Quinoa and Chia Modulate AMPK/ PPAR-ɣ Signaling in High-fat Diet-induced Obesity Rat Model

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Table S1:Primer sequence of FFAs and SREBP-1C genes

Gene	Primer sequence
FFAs	Forward :5-GCTTTGCTGCCGTGTCCTTCT-3 Reverse :5-GTGTCTGCTGGGGTCCTCGTT-3
SREBP-1c	Forward :5-TCCCAGAGTAGCCCCTTGTCC -3 Reverse :5-CCAGTCCCCATCCACGAATG-3
βeta actin	Forward primer 5′-TGTTTGAGACCTTCAACACC-3′ Reverse primer 5′-CGCTCATTGCCGATAGTGAT-3′

Table S2: Peak assignments using HPLC/PDA/ESI-MS/MS of metabolites detected in bioactive extract from quinoa (negative mode).

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Peak #	**Retention time (min)**	**Identified Compound**	**UV-Vis λmax**	**$[M-H]^-$ *(m/z)***	**Peak Area %**	**Fragment ions *(m/z)***	**Reference**
1	3.81	Quercetin malonyl) hexoside	255, 353	549.1	5.20	301	(Šuković et al., 2020)
2	4.16	Dihydroxybenzoic acid-dipentoside	253	417.7	7.88	241, 153	(Beelders et al., 2014)
3	5.40	*Ferulic acid hexoside	335, 295	355.5	7.90	193	(Tang et al., 2015)
4	6.81	Apigenin pentoside-hexoside	250, 354	563.2	0.88	503, 269	(Ağalar et al., 2018)
5	7.95	Apigenin	250, 330	269	0.37	-	tentative
6	9.56	Hydroxycinnamic acid	284	197.1	4.95	123	(Oliveira-Alves et al., 2017)

7	9.68	Caffeoyl-N-tryptophanrhamnoside	Ud	511.0	3.21	365, 161	(Llorent-Martinez et al., 2015)
8	10.2	Myricetin-*O*-β-Dgalactopyranoside	266, 350	479.3	0.97	316, 271	(Saldanha et al., 2013a)
9	10.40	Apigenin-hexosyl dihexoside	252, 353	755.1	0.27	269	(Ağalar et al., 2018)
10	10.9	Caffeic acid	256,336	786.2	2.01	191, 249	(Wu et al., 2009)
11	11.49	Apigenin (glucosyl glucosyl) pentosyl hexoside	270, 334	887.6	4.10	725, 353, 269	(Benyad et al., 2014)
12	11.45	*Quercetin-O-rutinoside	255, 353	609.1	1.80	301, 179, 153	(Brito et al., 2014)
13	11.67	Myricetin	221, 330	317.1	0.18	271, 179	(Saldanha et al., 2013a)

14	11.99	* Kaempferol dirhamnoside	260, 355	577.1	9.21	285	(Tang et al., 2015)
15	12.37	Quercetin O-(malonyl) hexoside	259, 356	549.1	3.53	505, 301, 179	(Šuković et al., 2020)
16	12.52	Feruloyl-caffeoylquinic acid	295	529.4	0.54	560, 191	(Ben Said et al., 2017)
17	12.60	Salvianolic acid F isomer	220, 333	313.1	2.13	269, 179	(Barros et al., 2013)
18	12.75	Salvianolic acid C	225, 336	491.1	13.79	311, 197	(Barros et al., 2013)
19	13.07	Dehydroxy amentoflavone	220, 335	521.4	0.23	375	(Yao et al., 2017)
20	13.45	(Iso)vitexin sulfate	273, 339	511.7	2.46	431, 269	(Ahmed et al., 2019)
21	15.22	*Quercetin	255,330	301.0	5.39	179, 153	(Tang et al., 2015)

22	15.84	Apigenin pentosyl hydroxyferuloy-pentoside	267, 335	725.1	7.2	443, 353, 269	(Benyad et al., 2014)
23	16.5	Apigenin-hexoside	267, 335	431.0	0.69	311, 269	(Ahmed et al., 2019)
24	16.85	Eriodictyol-neohesperidoside hexoside	258,332	757.2	0.48	595, 449, 287	(Brito et al., 2014)
25	17.6	Kaempferol	255, 334	285.0	1.31	151	(Brito et al., 2014)
26	18.2	Quercetin-*O*-xyloside-*O*-rutinoside	222, 336	741	0.66	609, 301, 179	(Simirgiotis et al., 2015a)
27	19.18	Coumaroyl-O-caffeoyl methylpentanoic acid hydroxyl quinate	220, 339	693.0	1.13	663, 353, 337	(Ben-Said et al., 2017)

28	20.05	*Vanillic acid hexoside	287	329.1	0.78	167	(Kramberger et al., 2020; Tang et al., 2015)
29	20.12	*Kaempferol hexoside	253, 352	447.3	0.81	285	(Brito et al., 2014)
30	20.44	Apigenin Acetyl-hexoside	268, 335	473.0	0.75	323, 221,161	(Brito et al., 2014)
31	21.71	*Kaempferol-apiofuranosyl-rhamnopyranosyl-hexoside	252, 352	725.1	0.89	593, 285	(Pereira et al., 2020)
32	21.75	Apigenin-dihexoside	268, 336	593.2	0.8	311, 269	(Ahmed et al., 2019)
33	22.25	Caffeoyl quinic acid	246, 310	353.2	0.4	191	(Simirgiotis et al., 2015a)

34	22.44	*Kaempferol(di-rhamnoside)-hexoside	252, 352	739.4	0.9	285	(Pereira et al., 2020)
35	26.20	Dicaffeoyl-quinic acid	310, 240	515.1	0.53	353, 191	(Simirgiotis et al., 2015a)
36	38.8	Apigenin *C*-hexoside (vicenin isomer)	28,336	431.0	0.73	-	-
37	39.12	*Quercetin-O-glucuronide	255, 352	477.4	0.33	301	(Pereira et al., 2020)
38	39.5	*Kaempferol-O-rutinoside	265,347	593.1	0.33	285	(Pereira et al., 2020)
39	40.6	Caffeoyl derivative hexose	285	499.4	0.86	277	(Kang et al., 2016)

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Table S3: Peak assignments using HPLC/PDA/ESI-MS/MS of metabolites detected in bioactive extract from chia (negative mode)

Peak #	**Retention time (min)**	**Identified Compound**	**UV-Vis λmax**	**$[M-H]^-$ *(m/z)***	**Peak area (%)**	**Fragment ions *(m/z)***	**Reference**
1	3.92	*Malic acid	225	133.0	7.49	133	(Tohma et al., 2016)
2	3.95	Caffeic acid derivative	226	377.1	4.15	341, 179	(Khole et al., 2014)
3	4.12	* Syringic acid- Hexoside	229	359.1	3.89	197, 182	(Guasch-Jané et al., 2006)
4	5.45	Myricetin galloyl galactoside	228, 336	631.0	1.03	317, 271, 179	(Saldanha et al., 2013b)
5	6.50	*Danshensu	285	197.0	3.87	179, 123	(Oliveira-Alves et al., 2017)
6	7.9	Quercetin-arabinoside	266, 350	433.2	6.69	301, 179, 151	(Tang et al., 2015)
7	8.9	Caffeic acid hexoside	220, 333	341.0	0.24	179	(Zhang et al., 2007)
8	9.316	Chrysophanol -*O*- hexoside	222, 335	415.0	-	253	(Ye et al., 2007)

9	10.37	*Danshensu methyl ester	285, 320	211.0	1.74	197, 179, 123	(Oliveira-Alves et al., 2017)
10	11.02	Ferulic acid	295, 332	193.0	0.61	149	(Tang et al., 2015)
11	11.79	*Rosmarinic acid-hexoside	220, 319	521.2	0.77	359,323, 161	(Oliveira-Alves et al., 2017)
12	12.10	Ferulic acid hexoside	225, 330	355.1	4.54	193	(Barros et al., 2013)
13	12.73	Rosmarinic acid	226, 336	359.1	14.95	197, 179, 161	(Elshamy et al., 2021)
14	13.19	Methyl Rosmarinic acid hexoside	284, 324	535.2	3.05	373, 179	(Oliveira-Alves et al., 2017)
15	14.55	Caffeic acid derivative	226, 330	339.1	22.59	211, 179	(Oliveira-Alves et al., 2017)
16	15.34	*Danshensu glucuronide	284, 328	373.0	13.83	(30), 197(10),	(Oliveira-Alves et al., 2017)

17	15.5	*Danshensu derivative	285, 327	295.0	1.02	135, 123, 105	(Oliveira-Alves et al., 2017)
18	17.59	*Danshensu rhamnoside hexoside	283, 339	321.0	0.81	323, 197	(Oliveira-Alves et al., 2017)
19	18.5	*Caffeoylquinic acid	220, 309	353.1	0.36	191	(Oliveira-Alves et al., 2017)
20	18.93	*Caffeic acid arabinoside	285, 330	311.3	0.49	179, 135	(Oliveira-Alves et al., 2017)
21	25.17	*Caffeoylquinic acid	220, 309	353.1	0.66	191	(Oliveira-Alves et al., 2017)
22	27.69	Feruloyl-caffeoylquinic acid derivative.	223, 336	365.0	-	353, 193	(Simirgiotis et al., 2015b)
23	29.6	*Ferulic truxilic acid	220, 336	387.1	0.089	193	(Oliveira-Alves et al., 2017)

24	33.2	Epicatechin	229, 339	289.3	0.06	245	
25	34.6	Binaringenin methyl ether	225, 394	555.0	-	387	(Yao et al., 2017)
26	35.6	Dihydroxydimethoxy flavonederivative	225, 332	375.1	-	316, 284	(Gouveia & Castilho, 2010)

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List of Captions (Supplementary data)

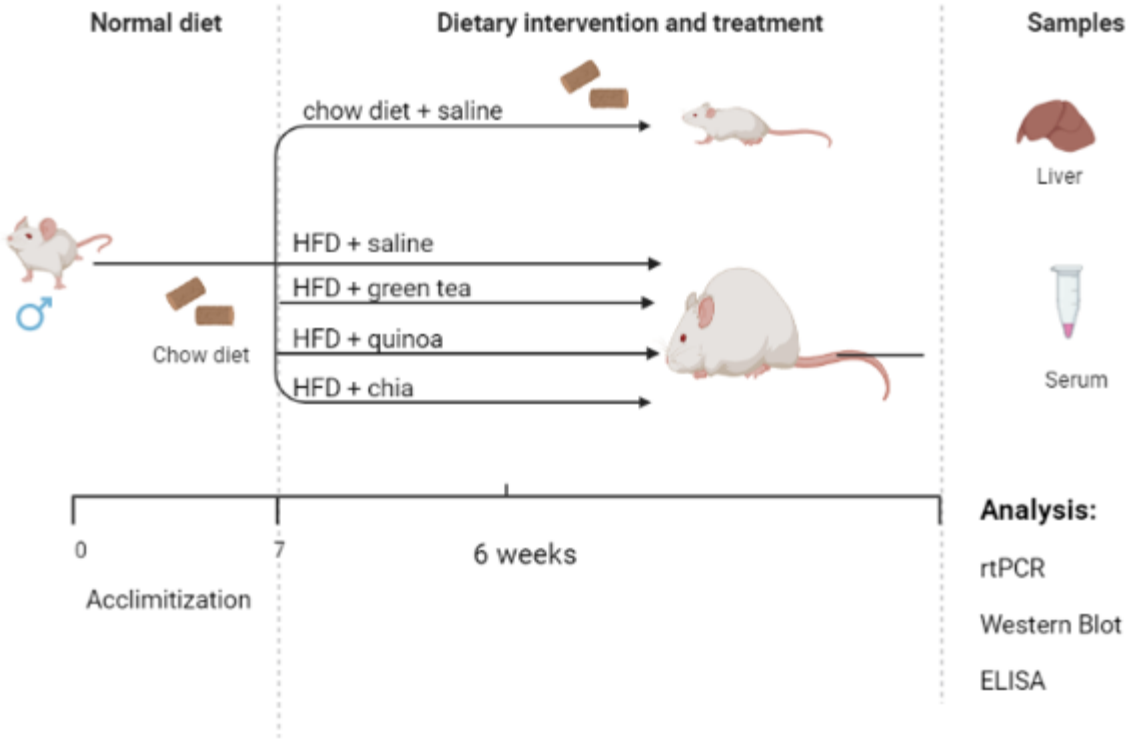

Fig. S1 : Schematic diagram of the experimental design

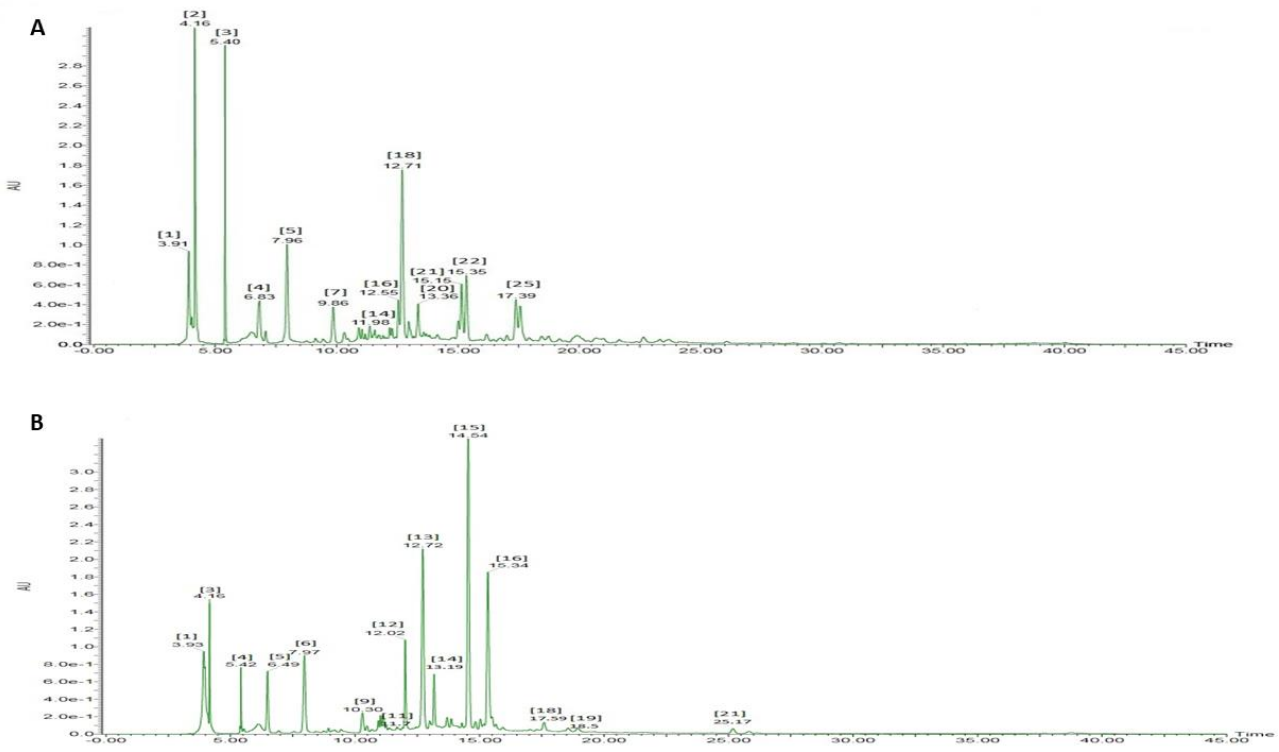

Fig. S2: HPLC/PDA/ESI-MS/MS chromatogram of metabolites detected in bioactive extract from (A) quinoa (B) chia (negative mode).

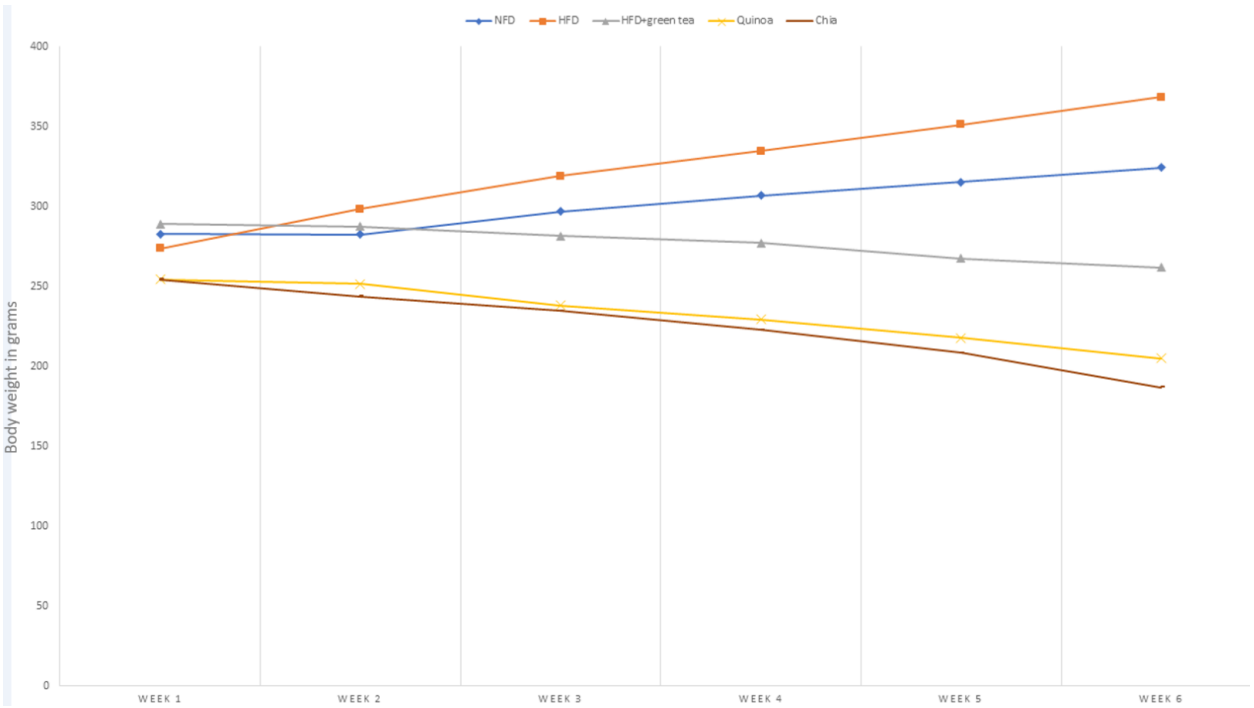

Fig. S3: Trend chart shows average of body weight (gms) in different study groups along the course of study (six weeks).

References

Ahmed RF, Elkhrisy EA, El-Kashak WA, Raey MA, Nassar MI, Aboutabl ESA (2019) Structural Characterization of Polyphenolics in Livistona chinensis Using HPLC-PDA-MS. J Adv Pharm Res *3*(1): 23–29. http://aprh.journals.ekb.eg/

Ağalar HG, Çiftçi GA, Göğer F, Kırımer N (2018) Activity guided fractionation of *Arum italicum* Miller tubers and the LC/MS-MS profiles. Rec Nat Prod 12: 64–75. https://doi.org/10.25135/rnp.06.17.05.089.

Barros L, Dueñas M, Dias MI, Sousa MJ, Santos-Buelga C, Ferreira ICFR (2012) Phenolic profiles of in vivo and in vitro grown Coriandrum sativum L. Food Chem *132* (2): 841–848. https://doi.org/10.1016/j.foodchem.2011.11.048.

Beelders T, De Beer D, Stander MA, Joubert E (2014) Comprehensive phenolic profiling of Cyclopia genistoides (L.) Vent. by LC-DAD-MS and -MS/MS reveals novel xanthone and benzophenone constituents. Molecules 19 (8): 11760–11790. https://doi.org/10.3390/molecules190811760.

Benayad Z, Gómez-Cordovés C, Es-Safi NE (2014) Characterization of flavonoid glycosides from fenugreek (Trigonella foenum-graecum) crude seeds by HPLC–DAD–ESI/MS analysis. Int J Mol Sci 15 (11): 20668-20685. https://doi.org/10.3390/ijms151120668

Ben Said R, Arafa I H, Usam AM, Abdullah AA, Kowalczyk M, Moldoch J, Oleszek W, Stochmal, A (2017) Tentative characterization of polyphenolic compounds in the male flowers of Phoenix dactylifera by liquid chromatography coupled with mass spectrometry and DFT. Int J Mol Sci *18* (3): 512. **https://doi.org/10.3390/ijms18030512**.

Brito A, Ramirez JE, Areche C, Sepúlveda, B, Simirgiotis, MJ (2014) HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in Northern Chile. *Molecules 19* (11): 17400-17421.

Elshamy S, Abdel Motaal A, Abdel-Halim M, Medhat D, Handoussa H (2021) Potential neuroprotective activity of Mentha longifolia L. in aluminum chloride-induced rat model of Alzheimer's disease. J Food Biochem 45 (4): 1770. https://doi.org/10.1111/jfbc.13644.

Gouveia SC, Castilho PC (2010a) Characterization of phenolic compounds in Helichrysum melaleucum by high-performance liquid chromatography with on-line ultraviolet and mass spectrometry detection. *Rapid Commun Mass Spectrom 24* (13): 1851–1868. https://doi.org/10.1002/rcm.4585

Guasch-Jané MR, Andrés-Lacueva C, Járegui O, Lamuela-Raventós RM (2006) The origin of the ancient Egyptian drink Shedeh revealed using LC/MS/MS. J Archaeol Sci *33*(1):98–101. https://doi.org/10.1016/j.jas.2005.06.013

Kang J, Price WE, Ashton J, Tapsell LC, Johnson S (2016) Identification and characterization of phenolic compounds in hydromethanolic extracts of. *Food Chemy 211*: 215–226.

Khole S, Chatterjee S, Variyar, P, Sharma A, Devasagayam TPA, Ghaskadbi S (2014) Bioactive constituents of germinated fenugreek seeds with strong antioxidant potential. *J Func Foods 6*(1): 270–279. https://doi.org/10.1016/j.jff.2013.10.016.

Kramberger K, Barlič-Maganja D, Bandelj D, Baruca Arbeiter A, Peeters K, Miklavčič Višnjevec A, Jenko Pražnikar Z (2020). HPLC-DAD-ESI-QTOF-MS determination of bioactive compounds and antioxidant activity comparison of the hydroalcoholic and water extracts from two Helichrysum italicum species. *Metabolites 10*(10): 403. https://doi: 10.3390/metabo10100403.

Oliveira-Alves SC, Vendramini-Costa DB, Cazarin CB, Júnior MR, Ferreira JP, Silva AB, Prado MA, Bronze MR (2017) Characterization of phenolic compounds in chia (Salvia hispanica L.) seeds, fiber flour and oil. Food Chem *232*: 295–305. https://doi.org/ 10.1016/j.foodchem.2017.04.002.

Pereira E, Cadavez V, Barros L, Encina-Zelada C, Stojković D, Sokovic M, Calhelha R C, Gonzales-Barron U, Ferreira ICFR (2020). Chenopodium quinoa Willd. (quinoa) grains: A good source of phenolic compounds. Int. Food Res *137*. https://doi.org/10.1016/j.foodres.2020.109574.

Saldanha LL, Vilegas W, Dokkedal AL (2013a) Characterization of flavonoids and phenolic acids in Myrcia bella cambess. Using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR. Molecules 18 (7): 8402–8416. https://doi.org/ 10.3390/molecules18078402.

Simirgiotis MJ, Benites J, Areche C, Sepu B (2015b). Antioxidant capacities and analysis of phenolic compounds in three endemic nolana species by HPLC-PDA-ESI-MS. *Molecules 20* (6): 11490–11507. https://doi.org/10.3390/molecules200611490

Šuković D, Knežević B, Gašić U, Sredojević M, Ćirić I, Todić S, Mutić J, Tešić Ž (2020) Phenolic profiles of leaves, grapes and wine of grapevine variety vranac (Vitis vinifera L.) from Montenegro. Foods 9 (2). https://doi.org/ 10.3390/foods9020138.

Tang Y, Li X, Zhang B, Chen PX, Liu R, Tsao R (2015) Characterisation of phenolics, betanins and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. *Food Chem 166*: 380–388. https://doi.org/10.1016/j.foodchem.2014.06.018.

Tohma H, Köksal E, Kılıç Ö, Alan Y, Abdullah Yılmaz M, Gülçin İ, Bursal E, Alwasel SH (2016) RP-HPLC/MS/MS analysis of the phenolic compounds, antioxidant and antimicrobial activities of salvia L. Species. *Antioxidants 5*(4): https://doi.org/10.3390/antiox5040038.

Wu ZJ, Ma XL, Fang DM, Qi HY, Ren WJ, Zhang GL (2009b) Analysis of caffeic acid derivatives from Osmanthus yunnanensis using electrospray ionization quadrupole time-of-flight mass spectrometry. Eur J Mass Spectrom *15* (3): 415–429. https://doi.org/ 10.1255/ejms.992.

Ye M, Han J, Chen H, Zheng J, Guo D (2007) Analysis of Phenolic Compounds in Rhubarbs Using Liquid Chromatography Coupled with Electrospray Ionization Mass Spectrometry. J Am Soc Mass Spectrom *18* (1): 82-91.https://doi.org/10.1016/j.jasms.2006.08.009

Yao H, Chen B, Zhang Y, Ou H, Li Y, Li S, Shi P, Lin X (2017) Analysis of the total biflavonoids extract from Selaginella doederleinii by HPLC-QTOF-MS and its in vitro and in vivo anticancer effects. *Molecules 22* (2). https://doi.org/10.3390/molecules22020325.