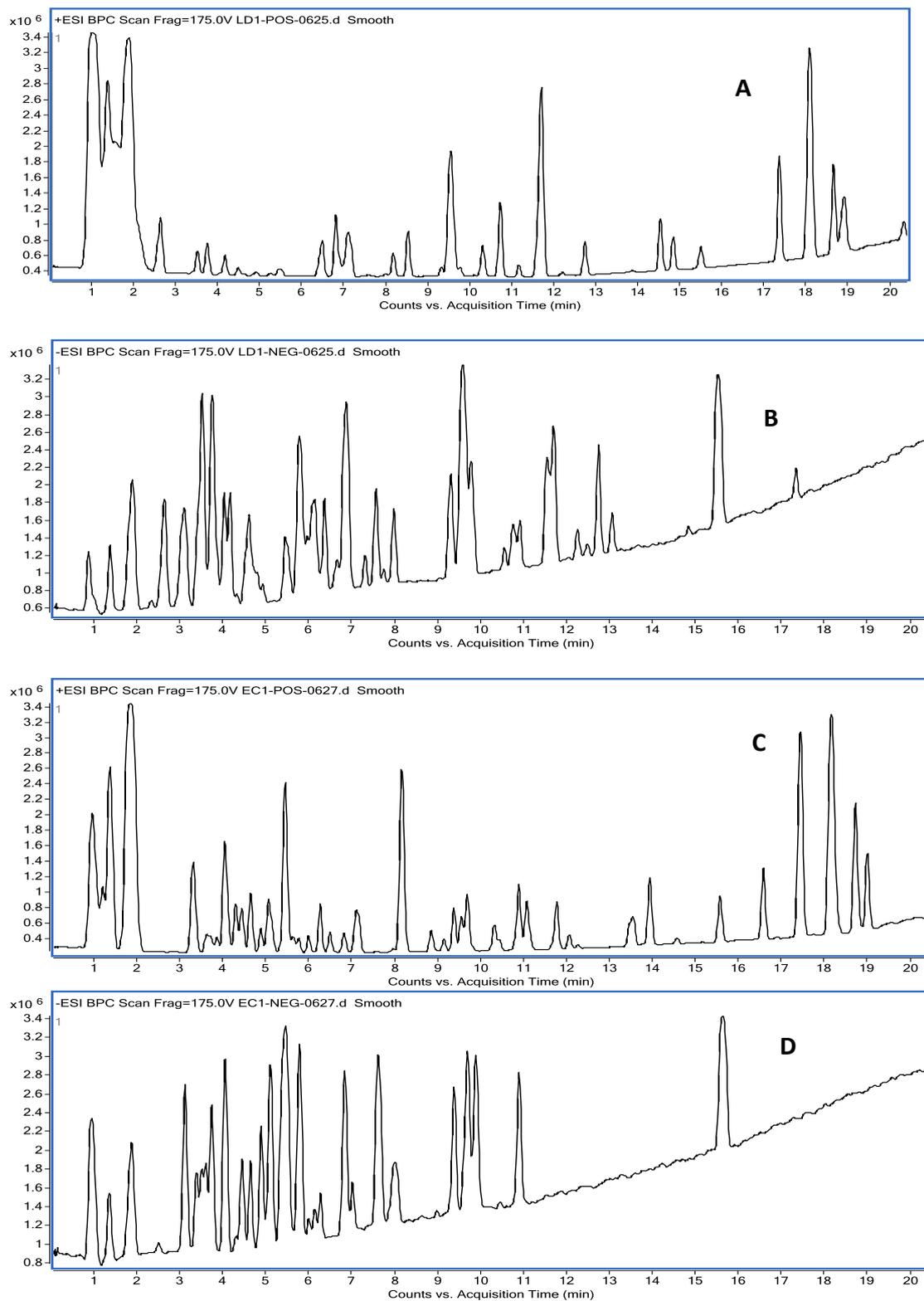
\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.

**Table S4.** Components identified using ESI- from the Medicago Polymorpha Linn in the second mowing crops (continued).

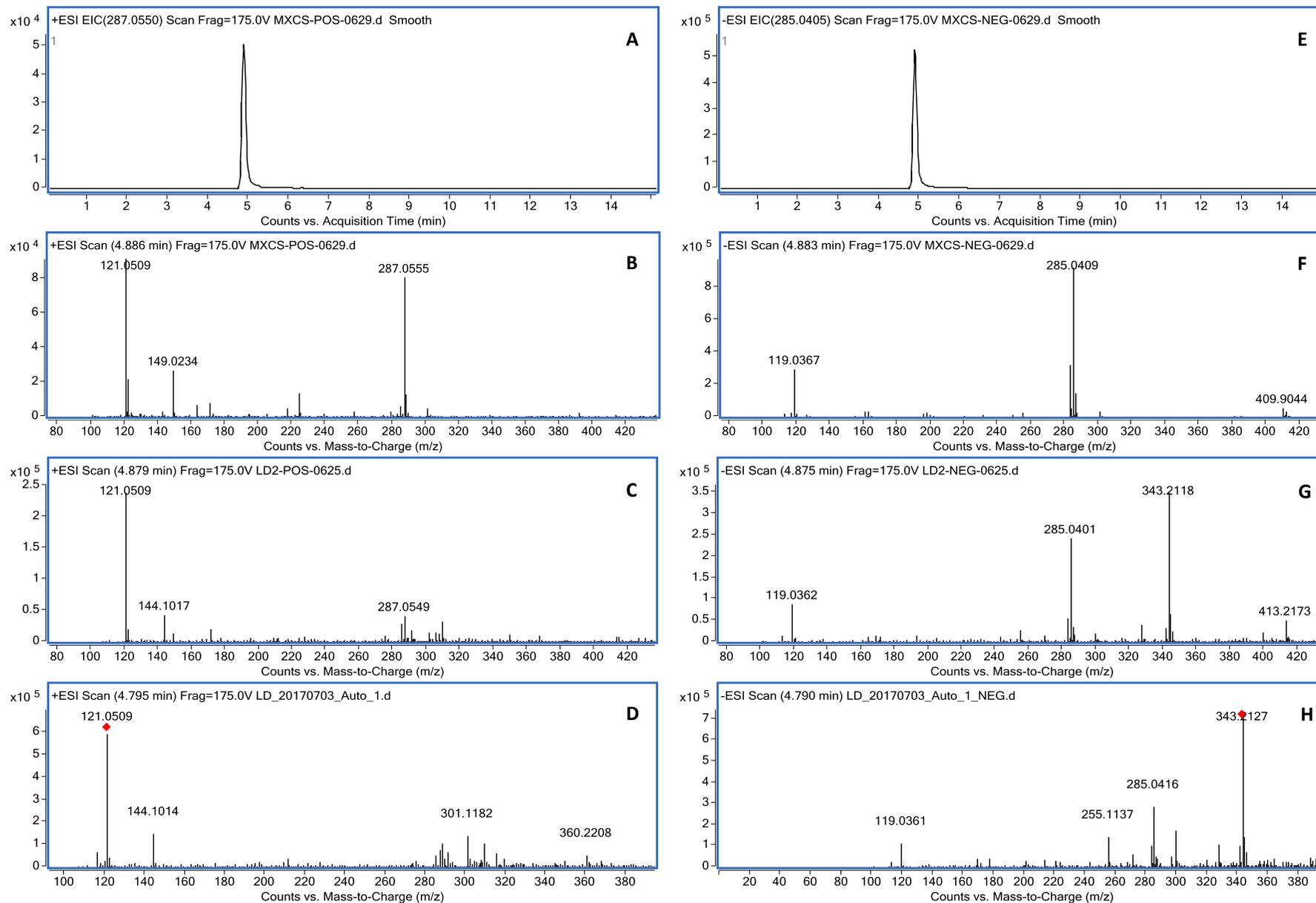
t <sub>R</sub>	Assigned identity	Molecular formula	[M-H] <sup>-</sup> m/z			Product ion	References
			Mean	Theoretical	Mass		

			measured mass (Da)	extract mass (Da)	Accuracy (ppm)		
7.97	$\alpha$ -Hederin <sup>*#</sup>	C <sub>41</sub> H <sub>66</sub> O <sub>12</sub>	751.4625	751.4627	0.27	705, 603, 471	Tava et al. (2011); Meng et al. (2013); Liu et al. (2016)
15.58	Oleanolic acid <sup>*#</sup>	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	457.364	457.3676	7.96	407, 391, 363, 295	Kinjo et al. (1994); Yu et al. (2016); Xiong et al. (2017)
19.01	Palmitic acid <sup>*</sup>	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	257.2476	257.2475	-0.36	238	Yang (2004); Wang et al. (2009)
19.51	Oleic acid <sup>*</sup>	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	283.2629	283.2632	0.91	264	Yang (2004)

\*represented these metabolites were significant changed in different mowing crops. # represented these metabolites were identified by reference compounds.



**Figure S1** The BPI chromatogram of Huaiyang Medicago polymorpha with the first mowing crops (A ESI+), (B ESI-), the second mowing crop (C ESI+), (D ESI-)



**Figure S2** Mass spectra of luteolin in the first mowing Medicago polymorpha compared to luteolin standard. A: EIC of luteolin standard in positive ion mode; B: mass spectra from luteolin standard in positive ion mode; C: mass spectra of luteolin in the first mowing Medicago polymorpha in positive ion mode; D: MS/MS of luteolin in the first mowing Medicago polymorpha in positive ion mode. E: EIC of luteolin

standard in negative ion mode.

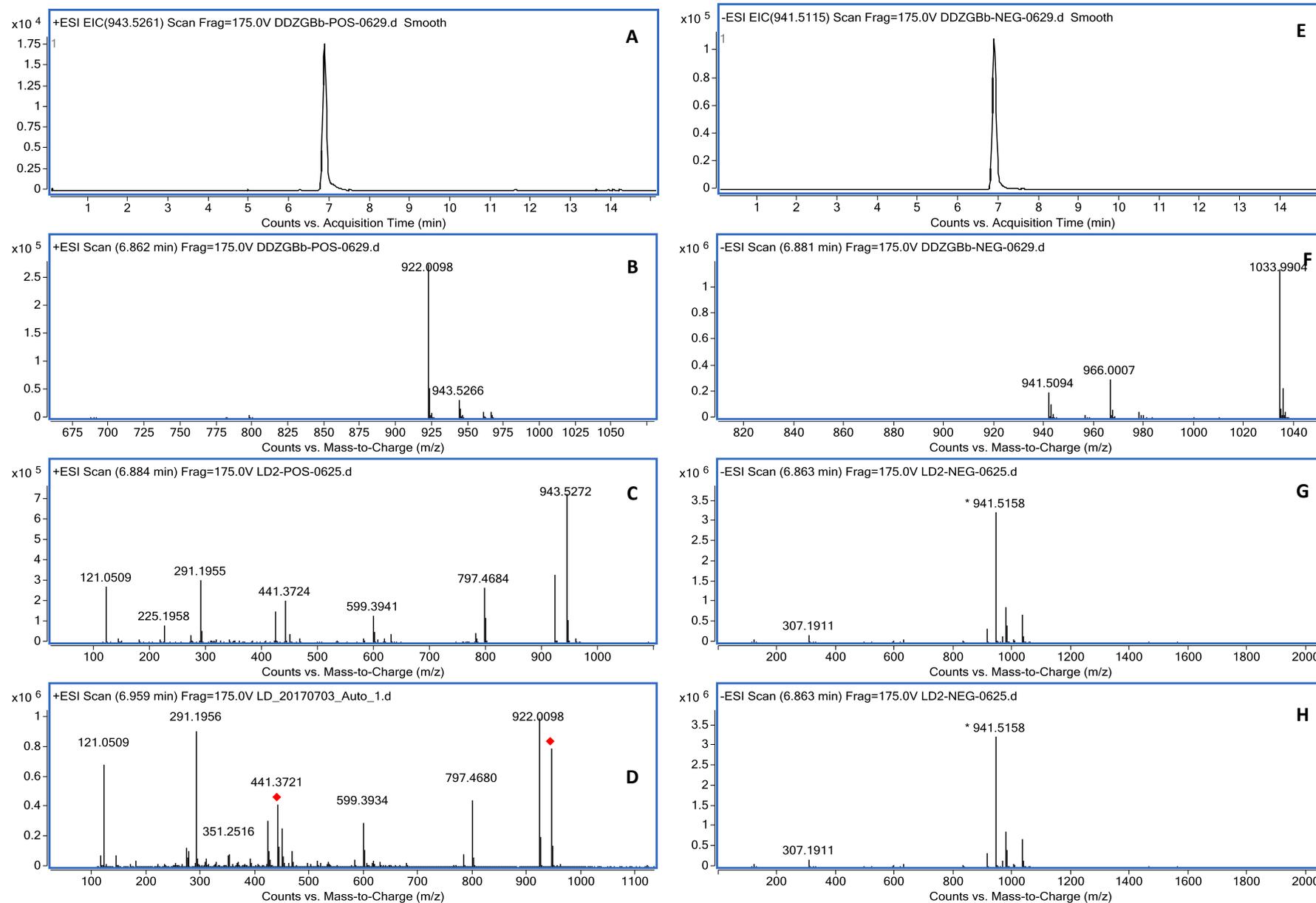
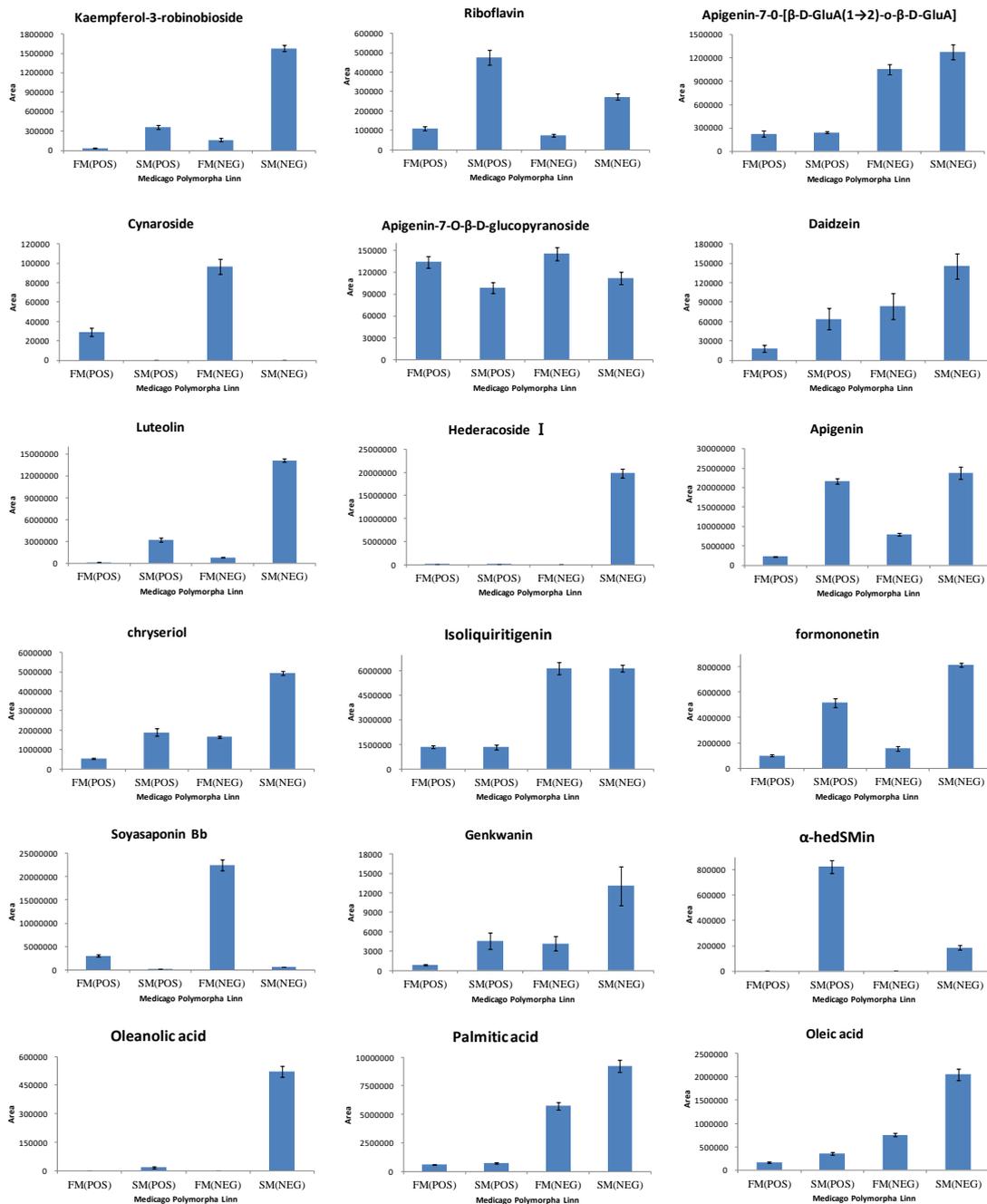


Figure S3 Mass spectra of soyasaponin Bb in the first mowing *Medicago polymorpha* compared to soyasaponin Bb standard. A: EIC of

soyasaponin Bb standard in positive ion mode; B: mass spectra from soyasaponin Bb standard in positive ion mode; C: mass spectra of soyasaponin Bb in the first mowing Medicago polymorpha in positive ion mode; D: MS/MS spectra of soyasaponin Bb in the first mowing Medicago polymorpha in positive ion mode. E: EIC of soyasaponin Bb standard in negative ion mode; F: mass spectra from soyasaponin Bb standard in negative ion mode; G: mass spectra of soyasaponin Bb in the first mowing Medicago polymorpha in negative ion mode; H: MS/MS spectra of soyasaponin Bb in the first mowing Medicago polymorpha in negative ion mode.



**Figure S4** The different levels of identified compounds in two mowing by ESI+ and ESI-



Family	Leguminosae		Local name	Yangcao
Scientific name	Medicago polymorpha Linn.		-	
Alias	Juhucun	Medicinal part	herba,se,f	
Collector	Yang Cao	Collector Number	YC-201801	
Date Collected	November 6th, 2018	Description	Herb	
Location	Xinxiang Village, Economic Development Zone, Yang Zhong			
Efficacy	heat-clearing and detoxifying and remove-dampness			
科名	豆科	植物名	秧草	
学名	Medicago polymorpha Linn.			
别名	金花菜、南苜蓿	药用部位	全草	
采集人	曹阳	采集号	YC-201801	
采集日期	2018-11-6	习性	草本植物	
采集地点	扬州市经济开发区新庄村			
用途	清热利湿			

**Figure S5** The appearance and sample number of *Medicago polymorpha*.