

***Lathyrus aureus* and *Lathyrus pratensis*: characterization of phytochemical profiles
by liquid chromatography-mass spectrometry, and evaluation of their enzyme
inhibitory and antioxidant activities**

E. J. Llorent-Martínez¹, P. Ortega-Barrales², G. Zengin^{3*}, S. Uysal³, R. Ceylan³,
G.O.Guler⁴, A.Mocan⁵, A. Aktumsek³

¹ University of Castilla-La Mancha, Regional Institute for Applied Chemistry Research (IRICA), Ciudad Real 13071, Spain

² Department of Physical and Analytical Chemistry, University of Jaén, Campus Las Lagunillas S/N, E-23071 Jaén, Spain

³ Selcuk University, Science Faculty, Department of Biology, Campus, 42250, Konya, Turkey

⁴ Necmettin Erbakan University, Ahmet Kelesoglu Education Faculty, Department of Biological Education, 42075, Konya, Turkey

⁵ Department of Pharmaceutical Botany, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8, V. Babes Street, Cluj-Napoca, Romania

*Corresponding author. Tel.: +90 332 223 27 81; Fax: +90 332 2410106 E-mail address: biyologzengin@yahoo.com (Dr. Gokhan ZENGIN)

Table 1. Characterization of the methanolic extract of aerial parts from *L. pratensis*

No.	t _R (min)	[M-H] ⁻ m/z	m/z (% base peak)	Assigned identification
1	1.1	378	MS ² [378]: 341 (100), 215 (11), 179 (9) MS ³ [378→341]: 282 (18), 179 (100), 161 (48), 131 (32), 119 (11), 107 (13) MS ⁴ [378→341→179]: 143 (84), 113 (100)	Oligosaccharide derivative
3	1.5	315	MS ² [315]: 153 (100), 109 (13), 108 (7)	Dihydroxybenzoic acid hexoside
4	2.8	353	MS ² [353]: 191 (100), 173 (30), 179 (21), 135 (4) MS ³ [353→191]: 191 (100), 173 (10), 163 (9)	Caffeoylquinic acid
6	3.1	595	MS ² [595]: 577 (5), 505 (19), 475 (48), 415 (26), 385 (83), 355 (100) MS ³ [595→355]: 327 (20), 261 (25), 249 (30), 235 (100), 209 (85), 167 (17), 143 (19)	Naringenin-6,8-di-C-hexoside
9	4	609	MS ² [609]: 447 (100), 285 (33) MS ³ [609→447]: 285 (74), 284 (100), 297 (81), 255 (74), 225 (36)	Kaempferol-di-O-hexoside
10	4.6	785	MS ² [785]: 623 (100) MS ³ [785→623]: 315 (100), 300 (28) MS ⁴ [785→623→315]: 300 (100), 271 (7), 201 (6), 191 (4)	Isorhamnetin-3-O-rutinoside-7-O-hexoside
11	4.7	931	MS ² [931]: 769 (100) MS ³ [931→769]: 769 (100), 315 (80), 300 (23), 271 (9), 255 (6) MS ⁴ [931→769→315]: 300 (100), 255 (5), 151 (6)	Isorhamnetin-O-hexoside-O-rhamnosylrutinoside
12	5.7	367	MS ² [367]: 191 (100), 173 (33)	5-Feruloylquinic acid

14	6.4	609	MS ² [609]: 463 (64), 447 (100), 301 (79) MS ³ [609→447]: 301 (100)	Quercetin-O-hexoside-O-rhamnoside
15	6.5	625	MS ² [625]: 317 (90), 316 (100) MS ³ [625→316]: 271 (100), 179 (60), 151 (25)	Myricetin-O-rutinoside
17	6.8	479	MS ² [479]: 317 (100), 316 (85), 271 (9), 179 (10) MS ³ [479→316]: 271 (100), 179 (57), 151 (21)	Myricetin-O-hexoside
18	7.0	367	MS ² [367]: 191 (23), 179 (100), 135 (48) MS ³ [367→179]: 135 (100)	Methyl-caffeoyl-quinic acid
19	7.4	739	MS ² [739]: 593 (45), 575 (70), 285 (100), 284 (96) MS ³ [739→285]: 255 (100), 229 (13), 151 (35)	Kaempferol-O-rhamnoside-O-rutinoside
20	7.5	623	MS ² [623]: 503 (18), 461 (100), 447 (38), 351 (36), 285 (87) MS ³ [623→461]: 286 (18), 285 (100)	Kaempferol-O-hexoside-O-glucuronide
22	7.7	593	MS ² [593]: 483 (13), 447 (100), 431 (35), 285 (78) MS ³ [593→447]: 327 (94), 285 (64), 255 (71), 284 (100)	Kaempferol-O-hexoside-O-rhamnoside
23	7.8	755	MS ² [755]: 609 (8), 301 (100) MS ³ [755→301]: 271 (13), 255 (17), 179 (95), 151 (100)	Quercetin-O-rhamnoside-O-rutinoside
25	7.9	655	MS ² [655]: 609 (9), 447 (100) MS ³ [655→447]: 285 (100), 151 (2)	Kaempferol-di-O-hexoside (formate adduct)
27	8.1	609	MS ² [609]: 302 (20), 301 (100), 300 (15), 271 (6)	Rutin

			MS ³ [609→301]: 273 (100), 271 (34), 255 (26), 179 (53), 151 (76) MS ⁴ [609→301→179]:151 (100), 107 (46)	
30	8.4	463	MS ² [463]: 302 (18), 301 (100) MS ³ [463→301]: 255 (4), 179 (51), 151 (100)	Quercetin-O-hexoside
32	8.7	739	MS ² [739]: 593 (1), 286 (18), 285 (100) MS ³ [739→285]: 257 (100), 241 (53), 213 (40), 151 (96)	Kaempferol-O-rhamnoside-O-rutinoside
34	9	479	MS ² [479]: 317 (199) MS ³ [479→317]: 179 (100), 151 (82)	Myricetin-O-hexoside
35	9.3	463	MS ² [463]: 302 (11), 301 (100) MS ³ [463→301]: 301 (100), 255 (53), 151 (47)	Quercetin-O-hexoside
37	9.5	769	MS ² [769]: 623 (4) 315 (100), 300 (8), 271 (10) MS ³ [769→315]: 300 (100), 271 (11)	Isorhamnetin rhamnosyl-rutinoside
38	9.5	593	MS ² [593]: 285 (100) MS ³ [593→285]: 257 (97), 243 (8), 241 (100), 229 (73), 213 (58), 199 (46), 151 (64)	Luteolin-O-rutinoside
39	9.8	623	MS ² [623]: 315 (100), 300 (16), 271 (16) MS ³ [623→315]: 301 (100), 300 (68), 271 (32), 163 (22) MS ⁴ [623→315→300]:271 (100)	Isorhamnetin-O-rutinoside (or isorhamnetin-O-neohesperidoside)
41	10.2	447	MS ² [447]: 285 (100) MS ³ [447→285]: 255 (100), 243 (12), 241 (36), 175 (53), 151 (8)	Luteolin-O-hexoside

44	10.9	515	MS ² [515]: 353 (100), 335 (7), 203 (13), 179 (7), 173 (13) MS ³ [515→353]: 191 (20), 179 (65), 173 (100), 135 (29)	3-4-dicaffeoylquinic acid
45	11.4	447	MS ² [447]: 285 (100) MS ³ [447→285]: 243 (96), 241 (82), 151 (100)	Luteolin-O-hexoside
46	13.1	753	MS ² [753]: 300 (12), 299 (100), 255 (15) MS ³ [753→299]: 284 (100), 240 (45), 165 (53) MS ⁴ [753→299→284]: 256 (52), 255 (100), 162 (28)	Methylkaempferol-O-(deoxyhexose-hexose-deoxyhexose)
48	14.2	285	MS ² [285]: 243 (24), 241 (100), 151 (29), 107 (43)	Luteolin
49	14.5	517	MS ² [517]: 471 (5), 311 (26), 293 (100), 233 (71), 191 (17), 149 (35) MS ³ [517→293]: 233 (51), 149 (100)	Unknown
52	15.3	431	MS ² [431]: 384 (22), 285 (40), 223 (100), 179 (54), 161 (34)	Unknown
55	19.5	327	MS ² [327]: 291 (36), 229 (100), 211 (74), 209 (22), 171 (60), 165 (14) MS ³ [327→229]: 211 (96), 209 (100), 165 (27), 155 (14), 125 (85)	Oxo-dihydroxy-octadecenoic acid
57	21.9	971	MS ² [971]: 953 (37), 927 (6), 909 (100), 825 (4), 763 (19), 645 (20), 627 (4), 601 (6), 555 (6), 487 (6), 469 (7) MS ³ [971→909]: 763 (100), 601 (21)	dHex-Hex-HexA-Bayogenin
59	25.0	329	MS ² [329]: 311 (19), 293 (20), 229 (100), 211 (78), 171 (30)	Trihydroxy-octadecenoic acid
61	26.6	1073	MS ² [1073]: 1055 (100), 1011 (36), 927 (9), 865 (26), 747 (20), 703 (46), 657 (20), 437 (8) MS ³ [1073→1055]: 657 (100)	Saponin

67	31	691	MS ² [691]: 673 (20), 415 (70), 397 (100), 293 (12) MS ³ [691→415]: 397 (53), 263 (49), 253 (100), 179 (71), 143 (70) MS ³ [691→397]: 305 (60), 287 (38), 235 (100), 221 (45), 161 (42)	Unknown
69	32.5	1027	MS ² [1027]: 983 (100) MS ³ [1027→983]: 965 (100), 913 (40), 863 (20), 775 (31), 567 (15), 479 (56)	Saponin
70	32.8	939	MS ² [939]: 921 (100) MS ³ [939→921]: 877 (100) MS ⁴ [939→921→877]: 731 (100)	Saponin
71	33.6	941	MS ² [941]: 923 (100), 879 (24), 795 (9), 751 (4), 795 (11), 733 (32), 633 (1), 615 (8), 597 (5), 525 (27), 457 (2) MS ³ 941[→923]: 879 (100), 733 (27), 597 (64), 525 (60)	3-Rha-Gal-GlcA-Soyasapogenol B
72	34.7	795	MS ² [795]: 633 (23), 615 (100), 525 (20), 457 (9) MS ³ [795→615]: 571 (88), 437 (100)	Unknown
75	36.2	939	MS ² [939]: 921 (100), 877 (11), 793 (5), 731 (17), 613 (9), 595 (18), 523 (11) MS ³ [939→921]: 877 (100), 731 (52), 595 (44), 525 (32), 523 (87)	dHex-Hex-HexA-Soyasapogenol E

HexA, uronic acid, such as glucuronic acid or galacturonic acid; Hex, hexose, such as glucose or galactose; dHex, 6-deoxyhexose, such as rhamnose or furanose; GlcA, glucuronic acid; Gal, galactose; Rha, rhamnose. * Comparison with analytical standard

Table 2. Characterization of the methanolic extract of aerial parts from *L. aureus*

No.	t_R (min)	[M-H] ⁻ m/z	m/z (% base peak)	Assigned identification
1	1.1	378	MS ² [378]: 341 (100), 215 (11), 179 (8), 143 (1) MS ³ [378→341]: 277 (100), 179 (100), 161 (57), 143 (33), 119 (81), 113 (62) MS ⁴ [378→341→179]: 161 (100), 143 (24), 119 (37)	Oligosaccharide derivative
2	1.1	191	MS ² [191]: 173 (32), 111 (100)	Citric acid
5	3.0	295	MS ² [295]: 163 (100), 149 (4), 119 (16), 113 (5) MS ³ [295→163]: 119 (100)	Coutaric acid
7	3.1	431	MS ² [431]: 329 (100), 203 (52), 125 (41), 165 (20), 143 (29) MS ³ [431→329]: 203 (93), 191 (76), 125 (100)	Unknown
8	3.6	325	MS ² [325]: 193 (100) MS ³ [325→193]: 178 (32), 149 (100), 134 (68)	Ferulic acid-O-pentoside
13	6.2	755	MS ² [755]: 300 (100), 301 (85) MS ³ [755→300]: 271 (100), 255 (50), 179 (22), 151 (20)	Quercetin derivative
16	6.7	447	MS ² [447]: 429 (16), 357 (66), 327 (100), 285 (5)	Luteolin-6-C-hexoside
19	7.2	739	MS ² [739]: 593 (40), 575 (100), 285 (92), 284 (85), 257 (31) MS ³ [739→575]: 429 (35), 339 (100), 327 (18), 285 (9) MS ³ [739→285]: 257 (21), 255 (100), 229 (8), 151 (22)	Kaempferol-O-rhamnoside-O-rutinoside
21	7.6	279	MS ² [279]: 163 (100), 133 (2), 119 (5), 115 (1) MS ³ [279→163]: 119 (100)	p-coumaroyl malate

24	7.8	769	MS ² [769]: 623 (15), 315 (100), 300 (32) MS ³ [769→315]: 300 (100), 285 (18), 151 (14) MS ⁴ [769→315→300]: 271 (100), 243 (37), 151 (51)	Isorhamnetin rhamnosyl-rutinoside
26	8.0	431	MS ² [431]: 341 (3), 311 (100), 283 (7) MS ³ [431→311]: 283 (100), 225 (8)	Apigenin-8-C-hexoside
28	8.1	873	MS ² [873]: 593 (60), 739 (20), 279 (100) MS ³ [873→279]: 163 (72), 133 (100), 119 (5), 115 (20)	p-coumaroyl malate derivative
29	8.2	593	MS ² [593]: 285 (93), 284 (100), 255 (37) MS ³ [593→284]: 257 (31), 255 (100), 107 (21), 241 (18), 151 (54)	Kaempferol-O- rutinoside
31	8.5	753	MS ² [753]: 691 (3), 651 (3), 609 (100) MS ³ [753→609]: 489 (29), 343 (16), 301 (73), 300 (100), 271 (46)	Brutieridin
33	8.8	593	MS ² [593]: 285 (100), 284 (45), 255 (16) MS ³ [593→285]: 257 (56), 255 (100), 241 (25), 151 (43)	Kaempferol-O-rutinoside
36	9.3	737	MS ² [737]: 675 (12), 635 (14), 593 (100) MS ³ [737→593]: 285 (69), 284 (100), 255 (29) MS ⁴ [737→593→284]: 255 (100), 151 (12)	Kaempferol O- α -l-rhamnopyranosyl-(1 → 2)-[(O-(3-hydroxy-3-methylglutaryl)- β -d-galactopyranoside]
40	10.1	737	MS ² [737]: 675 (10), 635 (10), 593 (100) MS ³ [737→593]: 429 (29), 327 (28), 285 (100), 179 (21) MS ⁴ [737→593→285]: 255 (100)	Kaempferol O- α -l-rhamnopyranosyl-(1 → 2)-[(O-(3-hydroxy-3-methylglutaryl)- β -d-galactopyranoside]
43	10.5	591	MS ² [591]: 447 (100)	Kaempferol-O-hexoside derivative

			MS ³ [591→447]: 285 (100), 284 (56), 255 (11), 151 (3) MS ⁴ [591→447→285]: 257 (45), 255 (100), 213 (21)	
47	13.7	987	MS ² [987]: 969 (96), 925 (20), 825 (100), 807 (24), 763 (45), 645 (52), 627 (20), 555 (45), 469 (54) MS ³ [987→825]: 763 (30), 645 (100), 583 (52)	Saponin
50	14.8	825	MS ² [825]: 763 (14), 645 (100), 601 (7), 487 (8) MS ³ [825→645]: 487 (32), 443 (100)	Saponin
51	15.2	795	MS ² [795]: 645 (100) MS ³ [795→645]: 557 (45), 487 (82), 443 (100)	Unknown
53	17.0	809	MS ² [809]: 747 (5), 629 (100), 539 (7), 471 (2) MS ³ [809→629]: 585 (100)	Saponin
54	18.5	795	MS ² [795]: 645 (100), 487 (6) MS ³ [795→645]: 487 (100)	Unknown
55	19.5	327	MS ² [327]: 291 (67), 229 (84), 211 (61), 209 (39), 171 (100), 165 (15) MS ³ [327→229]: 211 (100), 209 (17), 171 (27), 155 (31), 125 (27)	Oxo-dihydroxy-octadecenoic acid
56	21.0	941	MS ² [941]: 923 (5), 879 (100), 733 (1), 645 (12) MS ³ [941→879]: 733 (100), 715 (5)	Saponin
57	21.9	971	MS ² [971]: 953 (51), 927 (2), 909 (100), 825 (4), 763 (18), 645 (32), 627 (2), 601 (7), 555 (5), 487 (4), 469 (7) MS ³ [971→909]: 763 (100), 601 (34)	dHex-Hex-HexA-Bayogenin
58	24.1	825	MS ² [825]: 781 (8), 763 (30), 645 (100), 487 (5), 469 (7)	Saponin
59	24.8	329	MS ² [329]: 311 (8), 309 (35), 229 (100), 211 (31), 209 (21)	Trihydroxy-octadecenoic acid

60	24.9	795	MS ² [795]: 733 (21), 645 (100), 487 (7) MS ³ [795→645]: 585 (80), 487 (100) MS ⁴ [795→645→487]: 441 (75), 439 (100)	Unknown
62	26.8	971	MS ² [971]: 851 (2), 809 (100), 791 (1), 747 (2), 629 (8), 585 (2) MS ³ [971→809]: 747 (19), 629 (100), 603 (9), 585 (4), 553 (3), 471 (20), 469 (2)	Hex-Hex-HexA-Hederagenin
63	27.3	941	MS ² [941]: 779 (100), 629 (38), 585 (5) MS ³ [941→779]: 629 (100), 585 (9), 471 (13) MS ⁴ [941→779→629]: 585 (20), 439 (100)	Unknown
64	29.6	779	MS ² [779]: 629 (100), 439 (20) MS ³ [779→629]: 585 (67), 567 (55), 511 (100), 471 (78)	Unknown
65	30.1	837	MS ² [837]: 775 (8), 687 (100), 643 (6), 611 (5), 529 (10) MS ³ [837→687]: 643 (100), 625 (80), 481 (95), 463 (45)	Unknown
66	30.5	793	MS ² [793]: 643 (100), 599 (14), 485 (4) MS ³ [793→643]: 599 (100), 597 (25), 583 (36), 527 (16), 485 (34)	Unknown
68	32.1	823	MS ² [823]: 673 (100), 515 (22), 451 (10) MS ³ [823→673]: 629 (96), 611 (100), 537 (83), 515 (70), 495 (58)	Unknown
71	33.6	941	MS ² [941]: 923 (100), 879 (34), 795 (7), 751 (4), 733 (27), 633 (1), 615 (14), 597 (8), 525 (18), 457 (1) MS ³ [941→923]: 879 (70), 733 (62), 597 (100), 525 (48) MS ⁴ [941→923→879]: 733 (100)	3-Rha-Gal-GlcA-Soyasapogenol B
72	34.7	795	MS ² [795]: 615 (100), 457 (18)	Unknown

			MS ³ [795→615]: 571 (32), 553 (45), 457 (100), 423 (28)	
73	35.2	765	MS ² [765]: 615 (100), 457 (30) MS ³ [765→615]: 571 (63), 567 (38), 553 (100), 497 (50), 375 (67) MS ⁴ [765→615→553]: 497 (100)	Unknown
74	35.9	809	MS ² [809]: 747 (13), 647 (6), 629 (100), 603 (8), 585 (15), 567 (1), 553 (11), 539 (5), 471 (19), 469 (1), 453 (10), 451 (4), 423 (4)	Hex-HexA-Hederagenin
75	36.3	939	MS ² [939]: 921 (100), 877 (32), 793 (10), 749 (4), 731 (22), 613 (16), 595 (6), 523 (15), 455 (1) MS ³ [939→921]: 877 (100), 523 (59)	dHex-Hex-HexA-Soyasapogenol E
76	37.4	909	MS ² [909]: 891 (100), 847 (36), 701 (12), 613 (34), 595 (6), 523 (7) MS ³ [909→891]: 847 (100), 595 (78), 523 (61)	Soyasapogenol E-3-O-rhamnosyl arabinosyl glucuronide
77	38.1	763	MS ² [763]: 613 (100), 595 (18) MS ³ [763→613]: 569 (100), 553 (69), 455 (74), 407 (73) MS ⁴ [763→613→569]: 551 (100)	Unknown

HexA, uronic acid, such as glucuronic acid or galacturonic acid; Hex, hexose, such as glucose or galactose; dHex, 6-deoxyhexose, such as rhamnose or furanose; GlcA, glucuronic acid; Gal, galactose; Rha, rhamnose.

Table 3Extraction yields, total phenolic and flavonoid contents of *Lathyrus* species (mean±SD)*

Assay	<i>L. aureus</i>			<i>L. pratensis</i>		
	Ethyl acetate	Methanol	Water	Ethyl acetate	Methanol	Water
Extraction yields (%)	0.31	14.92	15.72	0.86	16.49	22.12
Total phenolics (mg GAEs/g extract) **	21.34±0.18b	22.74±0.08a	19.42±0.51c	13.33±0.15c	40.54±0.24b	66.69±0.21a
Total flavonoids (mg REs/g extract) ***	0.89±0.15c	5.32±0.13b	15.77±0.28a	0.08±0.07c	26.16±0.16b	38.90±0.77a

* Data marked with different letters within the same row indicate statistically significant differences for each sample ($p < 0.05$)

** GAEs, gallic acid equivalents.

*** REs, rutin equivalents.

Table 4Radical scavenging activities of *Lathyrus* species (mean±SD)*

Samples	<i>L. aureus</i>		<i>L. pratensis</i>	
	DPPH (mg TEs/g extract)**	ABTS cation (mg TEs/g extract)**	DPPH (mg TEs/g extract)**	ABTS cation (mg TEs/g extract)**
Ethyl acetate	6.92±0.62ab	3.39±0.01c	12.39±0.67c	5.14±0.96c
Methanol	4.09±1.38b	34.37±0.75b	126.68±0.58b	67.85±0.25b
Water	9.77±1.77a	67.38±0.99a	259.00±1.28a	166.09±0.28a

* Data marked with different letters within the same column indicate statistically significant differences for each sample ($p < 0.05$)

** TEs, trolox equivalents.

Table 5Reducing power of *Lathyrus* species (mean±SD)*

Samples	<i>L. aureus</i>		<i>L. pratensis</i>	
	FRAP (mg TEs/g extract)**	CUPRAC (mg TEs/g extract)**	FRAP (mg TEs/g extract)**	CUPRAC (mg TEs/g extract)**
Ethyl acetate	14.89±0.46c	37.47±1.76c	13.32±0.06c	35.33±0.38c
Methanol	31.98±0.02b	71.22±0.08a	82.02±2.37b	117.37±1.90b
Water	39.22±0.91a	54.17±0.57b	154.69±0.21a	221.61±0.44a

* Data marked with different letters within the same column indicate statistically significant differences for each sample ($p < 0.05$)

** TEs, trolox equivalents.