AN INTEGRATED HIGH RESOLUTION MASS SPECTROMETRIC AND INFORMATICS APPROACH FOR THE RAPID IDENTIFICATION OF FLAVONOIDS IN PLANT EXTRACT

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Introduction

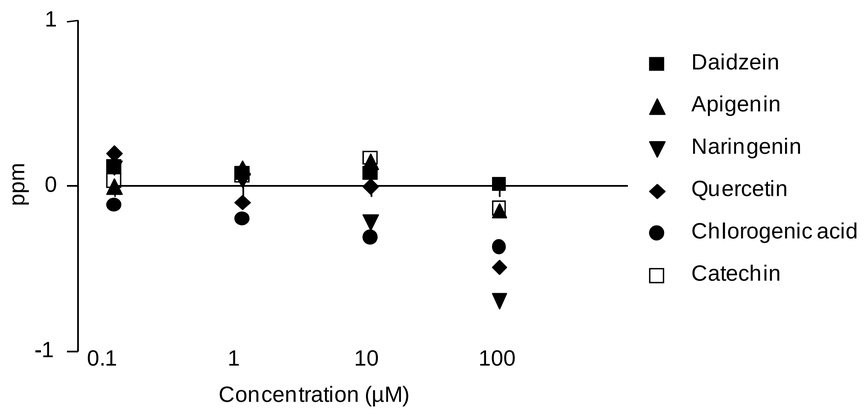
The identification and characterization of phenol compounds in plants and manufactured foods represent an important analytical issue, which has blossomed during the last decades.

Currently, HPLC coupled to mass spectrometry by APCI or ESI source represents the most selective analytical technique for the identification and quantification of phenol compounds from plants and foods.

Here we report an integrated approach based on high resolution MS analysis (orbitrap) and database utilization for the rapid identification of plant phenols.

This approach is firstly validated by using a mixture of phenolic standards and then applied for the rapid identification of phenols from ***Angelica Keiskei* extract**

Mass accuracy of standard plant polyphenols at four different concentrations (0.1 – 100 µM)



Since in a plant extract the polyphenols are contained in a wide concentration range, we firstly investigated the effect of concentration on mass accuracy, thus to establish the tolerance value to be set in db searching parameters. 1 ppm was selected as a default tolerance value in db searching.

In order to simplify the data mining process of flavonoid databases, a specific plug-in for the VEGA ZZ package [1] was developed in C++ programming language. The software is freely available for academic non-profit use at http://www.vegazz.net. Two different flavonoid databases were used; one was created by importing the polyphenols listed in the Phenol-Explorer data base and containing 502 polyphenols [2]; the second was built from 6850 molecules downloaded as 2D MOL files from http://www.metabolome.jp [3]

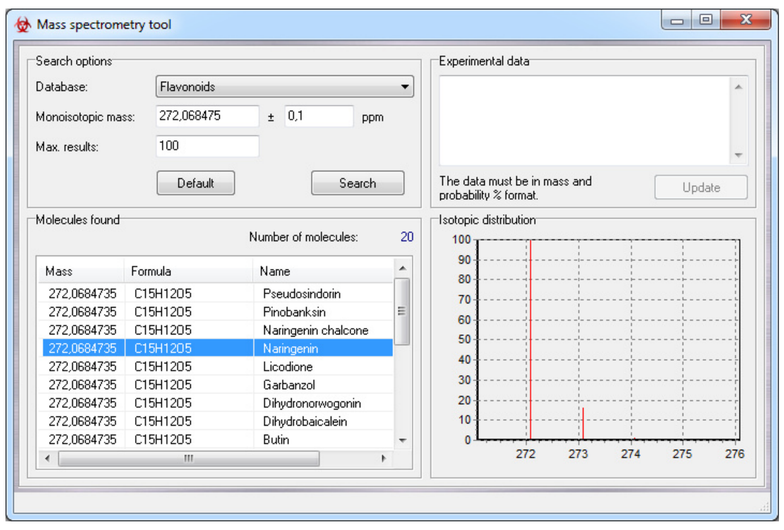


Table 1: The method was firstly validated by analyzing a mixtures of standards belonging to different polyphenol classes (hydroxycynnamics acids, flavones, flavonols, flavanones, flavanols, isoflavonoids); Db searching, Uv-Vis data and experimental and predicted MS/MS data for flavonoids and phenolic acid standards are reported in the Table

Peak	Mono-isotopic mass value (Da)	List 1	UV-Vis (nm)	List 2	Matched experimental/simulated MS/MS fragments	Hit compound
S1	290.07903	• (+)-Catechin • (-)-Epicatechin	234, 279	• (+)-Catechin • (-)-Epicatechin	• 119, 123, 139, 151, 165, 249, 273 • 119, 123, 139, 151, 165, 249, 273	(+)-Catechin (-)-Epicatechin
S2	354.09508	• chlorogenic acid	240, 298 sh, 326	• chlorogenic acid	• 163	chlorogenic acid
S3	254.05791	• 7,4'-Dihydroxyflavone • Chrysin • Daidzein	261(sh), 301	• Daidzein	• 119, 121, 137, 227, 237, 199	Daidzein
S4	302.04265	• 6-Hydroxyluteolin • Morin • Quercetin	256,266(sh)370	• Morin • Quercetin	• 109,137,153,165,247,275, 285 • 109,137,153,165,247,275, 285	Quercetin
S5	272.06847	• Butein • Naringenin	288, 331(sh)	• Naringenin	• 119, 147,153, 163, 179, 189, 231, 255	Naringenin
S6	270.05282	• Galangin • Genistein • 7,3',4'-Trihydroxyflavone • Apigenin • Baicalein	267, 336	• 7,3',4'-Trihydroxyflavone • Apigenin • Baicalein	• 243 • 119, 153, 243 • 243	Apigenin

Conclusions

The proposed approach represents a new, reliable and potent tool for a facile and rapid identification of plant polyphenols. Moreover, such an approach can be successfully used without a strong background in polyphenol/flavonoid chemistry and MS/MS fragmentation knowledge, thus to greatly facilitate the phytochemical characterization of plant extracts.

Overview of the integrated strategy

The LC/UV/ESI-MS analysis represents the first step, which is performed by using an orbitrap as a MS analyzer thus to retrieve accurate monoisotopic mass and isotopic mass distribution of the unknown compounds. The monoisotopic mass is then searched in a database (db) of polyphenols and the output is a list of compounds (**list 1**), which is then filtered on the basis of the chemical class to which the unknown compound belongs, as determined on the basis of the UV-Vis spectrum and on the well established UV absorption bands of flavonoids. For each entry belonging to the filtered list (**list 2**), simulated MS/MS fragments are predicted using the Mass Frontier software and the values are compared with the experimental fragments. The compounds are then ranked on the basis of the matched predicted/experimental fragments and the hit compound at the top of the list identifies the unknown. Final confirmation is then achieved by comparing experimental and simulated isotopic patterns.

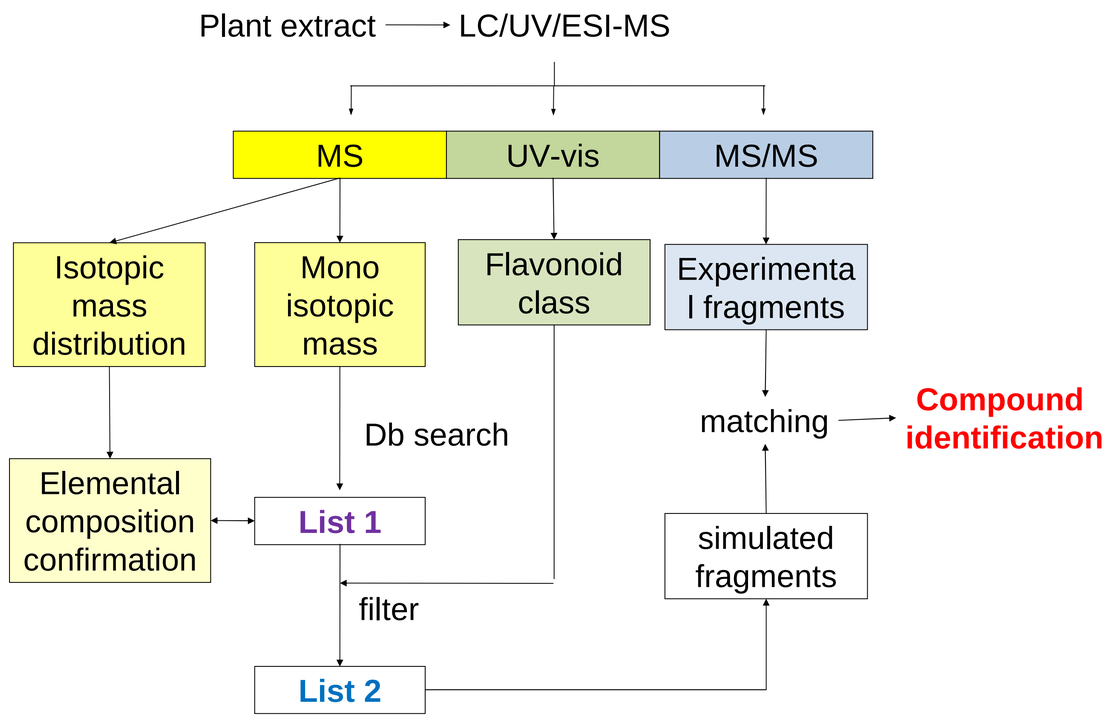


Table 2: The method was then applied for the identification of flavonoids contained in an EtOH extract of *Angelica keiskei*.

Peak	Mono-isotopic mass value (Da)	List 1	UV-Vis (nm)	List 2	Matched experimental/ simulated MS/MS fragments	Hit compound
a1	354.09510	• chlorogenic acid	240, 298 sh, 326	• chlorogenic acid	• 163	Chlorogenic Acid
a2	594.15825	• Chrysoeriol 7-O-apiosyl-glucoside • Luteolin 7-O-rutinoside • Apigenin 6,8-di-C-glucoside • Kaempferol 3-O-galactoside 7-O-rhamnoside • Kaempferol 3-O-rutinoside	251, 268, 346	• Chrysoeriol 7-O-apiosyl-glucoside • Luteolin 7-O-rutinoside • Apigenin 6,8-di-C-glucoside	• 449 • 287, 449 • -	Luteolin 7-O-rutinoside
a3	464.09565	• Ellagic acid glucoside • Myricetin 3-O-rhamnoside • Quercetin 3-O-galactoside • Quercetin 3-O-glucoside	255, 266 sh, 352	• Myricetin 3-O-rhamnoside • Quercetin 3-O-galactoside • Quercetin 3-O-glucoside	• - • 303 • 303	Quercetin 3-O-galactoside Quercetin 3-O-glucoside
a4	448.10084	• 6-Hydroxyluteolin 7-O-rhamnoside • Luteolin-C-glucoside • Kaempferol-O-galactoside • Kaempferol-O-glucoside • Quercetin 3-O-rhamnoside	255, 268 sh, 346	• 6-Hydroxyluteolin 7-O-rhamnoside • Luteolin-C-glucoside	• - • 287	Luteolin-C-glucoside
a5	338.15189	• 4-Hydroxyderricin • 2',4'-Dihydroxy-6'-methoxy-3'-prenylchalcone • 2'-Hydroxy-6'-methoxy-4'-prenyloxychalcone • Licochalcone C • Licochalcone A • Crotaramin • Bavachinin • 4'-Hydroxyisoderricin • Falciformin • 5-Hydroxy-7-methoxy-6-C-prenylflavanone • Tephrinone • 5-Methoxy-7-prenyloxyflavanone • 6-C-Prenyl-8-C-methylpinocembrin	239, 366	• 4-Hydroxyderricin • 2',4'-Dihydroxy-6'-methoxy-3'-prenylchalcone • 2'-Hydroxy-6'-methoxy-4'-prenyloxychalcone • Licochalcone C • Licochalcone A	• 119, 147, 219, 283 • 283 • 283 • 147, 283 • 147	4-Hydroxyderricin
a6	392.19895	• Abyssinone VI • Kanzonol C • 4,2',4'-Trihydroxy-3',5'-diprenylchalcone • 4'-Geranyloxy-4,2'-dihydroxychalcone • Xanthoangelol • Stipulin • Artoindonesianin J • Bis(6'',6''-dimethyl-4'',5''-dihydropyrano) • [2'',3'':4',5'][2'',3'':4,3]-2'-hydroxychalcone • Flemiwallichin E • 3'-Geranyl-2',4',6'-trihydroxychalcone • 3'-Neryl-2',4',6'-trihydroxychalcone • Linderachalcone • 7-Prenyloxy-8-C-(3-hydroxy-3-methyl-trans-buten-1- yl)flavanone • 7,4'-Dihydroxy-6,8-di-C-prenylflavanone • Abyssinone IV • Glabrol • Prostratol F • Euchrenone a17 • Dorsmanin B • 5,7-Dihydroxy-8-C-geranylflavanone • 5-Hydroxy-7-prenyloxy-8-C-prenylflavanone • 5,7-Dihydroxy-6,8-di-C-prenylflavanone • 5-Hydroxy-7-O-nerylflavanone • Linderatone • Kazinol B • Hispaglabridin A • Lespein • Lespedezin • Ficifolinol • Erythrabyssin II • Erybraedin C	239, 366	• Abyssinone VI • Kanzonol C • 4,2',4'-Trihydroxy-3',5'-diprenylchalcone • 4'-Geranyloxy-4,2'-dihydroxychalcone • Xanthoangelol • Stipulin • Artoindonesianin J • Bis(6'',6''-dimethyl-4'',5''-dihydropyrano)[2'',3'':4',5'][2'',3'':4,3]-2'-hydroxychalcone • Flemiwallichin E • 3'-Geranyl-2',4',6'-trihydroxychalcone • 3'-Neryl-2',4',6'-trihydroxychalcone • Linderachalcone	• 283, 337 • 337 • 273, 337 • 269, 273, 283, 337 • 269, 273, 283, 337 • 337 • 269.337 • 269, 283, 337 • 269 • 269, 283. 337 • 269, 283. 337 • -	Xanthoangelol

a7: xanthangelol b **a8**: xanthangelol f

References

[1] A. Pedretti, et al. J. Comput. Aided Mol Des. 18 (2004) 167.

[2] V. Neveu, et al. Database (2010) doi: 10.1093/database/bap024.

[3] M. Arita, et al. BioData Min. 1(2008) 7.