Supplementary information

Polyphenolic Profiling of Green Waste Determined by UPLC-HDMS^E

Colin M. Potter[1,*] and David. L. Jones[1,2]

[1] Centre for Environmental Biotechnology, School of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK

[2] UWA School of Agriculture and Environment, The University of Western Australia, Perth, WA 6009, Australia

[*]Correspondence: colinmpotter@gmail.com

S1. Description of the four plants used in the study

S1.1. Smyrnium olusatrum

Alexanders (*Smyrnium olusatrum*) is a very common coastal hedgerow plant and is a member of the carrot family (*Umbelliferae*) of Macedonian origin. It typically grows within 1.5 km of the sea in Britain. This biennial plant was initially cultivated by the Romans as horse fodder when little else was available and appears from winter onwards in Britain. Its historical popularity was not only based on its nutritional value but also its herbal and apparent medicinal value [1]. Although there is limited information available on the polyphenol content of alexanders [2] much of the research focuses on the volatile constituents such as sesquiterpenes [3], isofuranodiene [4] and its stimulation of neuritogenesis, isofuranodiene and germacrone as a pesticide against spider mite [5] and also as a promising inhibitor of the protozoan parasite causing human African trypanosomiasis (sleeping sickness) [6].

S1.2. Urtica dioica

Stinging Nettle (*Urtica dioica*) is a widespread perennial, abundant throughout Europe, which is highly nutritious and has a long history in traditional medicine [7]. Stinging nettle

has an extensive range of uses, being of economic importance to many industries, for example the textile, paper, food and cosmetic industries [8, 9]. Overarching studies of the chemical composition of stinging nettle have been published relating to such components as polyphenols, terpenes, sterols, lectins and elemental analysis by inductively coupled plasma MS [10,11]. Progress has been achieved in the analysis of the phenolic composition of stinging nettle and related species by the use of a liquid chromatography coupled to a low resolution ion trap and a diode array detector [12]. Useful information was provided in a study of polyphenol content and how this varied through changes in season and location [13]. A review of the pharmacological uses of stinging nettle highlights its nutritional wealth and medicinal potential in terms of anti-inflammatory, astringent and anti-histamine qualities [14].

S1.3. Allium ursinum

Wild garlic (*Allium ursinum*) is a bulbous perennial, which is abundant in shaded woods and hedgerows and is a wild relative of the onion. Wild garlic is commonly used in traditional medicine and is claimed to have antibiotic and antiviral properties. Studies have also shown evidence of cardioprotective action and hence aiding in the reduction of the risk of stroke and heart disease [15]. Wild garlic has been shown to have allelopathic properties (the chemical inhibition of one plant by another) which causes the inhibition of seed germination and surrounding plant growth. The mechanism by which this occurs is thought to include the action of polyphenolics [16]. Numerous biologically active compounds are presented in this herb such as organo-sulphur compounds, polyphenolics and steroidal glycosides lectins, polysaccharides, fatty acids (palmitic, linoleic, oleic, palmitoleic, stearic, α-linolenic, and myristic acid), amino acids (alanine, asparagine, aspartic acid, glutamic acid, arginine, glycine, threonine, glutamine) although much of the pharmacological activity is attributed to volatile sulphur compounds and so therefore much of the analytical focus lies here [17]. Antimicrobial activity and prevention

and treatment of cardiovascular disease are the main health claims that are made about this wild hedgerow plant. Only the leaf was taken for the analysis of wild garlic in this study.

S1.4. Ulex europaeus

Gorse (*Ulex europaeus*) is a species of flowering plant in the family *Fabaceae*. It is an evergreen used for hedging and boundary definition and is able to fix nitrogen. Gorse flowers throughout the year. In traditional medicine, flowers have been used in the treatment of jaundice and as a treatment for scarlet fever in children. Planted for soil stabilization on sandy substrates, it is used for stabilizing roadside banks as gorse is an excellent pioneer species for poor soils and areas with maritime exposure. In former times, gorse was valued as fodder for horses and cattle as it was abundant on land where little else of nutritional value grew. The gorse was bruised, crushed or milled prior to using as animal feed [18]. Extracts from gorse have been shown to be very rich in flavonoids and were evenly distributed as flavonols, flavones, isoflavones and flavanones [19]. A detailed LC-MS characterisation shows a vast array of polyphenols of nutritional and health benefits. In particular, liquiritigenin is highlighted which, in a review article, is shown to exhibit antioxidant, anti-inflammatory, antitumor activities, neuroprotective effects plus many other significant pharmacological properties [20].

References

1. Wright, J. *Edible Seashore*; Bloomsbury Publishing PLC, 2009; ISBN 0747595313.
2. Wafa, N.; Sofiane, G. Antioxidant, antimicrobial and anti-inflammatory activities development of methanol extracts of some species growth in the massif of Boutaleb Setif Algeria. *Int. J. Pharm. Nat. Med.* **2018**, *6*, 15–20.
3. Youssefi, M.R.; Nikpay, A.; Hassanpour, N.; Mirzapour, A. In vitro scolicidal activity

of the Sesquiterpenes. *Molecules* **2020**, *25*, 1–9, doi:10.3390/molecules25163593.

4. Mustafa, A.M.; Maggi, F.; Papa, F.; Kaya, E.; Dikmen, M.; Öztürk, Y. Isofuranodiene: A neuritogenic compound isolated from wild celery (*Smyrnium olusatrum* L., *Apiaceae*). *Food Chem.* **2016**, *192*, 782–787, doi:10.1016/j.foodchem.2015.07.079.
5. Benelli, G.; Pavela, R.; Canale, A.; Nicoletti, M.; Petrelli, R.; Cappellacci, L.; Galassi, R.; Maggi, F. Isofuranodiene and germacrone from S*myrnium olusatrum* essential oil as acaricides and oviposition inhibitors against *Tetranychus urticae*: impact of chemical stabilization of isofuranodiene by interaction with silver triflate. *J. Pest Sci.* **2017**, *90*, 693–699, doi:10.1007/s10340-016-0829-5.
6. Petrelli, R.; Ranjbarian, F.; Dall'Acqua, S.; Papa, F.; Iannarelli, R.; Ngahang Kamte, S.L.; Vittori, S.; Benelli, G.; Maggi, F.; Hofer, A.; et al. An overlooked horticultural crop, *Smyrnium olusatrum*, as a potential source of compounds effective against African trypanosomiasis. *Parasitol. Int.* **2017**, *66*, 146–151, doi:10.1016/j.parint.2017.01.001.
7. Guil-guerrero, J.L.; Rebolloso-fuentes, M.M.; Isasa, M.E.T. Fatty acids and carotenoids from Stinging Nettle (*Urtica dioica* L.). *J. Food Comp. Anal.* **2003**, *16*, 111–119, doi:10.1016/S0889-1575(02)00172-2.
8. Upton, R. Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. *J. Herb. Med.* **2013**, *3*, 9–38, doi:10.1016/j.hermed.2012.11.001.
9. Di Virgilio, N.; Papazoglou, E.G.; Jankauskiene, Z.; Di Lonardo, S.; Praczyk, M.; Wielgusz, K. The potential of stinging nettle (*Urtica dioica* L.) as a crop with multiple uses. *Ind. Crops Prod.* **2015**, *68*, 42–49, doi:10.1016/j.indcrop.2014.08.012.
10. El Haouari, M Rosado, J.A. Phytochemical, anti-diabetic and cardiovascular properties of *Urtica dioica* L. (Urticaceae): A review. *Mini-Reviews Med. Chem.* **2019**, *19*, 63–71.
11. Raimova, K. V.; Abdulladjanova, N.G.; Kurbanova, M.A.; Makhmanov, D.M.;

Kadirova, S.O.; Tashpulatov, F.N.; Juraev, S.S.; Matchanov, A.D.; Rakhimov, R.N. Comprehensive study of the chemical composition of *Urtica dioica* L. *J. Crit. Rev.* **2020**, *7*, 750–755, doi:10.31838/jcr.07.05.153.

12. Carvalho, A.R.; Costa, G.; Figueirinha, A.; Liberal, J.; Prior, J.A.V.; Lopes, M.C.; Cruz, M.T.; Batista, M.T. *Urtica spp*.: Phenolic composition, safety, antioxidant and anti-inflammatory activities. *Food Res. Int.* **2017**, *99*, 485–494, doi:10.1016/j.foodres.2017.06.008.
13. Gautam, V.K.; Datta, M.; Baldi, A. Effect of geographical and seasonal variations on phenolic contents and antioxidant activity of aerial parts of *Urtica diocia* L. *Curr. Tradit. Med.* **2019**, *5*, 159–167.
14. Dhouibi, R.; Affes, H.; Ben Salem, M.; Hammami, S.; Sahnoun, Z.; Zeghal, K.M.; Ksouda, K. Screening of pharmacological uses of *Urtica dioica* and others benefits. *Prog. Biophys. Mol. Biol*. **2020**, *150*, 67–77, doi:10.1016/j.pbiomolbio.2019.05.008.
15. Rietz, B.; Isensee, H.; Strobach, H.; Makdessi, S.; Jacob, R. Cardioprotective actions of wild garlic (*Anium ursinum*) in ischemia and reperfusion. *Mol. Cell. Biol*. **1993**, *119*, 143–150.
16. Djurdjevic, L.; Dinic, A.; Pavlovic, P.; Mitrovic, M.; Karadzic, B.; Tesevic, V. Allelopathic potential of *Allium ursinum* L. *Biochem. Syst. Ecol*. **2003**, *32*, 533–544, doi:10.1016/j.bse.2003.10.001.
17. Krivokapić, M.; Bradić, J.; Petković, A.; Popović, M. Phytochemical and Pharmacological Properties Of *Allium ursinum*. *Revijalni Rad.* **2018**, *1*, 1–6, doi:10.2478/SJECR.
18. Plant Lore - Gorse as horse fodder Available online: https://www.plant-lore.com/plantofthemonth/gorse-as-cattle-fodder/

19. Spínola, V.; Llorent-Martínez, E.J.; Gouveia-Figueira, S.; Castilho, P.C. *Ulex europaeus*: From noxious weed to source of valuable isoflavones and flavanones. *Ind. Crops Prod.* **2016**, *90*, 9–27, doi:10.1016/j.indcrop.2016.06.007.

20. Peng, F.; Du, Q.; Peng, C.; Wang, N.; Tang, H.; Xie, X.; Shen, J.; Chen, J. A review: The pharmacology of isoliquiritigenin. *Phyther. Res.* **2015**, *29*, 969–977, doi:10.1002/ptr.5348.

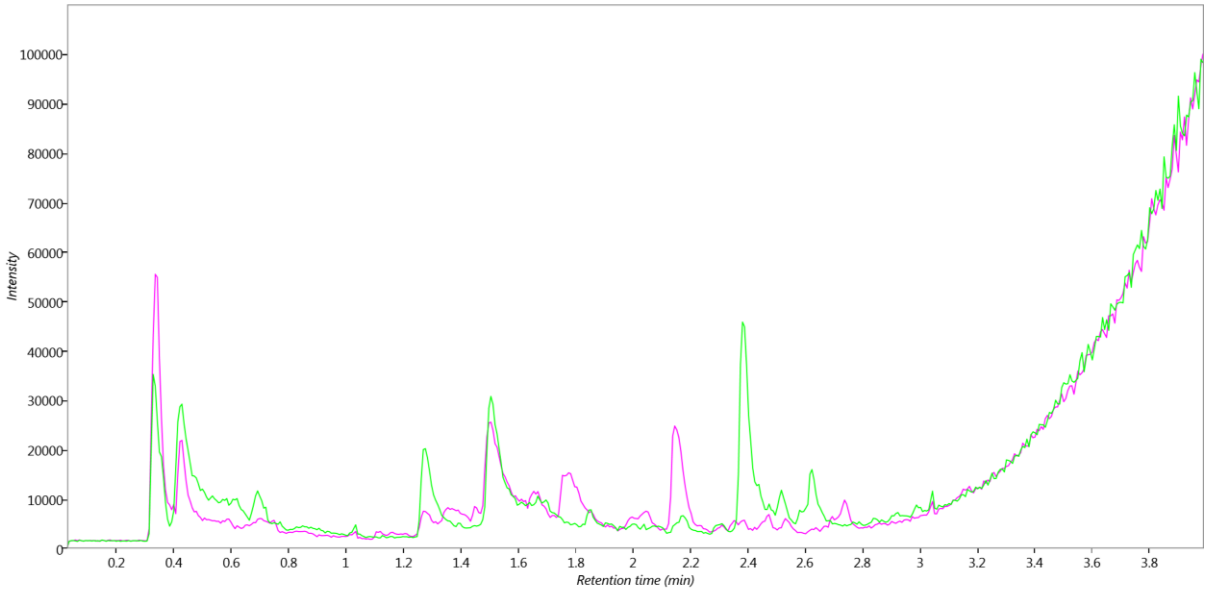


Figure S1. Total ion chromatogram of Alexanders (green) and the alignment reference (magenta).

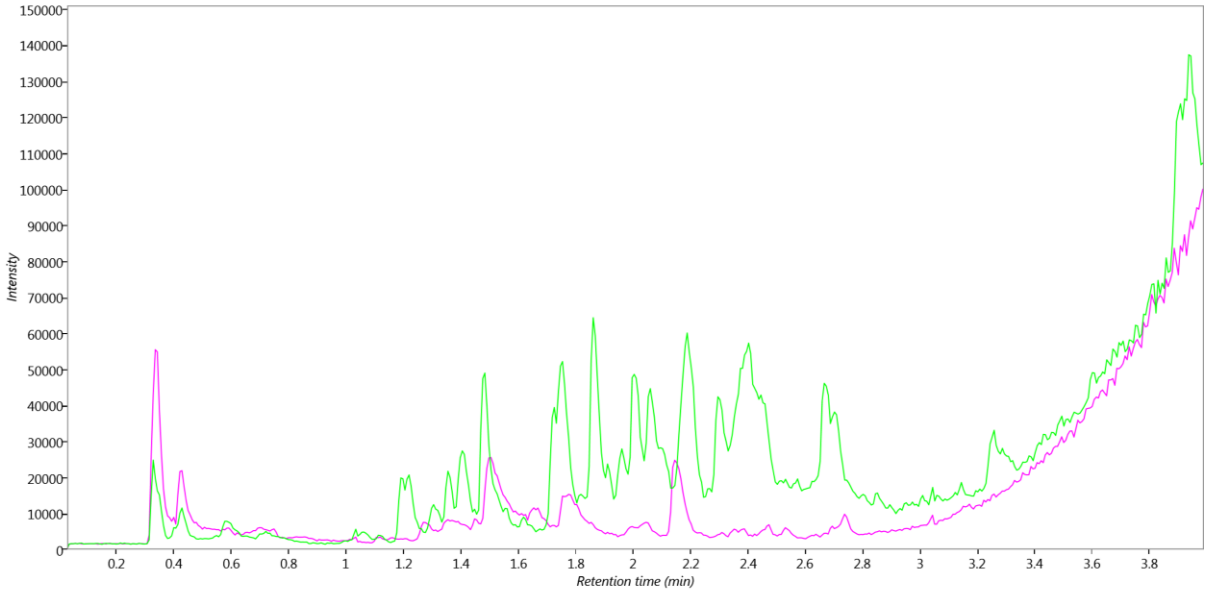


Figure S2. Total ion chromatogram of Gorse Flowers (green) and the alignment reference (magenta).

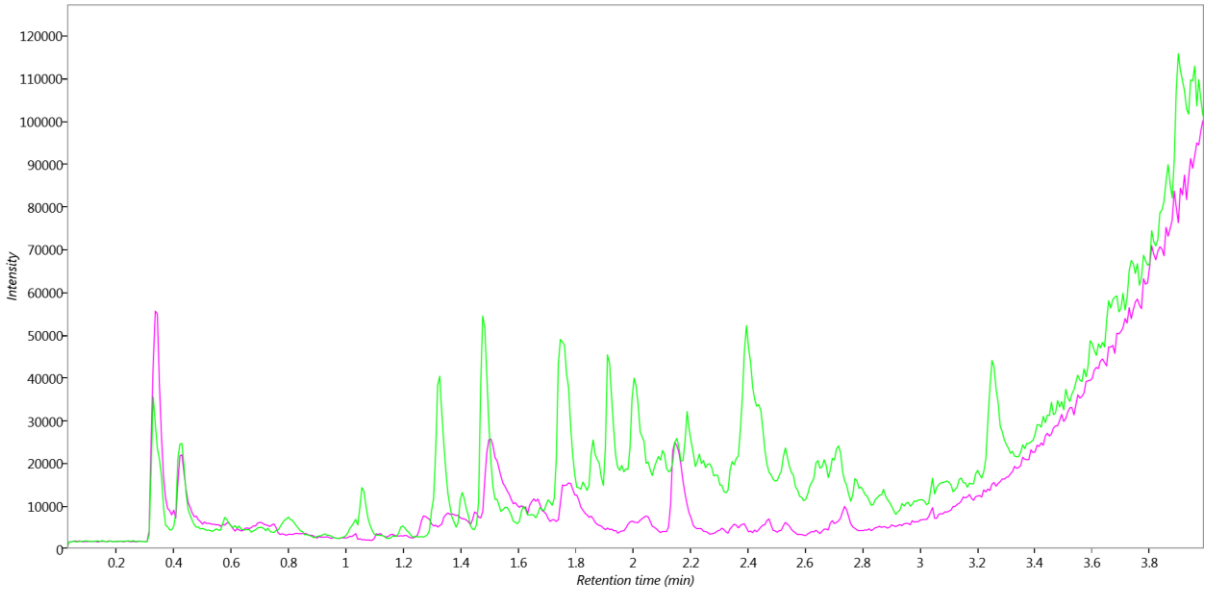


Figure S3. Total ion chromatogram of Gorse Stem (green) and the alignment reference (magenta).

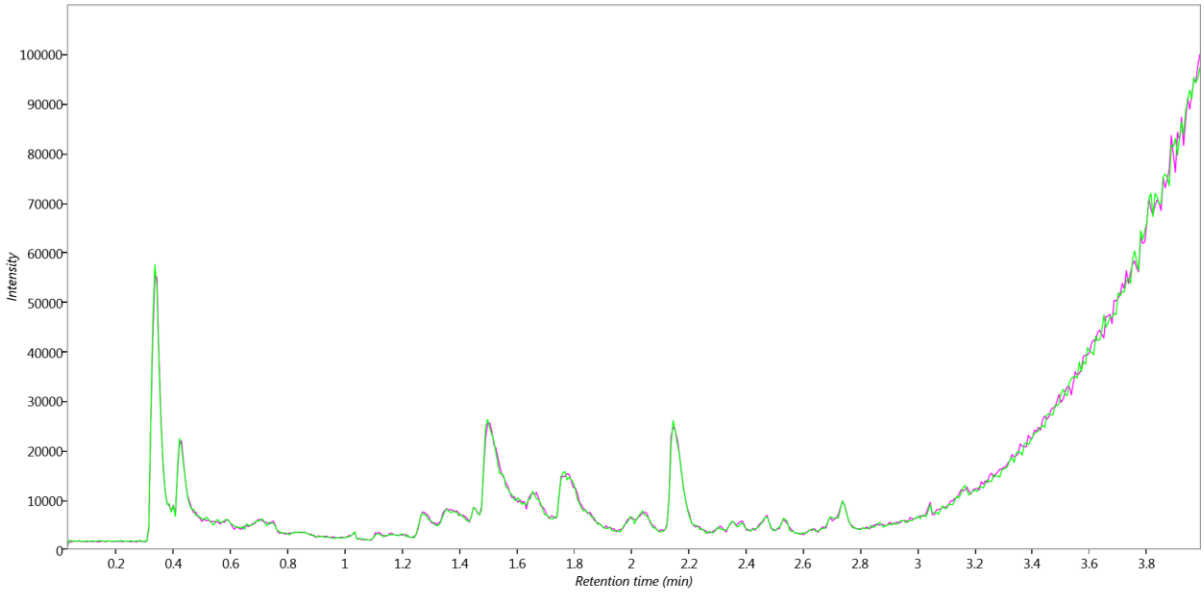


Figure S4. Total ion chromatogram of Nettle (green) and the alignment reference (magenta).

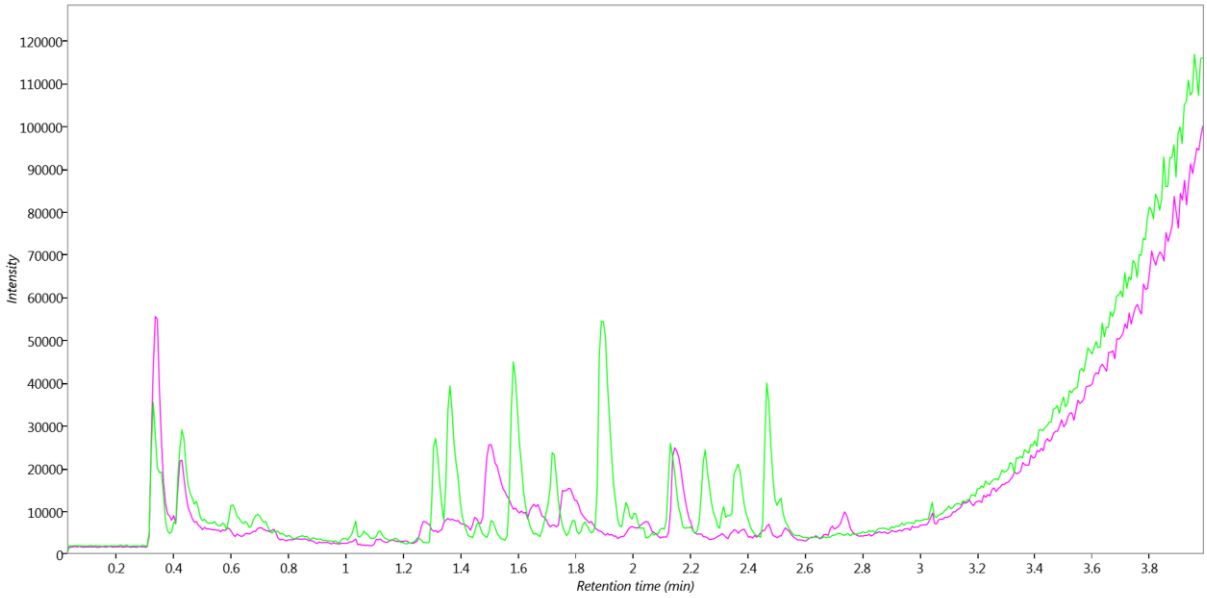


Figure S5. Total ion chromatogram of Wild Garlic Leaf (green) and the alignment reference (magenta).

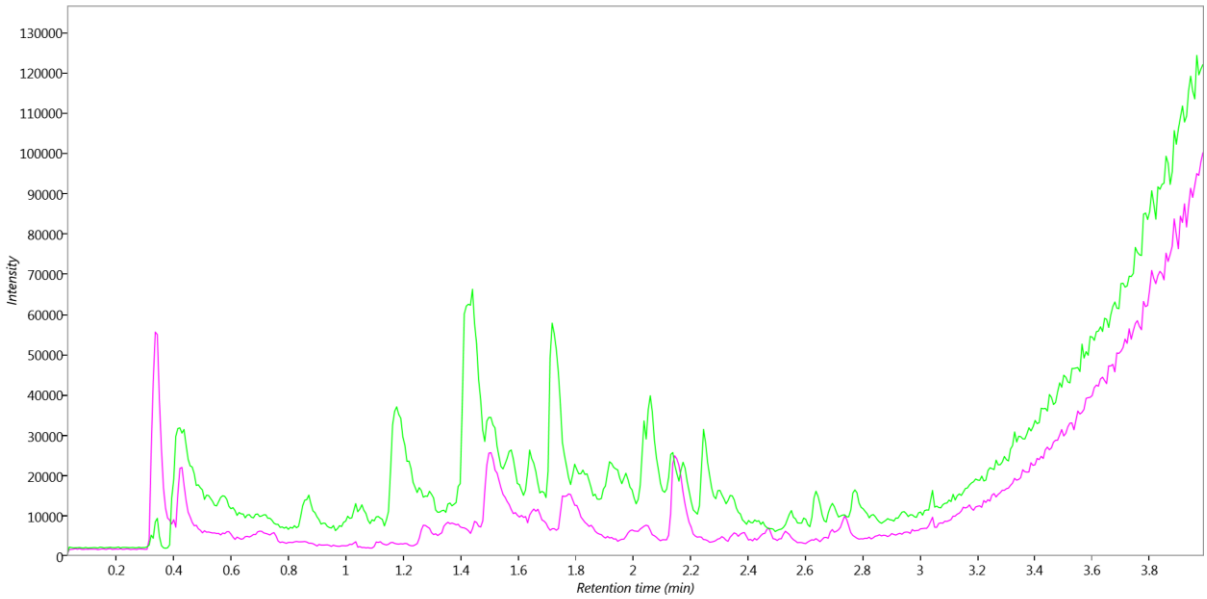
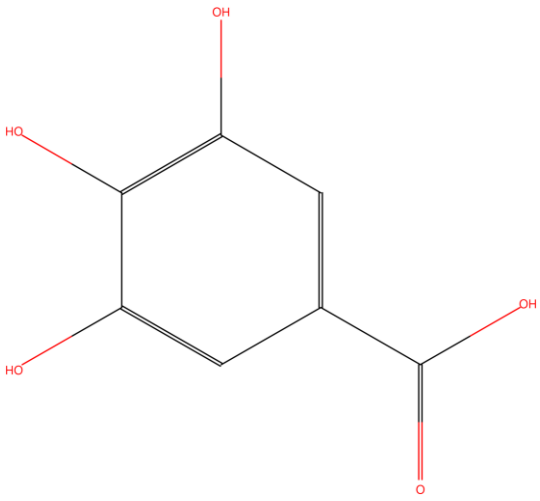
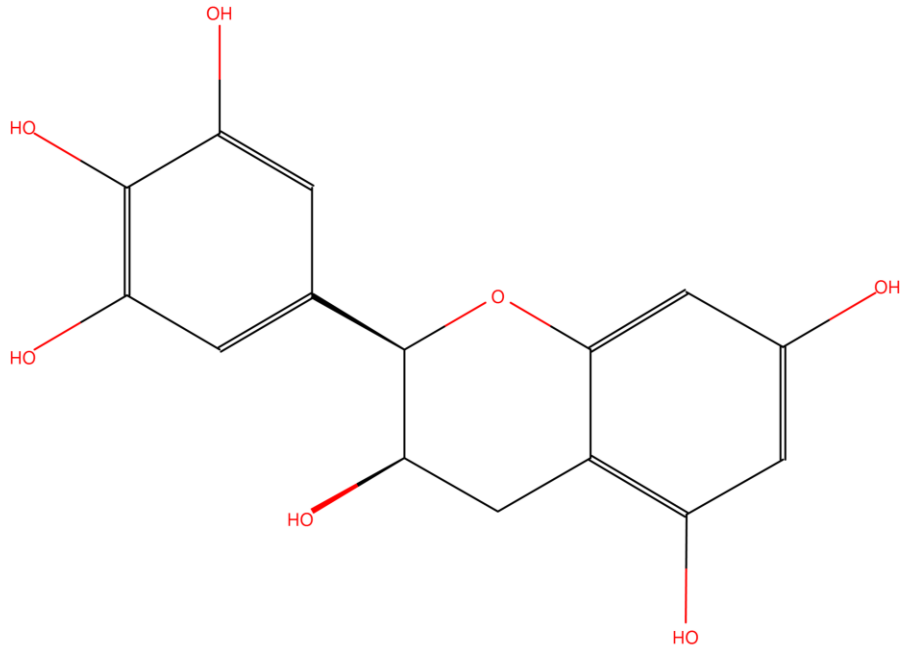


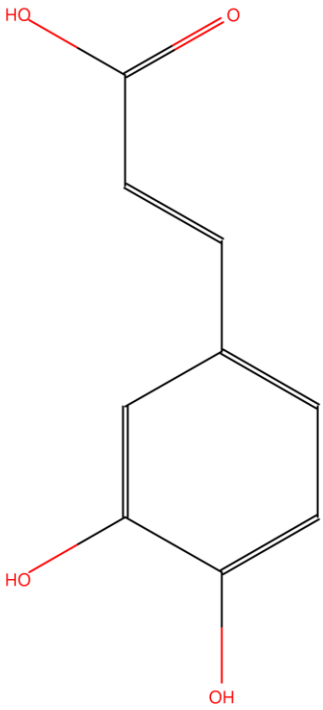
Figure S6. Total ion chromatogram of Green Tea (green) and the alignment reference (magenta).



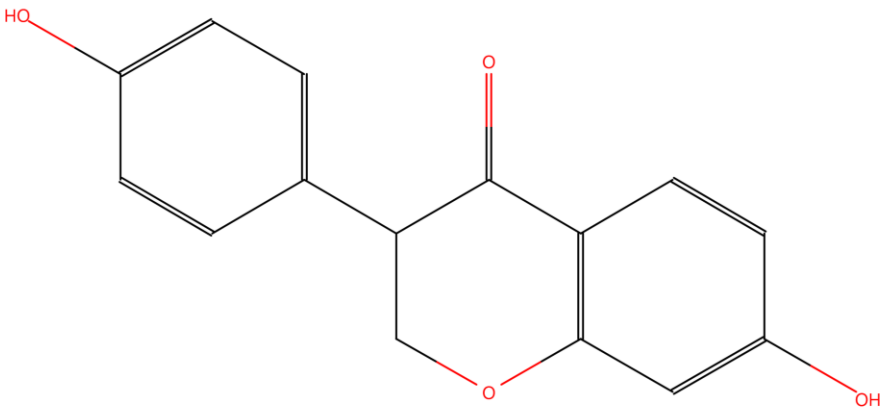
Gallic acid - CSID361



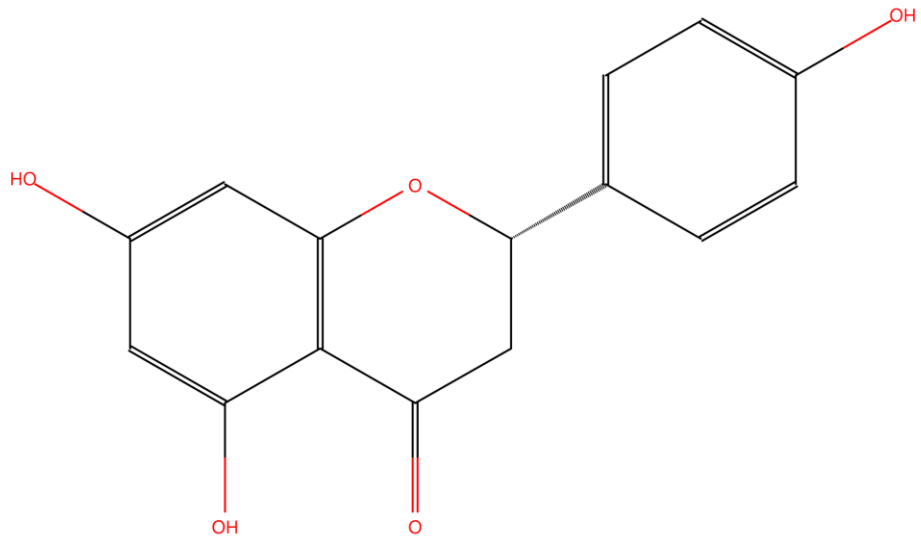
(-)-Epigallocatechin - CSID65231



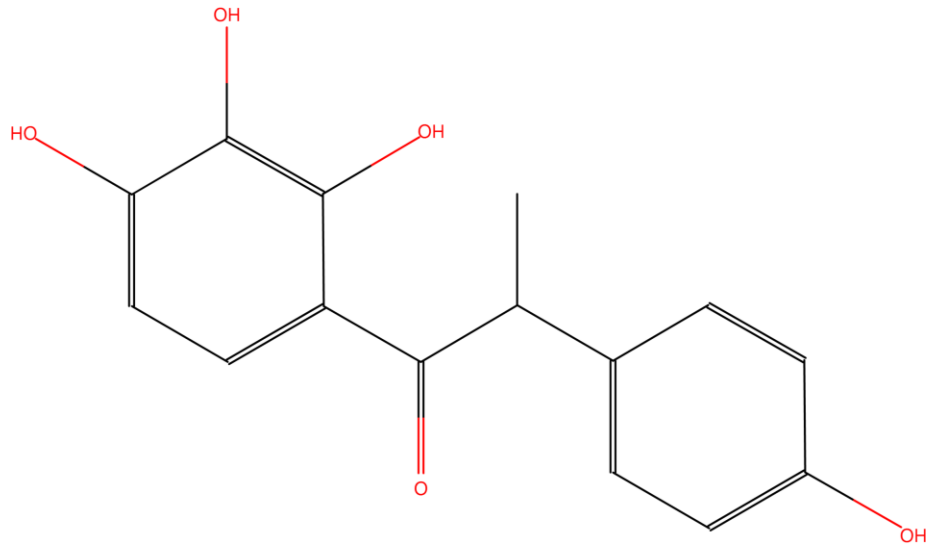
trans-Caffeic acid - CSID600426



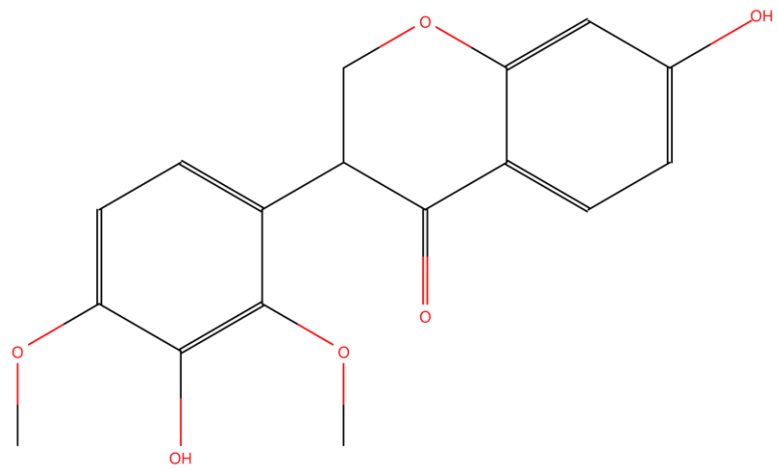
Dihydrodaidzein - CSID154076



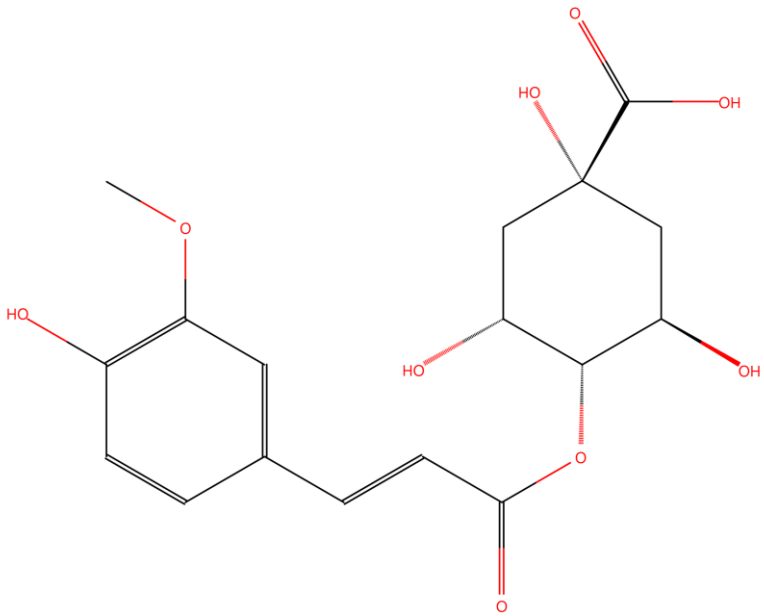
Naringenin – CSID388383



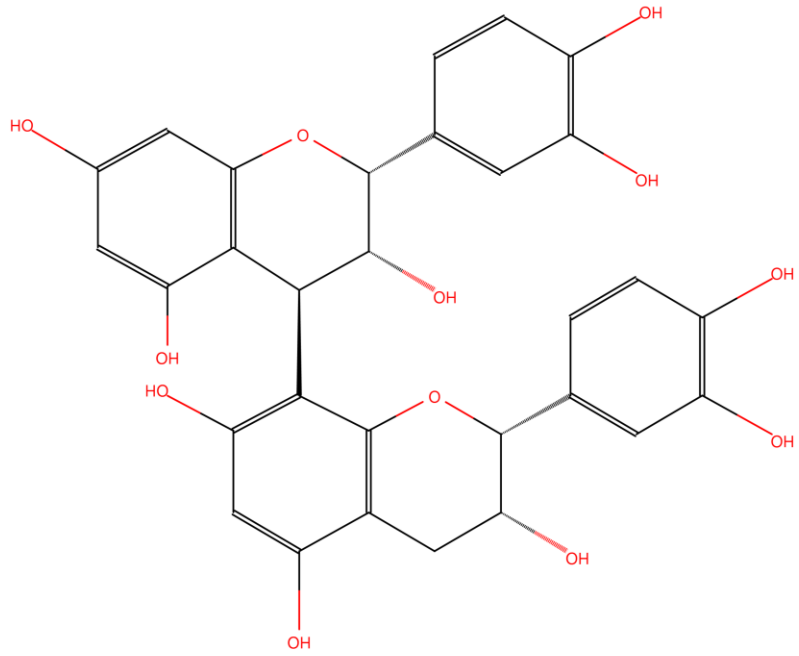
3'-Hydroxy-O-desmethylangolensin – CSID26369127



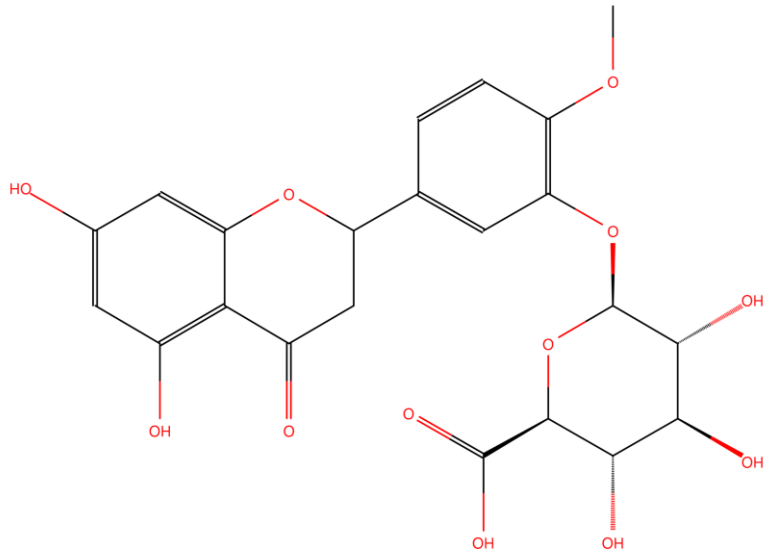
Violanone - CSID24842912



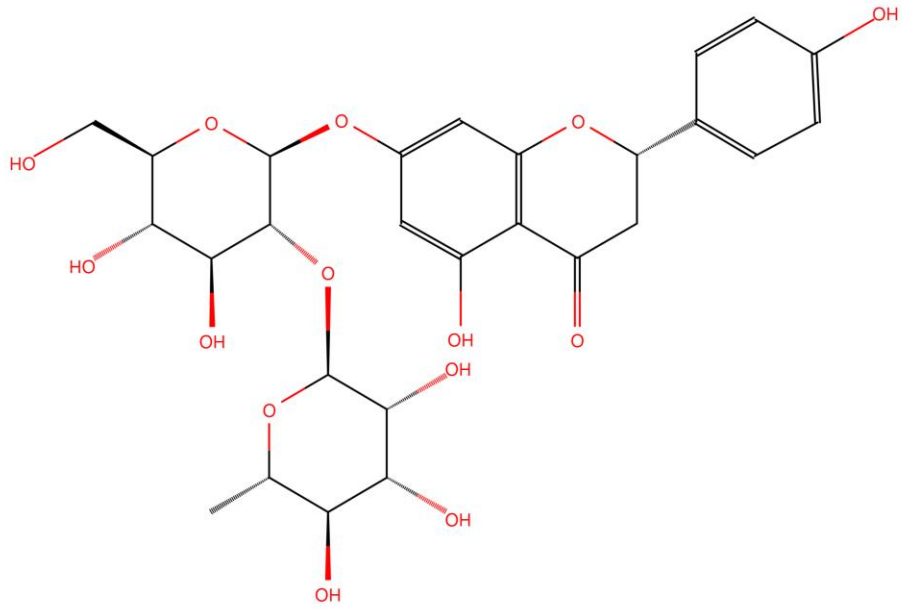
4-*O*-feruloyl-D-quinic acid - CSID10392638



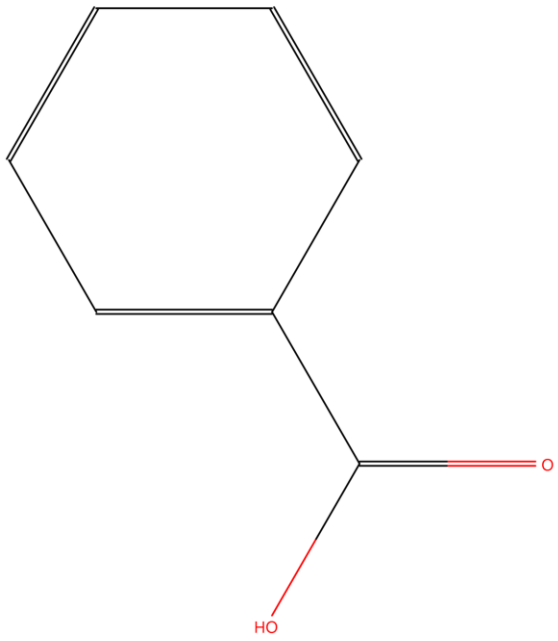
Procyanidin B2 – CSID109417 (given as an example of Procyanidin B1, B2, B3 or B4)



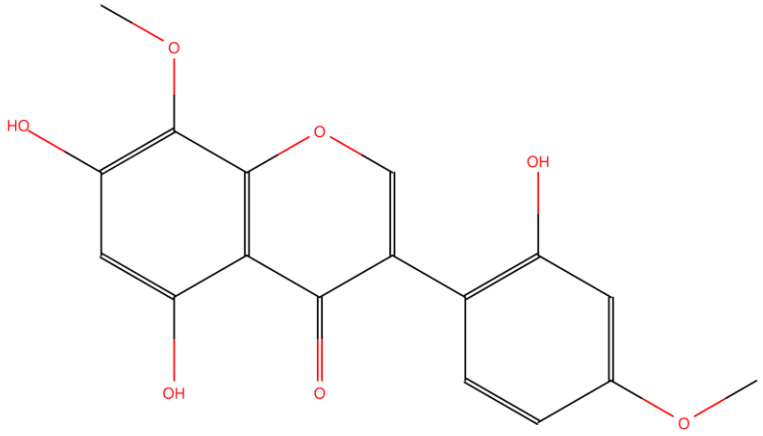
Hesperetin 3'-*O*-glucuronide – CSID32056973



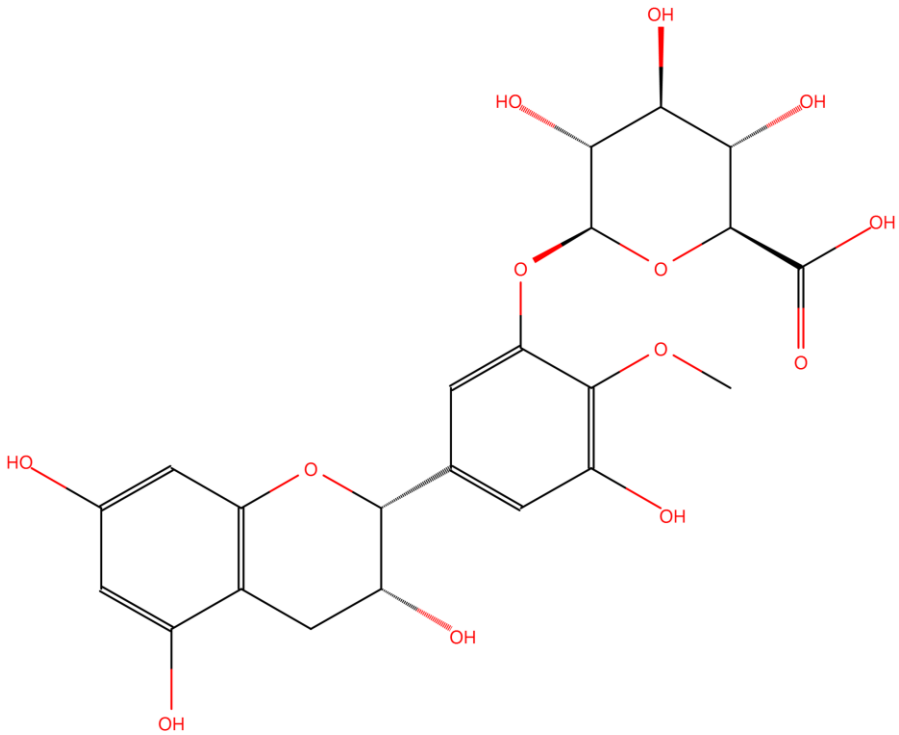
Naringen – CSID390868



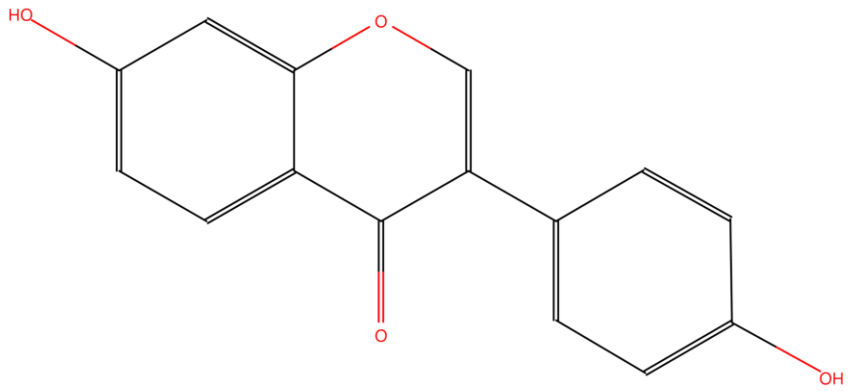
Benzoic acid – CSID238



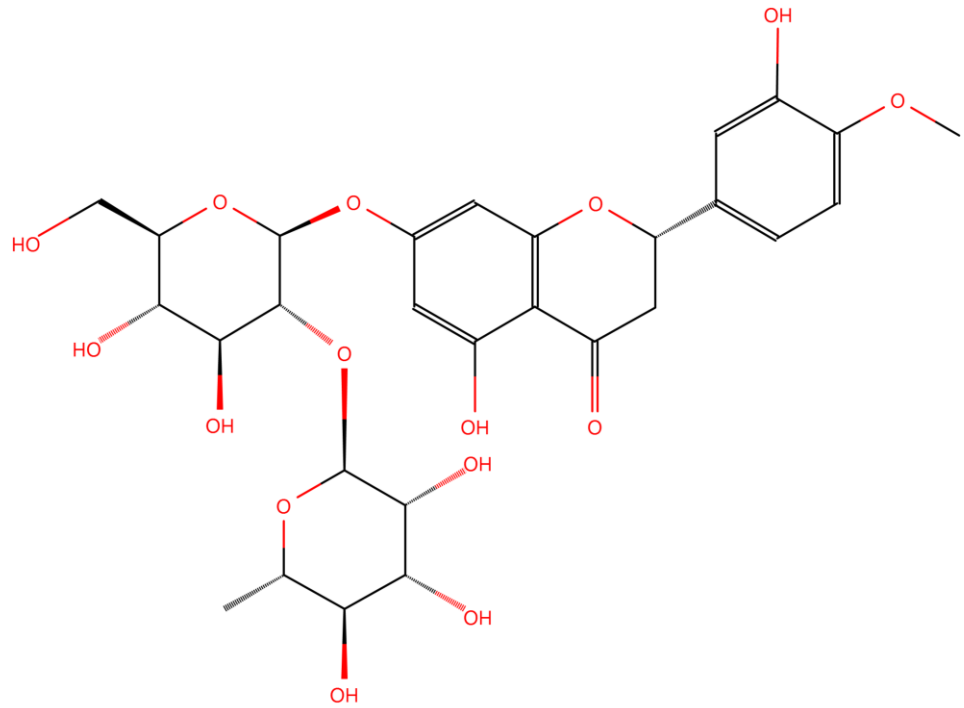
5,7-Dihydroxy-8,4'-dimethoxyisoflavone - CSID30777598



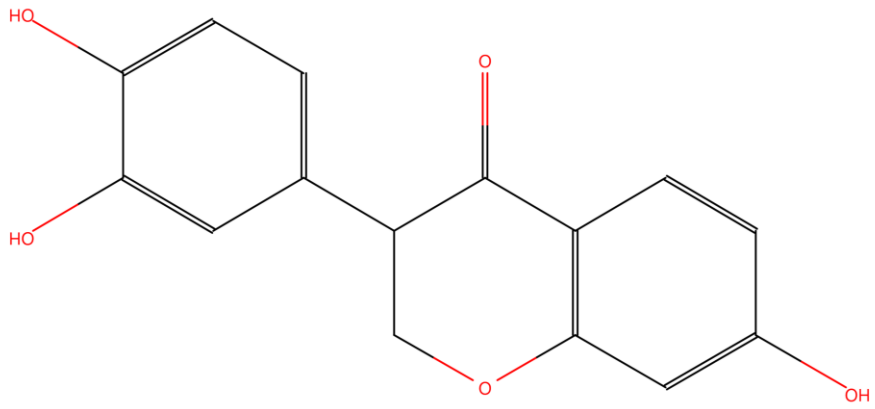
4'-Methyl-epigallocatechin-3'-glucuronide – CSID30777594



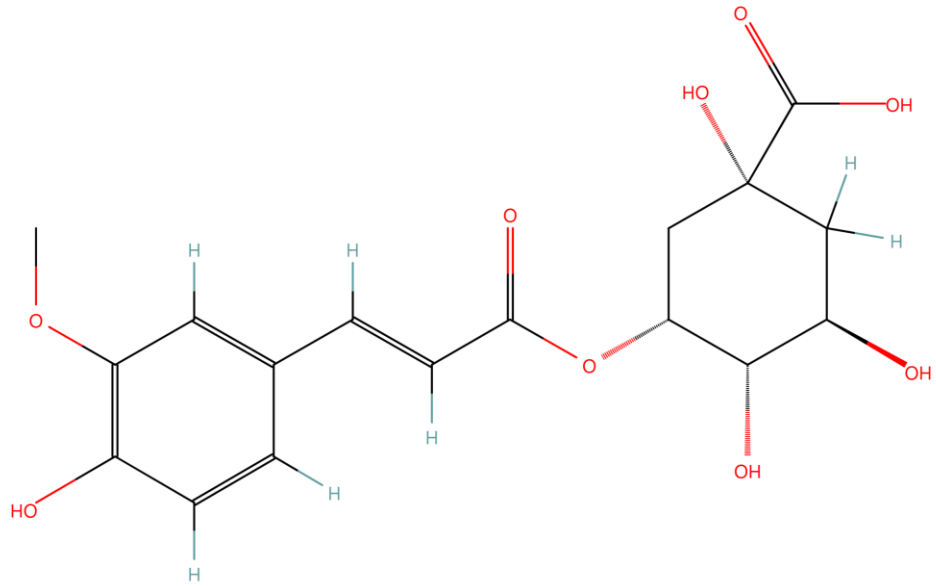
Daidzein - CSID4445025



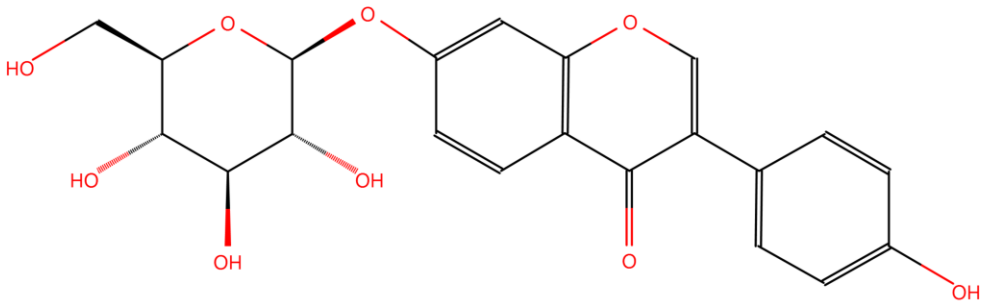
Neohesperidin - CSID390879



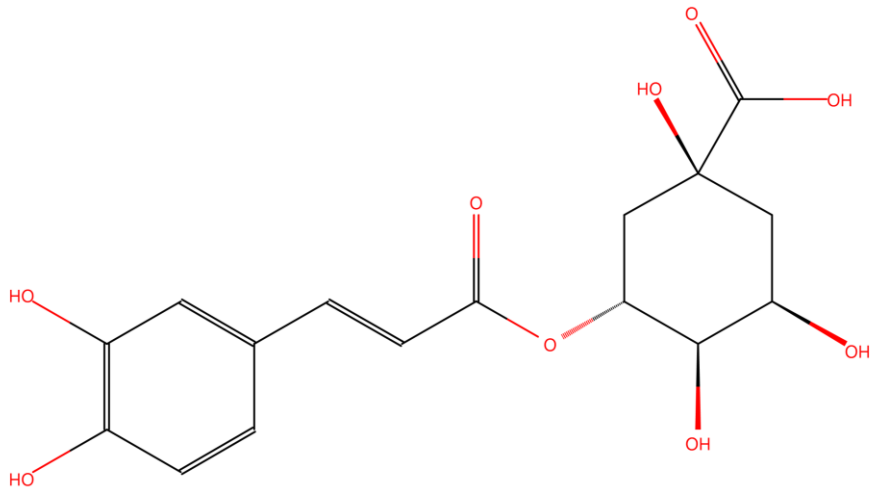
3',4',7-Trihydroxyisoflavanone – CSID10820100



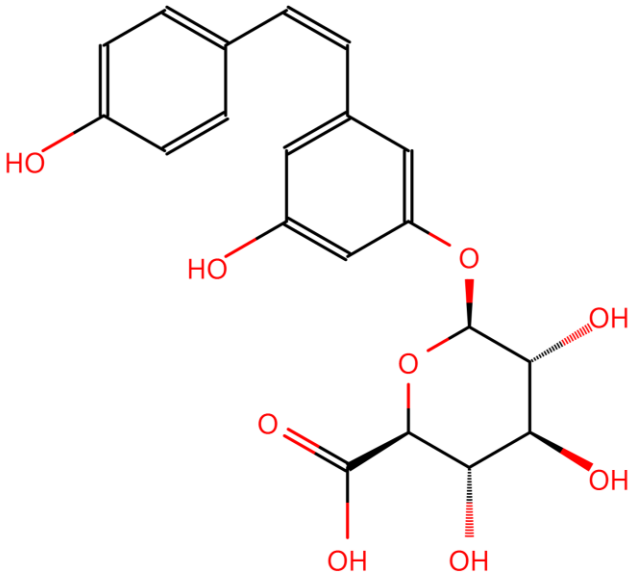
3-Feruloylquinic acid – CSID8309124



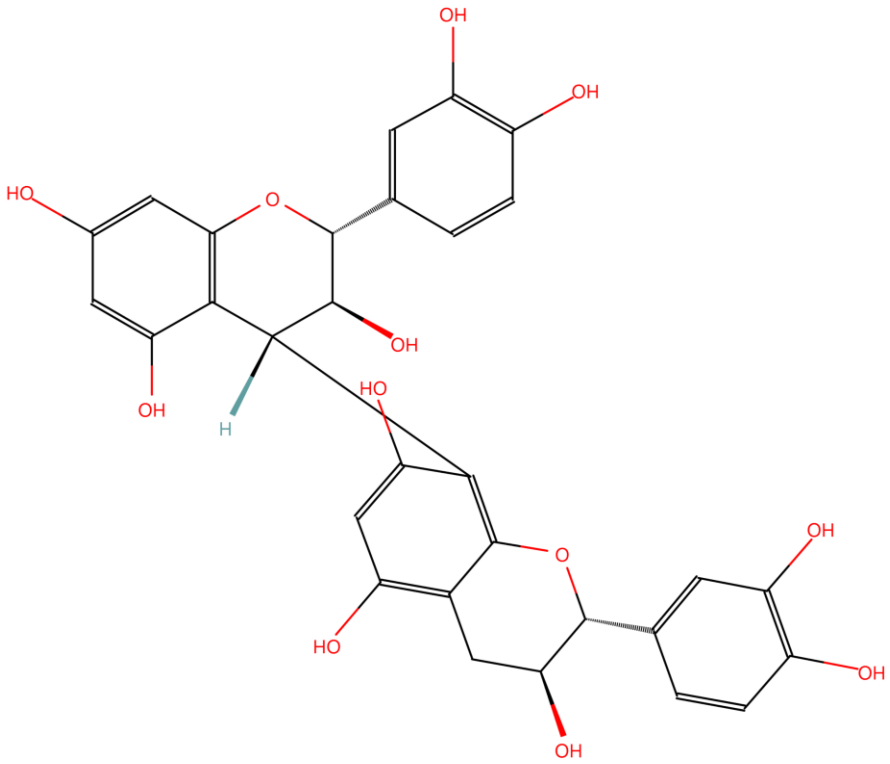
Daidzin - CSID97088



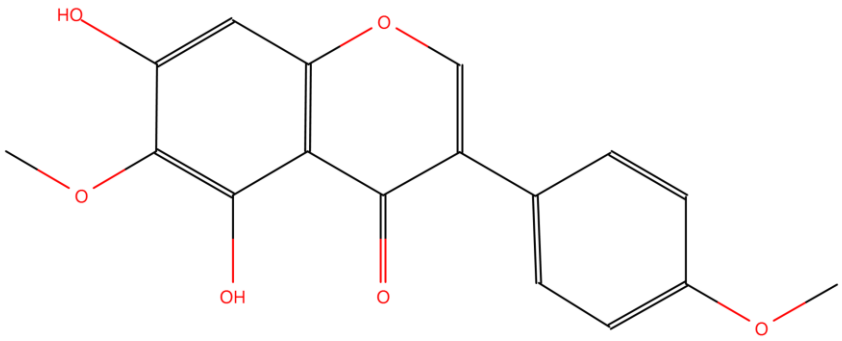
Chlorogenic acid - CSID1405788



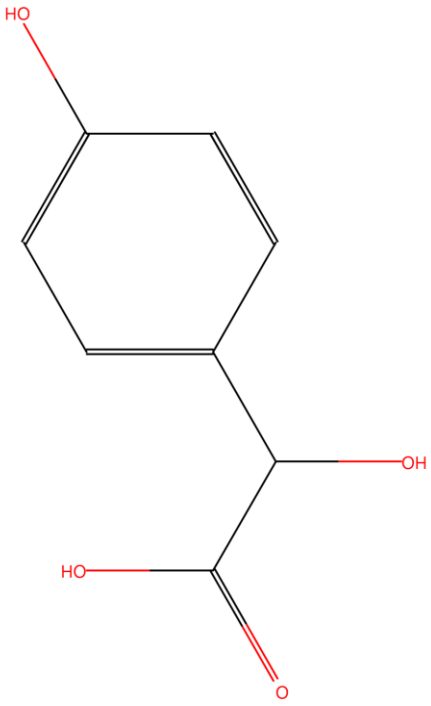
cis-Resveratrol 3-O-glucuronide – CSID30777610



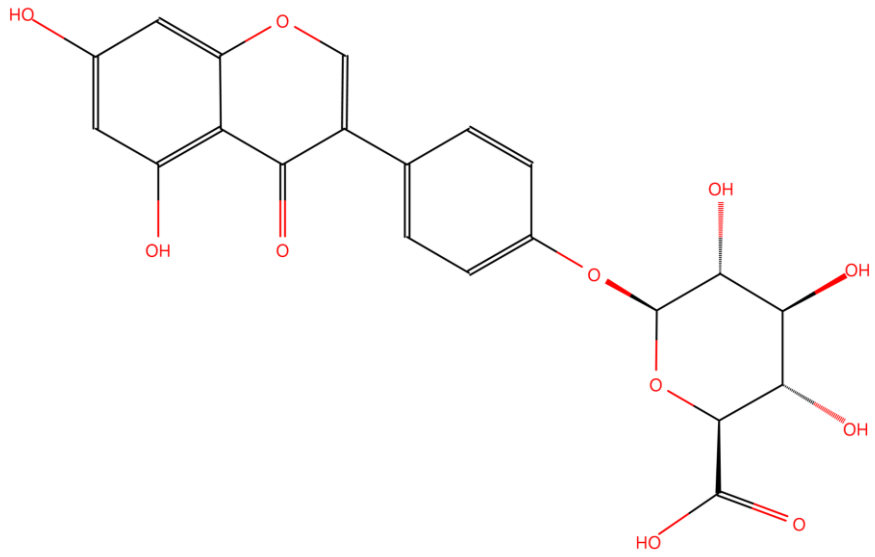
Procyanidin B3 - CSID129476 (given as an example of Procyanidin B1, B2, B3 or B4)



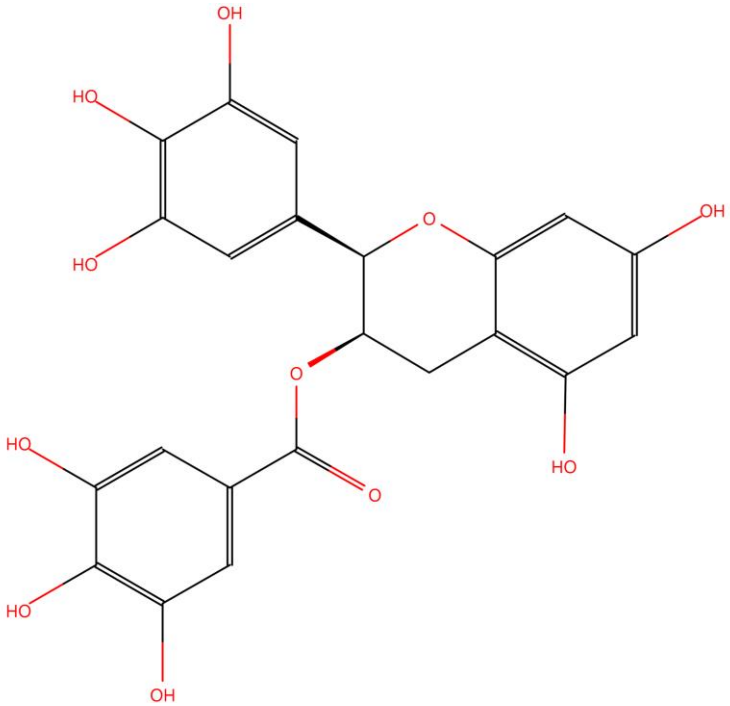
Irisolidone - CSID4445094



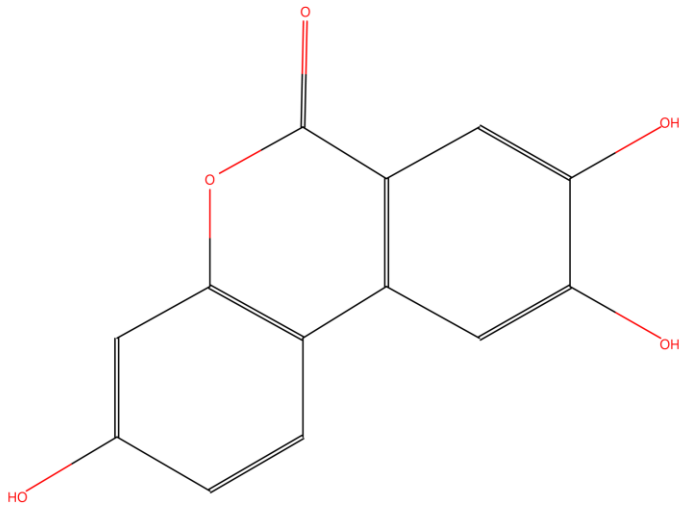
p-Hydroxymandelic acid – CSID321



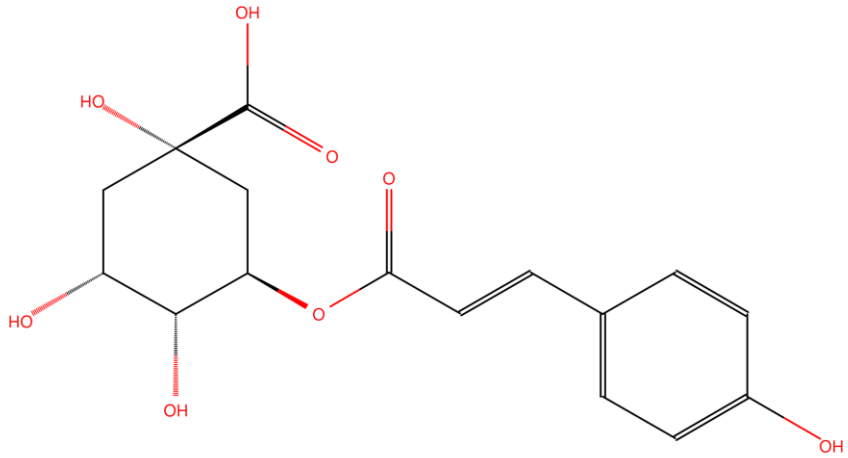
Genistein 4'-*O*-glucuronide - CSID29813968



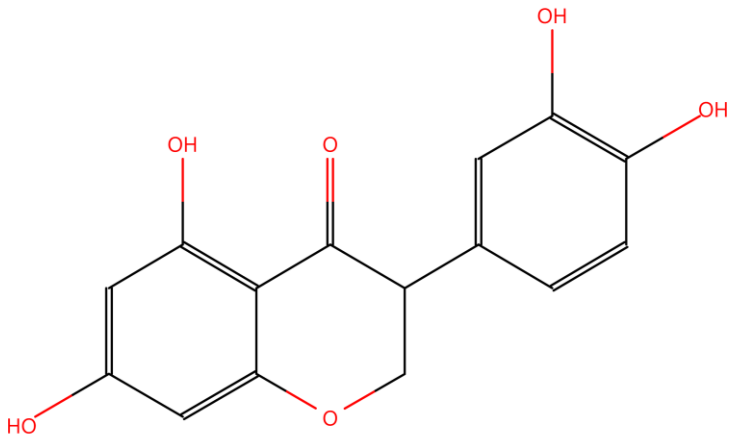
(-)-Epigallocatechin gallate – CSID58575



Urolithin C - CSID25941971

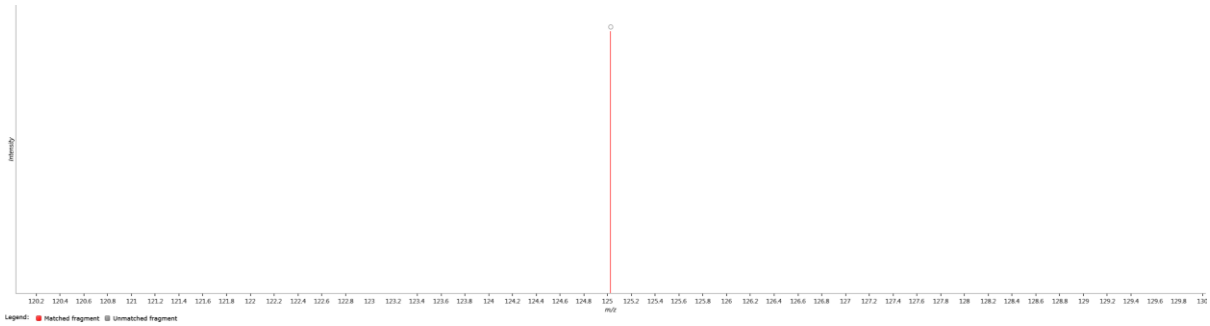


p-Coumaroyl quinic acid - CSID4945466

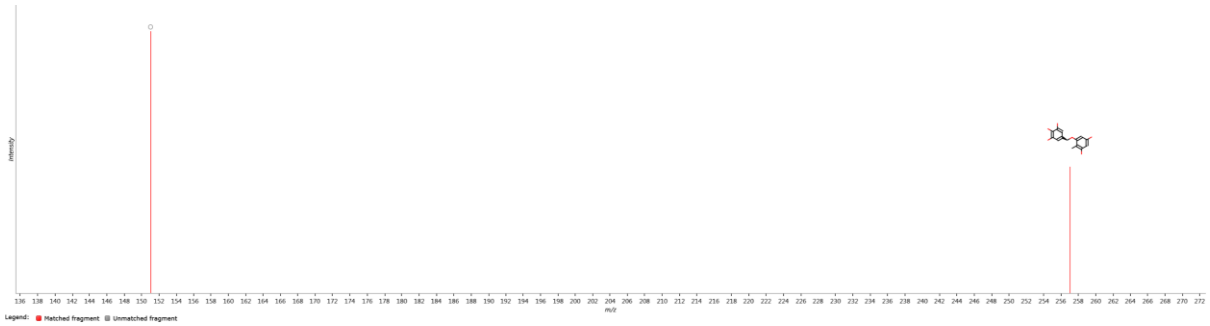


3',4',5,7-Tetrahydroxyisoflavanone - CSID35015212

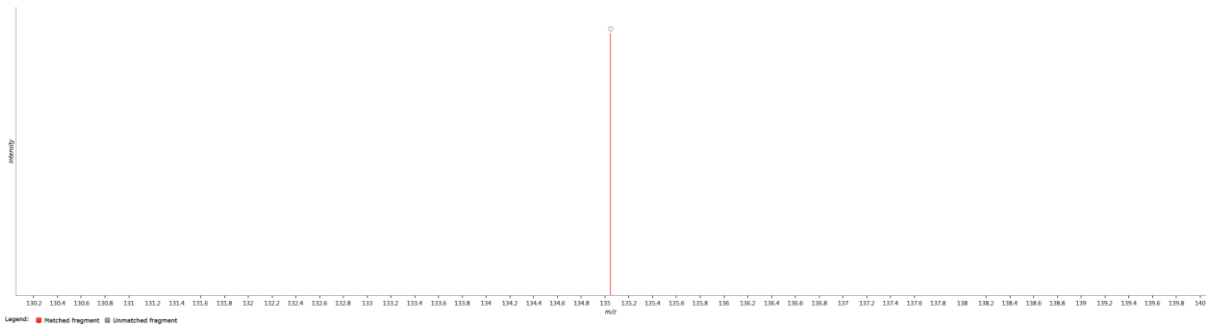
Figure S7. Molecular structures of identified polyphenolic components.



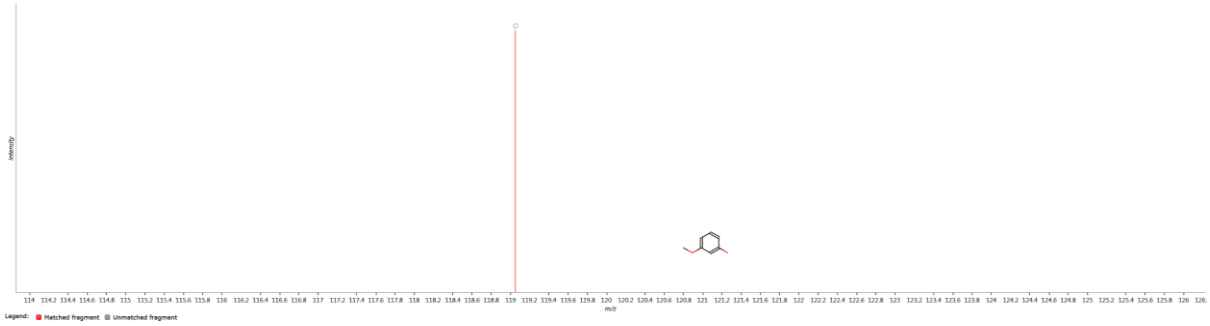
Fragmentation trace for compound 1.43min, 169.0140m/z



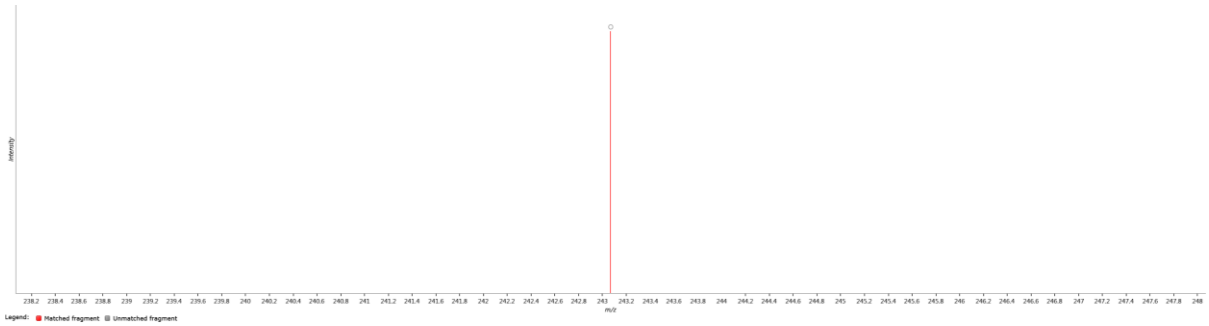
Fragmentation trace for compound 1.41min, 287.0562m/z



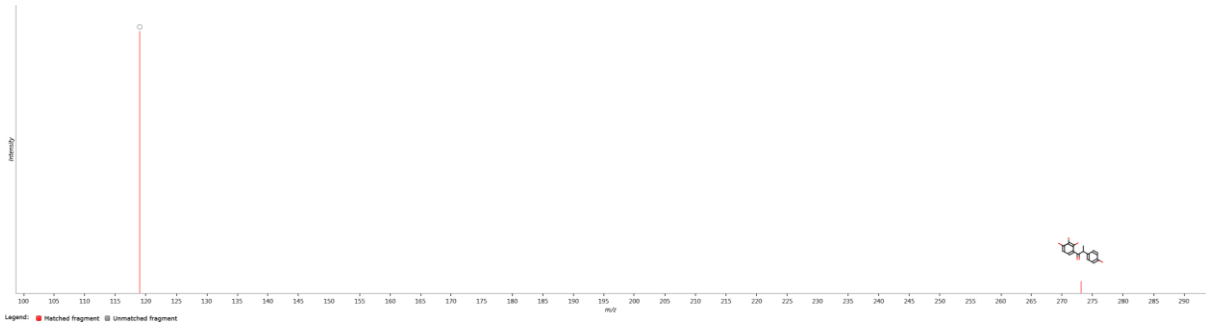
Fragmentation trace for compound 1.76min, 179.0343m/z



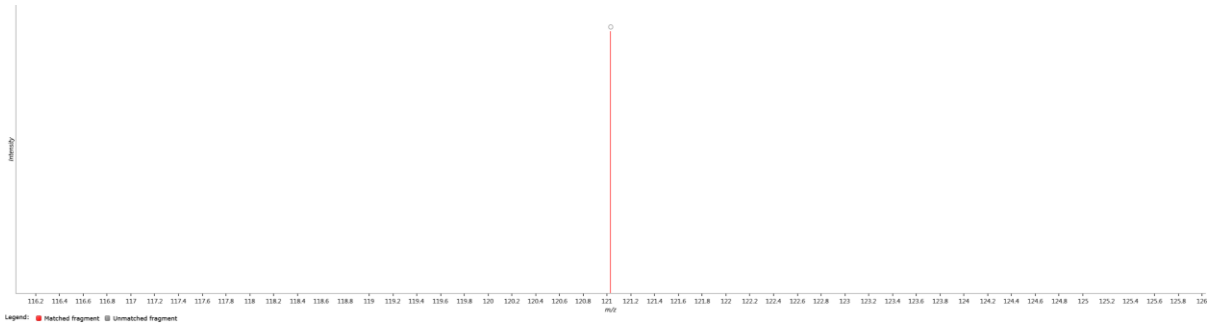
Fragmentation trace for compound 1.87min, 255.0663m/z



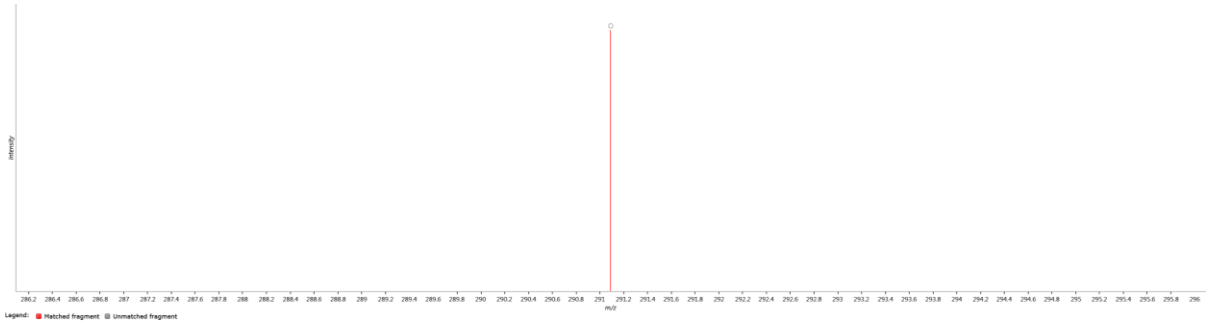
Fragmentation trace for compound 1.31min, 271.0617m/z



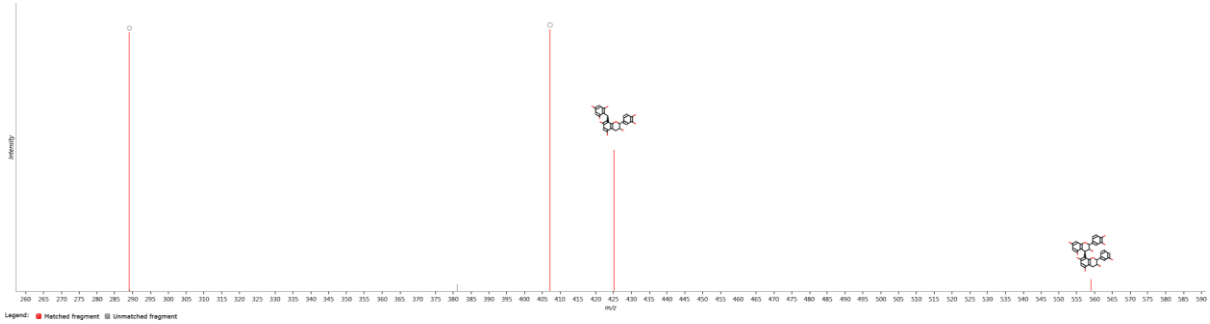
Fragmentation trace for compound 1.95min, 273.0770m/z



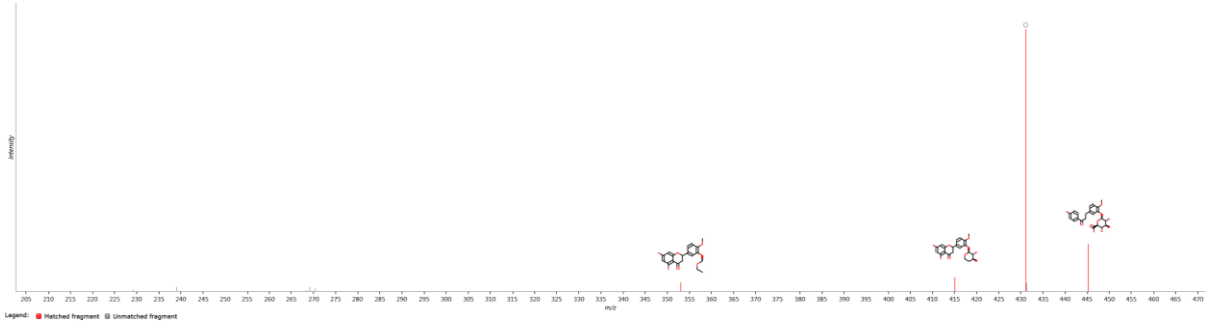
Fragmentation trace for compound 1.87min, 297.0759m/z



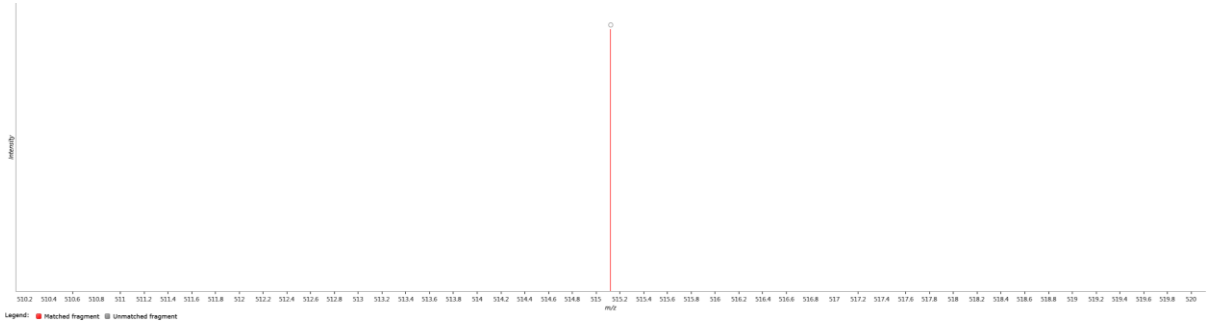
Fragmentation trace for compound 0.43min, 367.1050m/z



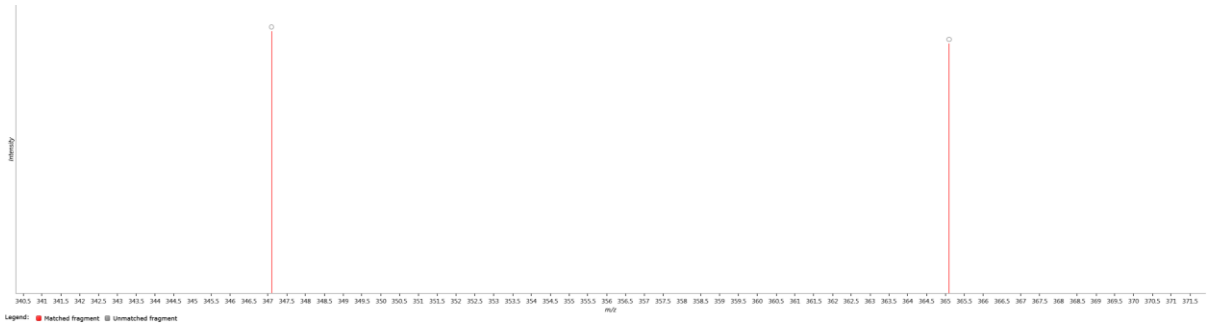
Fragmentation trace for compound 1.18min, 577.1376m/z



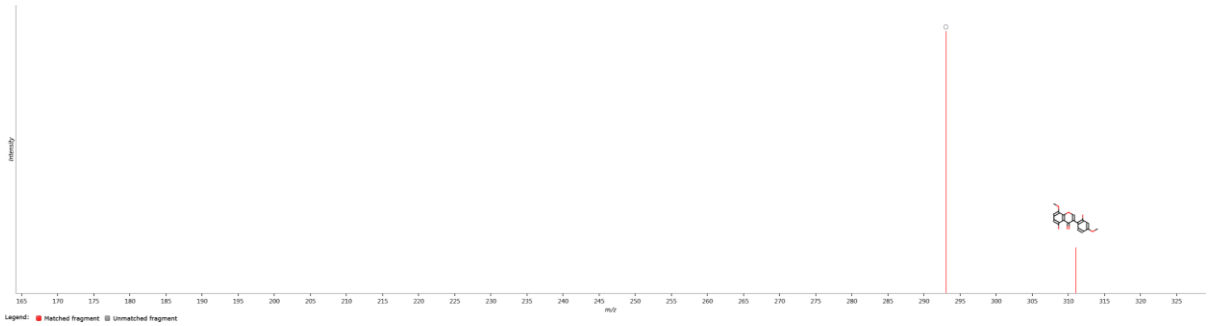
Fragmentation trace for compound 2.01min, 477.1044m/z



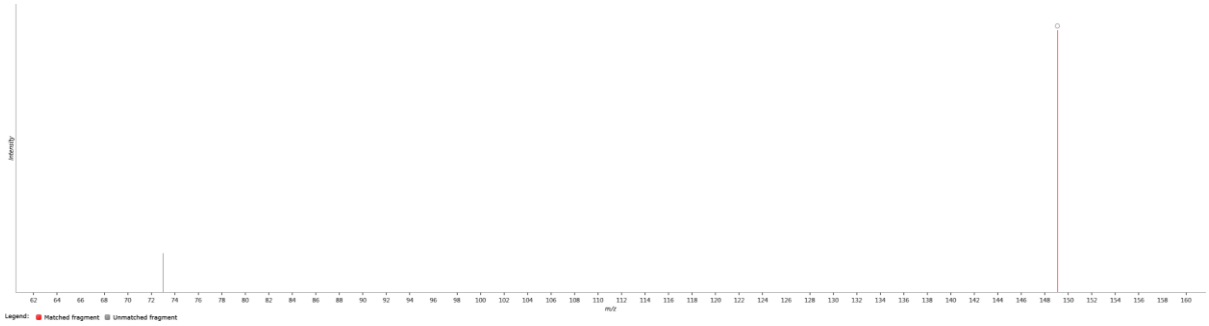
Fragmentation trace for compound 2.00min, 561.1596m/z



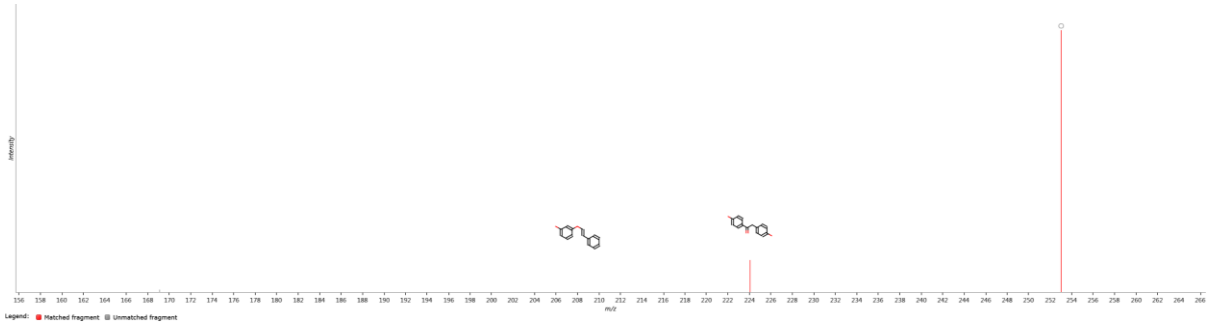
Fragmentation trace for compound 3.66min, 365.1031m/z



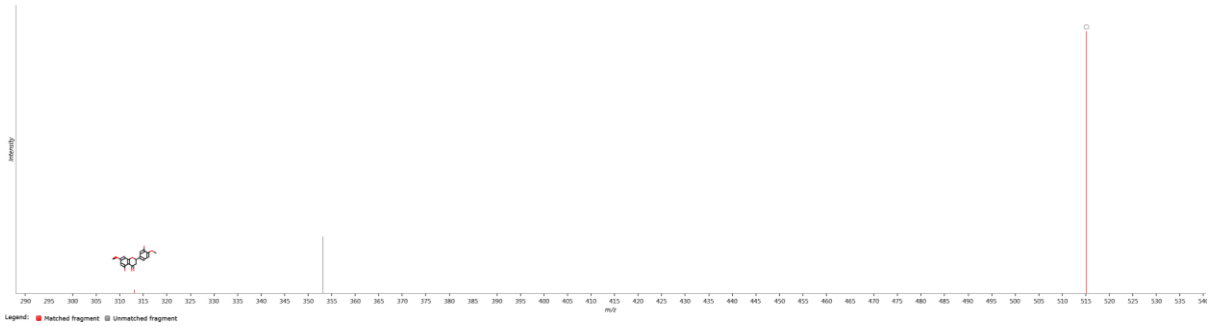
Fragmentation trace for compound 2.89min, 329.0660m/z



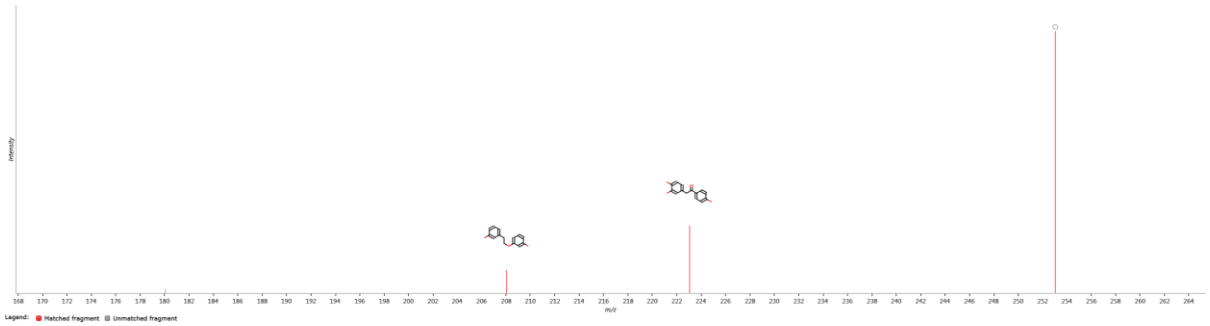
Fragmentation trace for compound 1.85min, 495.1132m/z



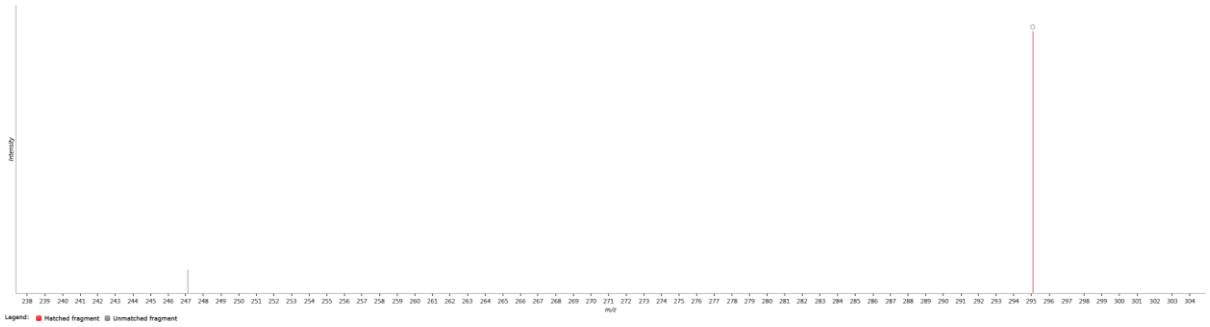
Fragmentation trace for compound 2.58min, 253.0515m/z



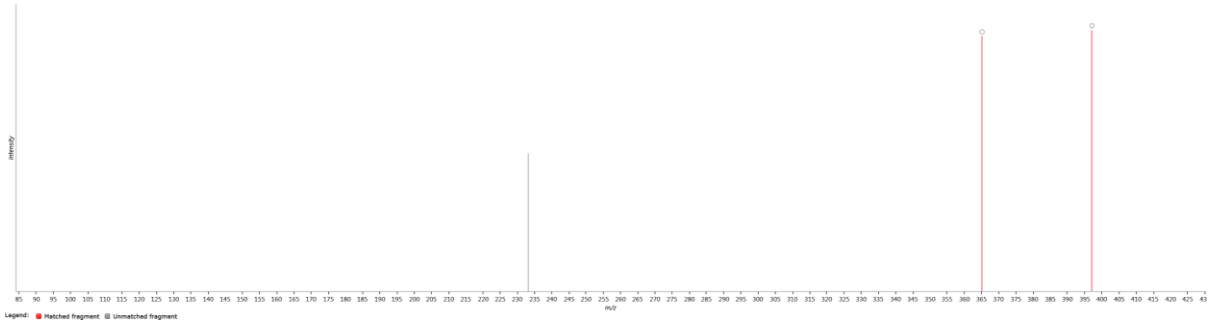
Fragmentation trace for compound 2.77min, 591.1716m/z



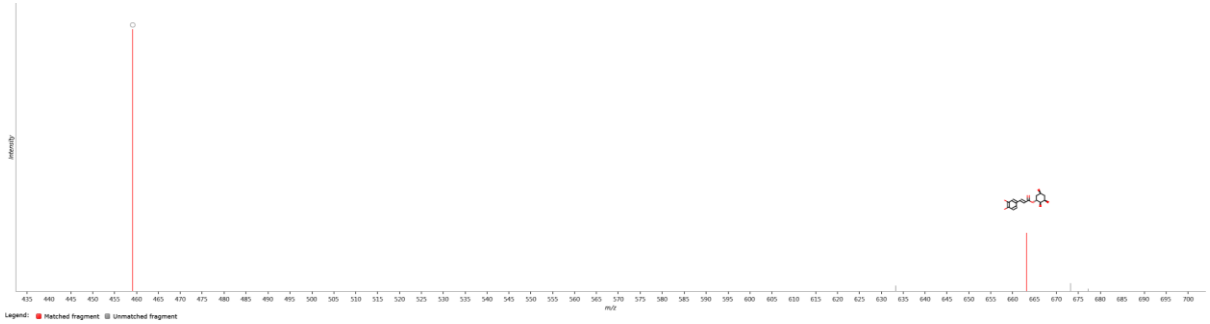
Fragmentation trace for compound 1.79min, 253.0518m/z



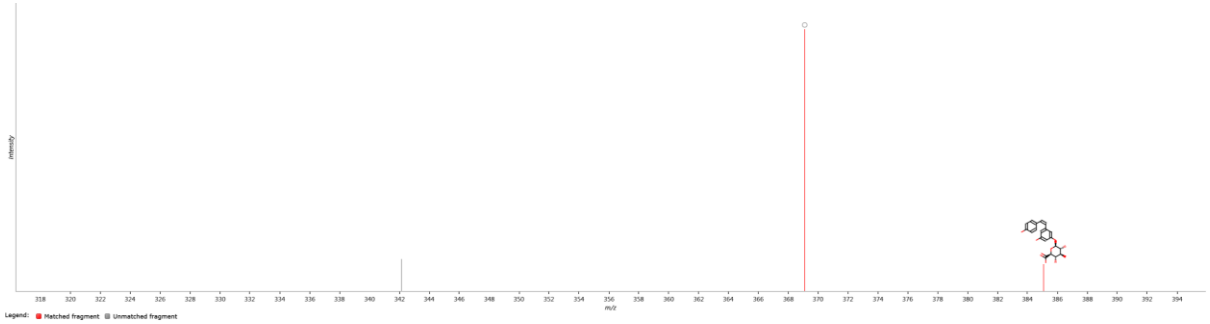
Fragmentation trace for compound 1.60min, 367.1024m/z



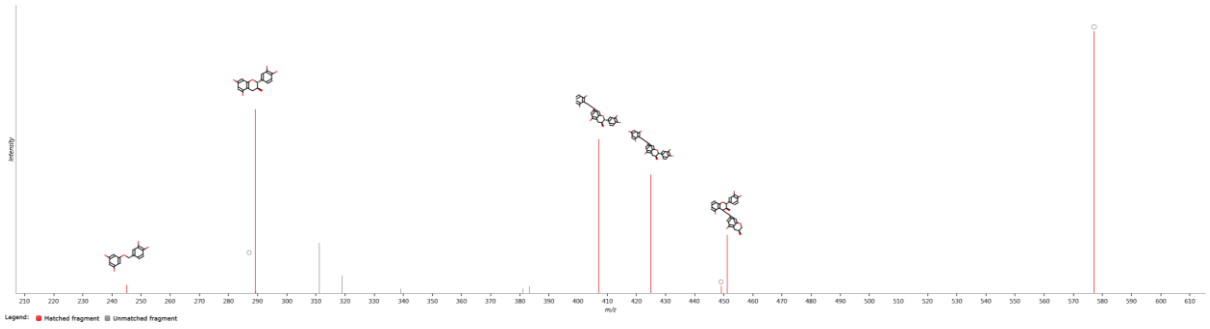
Fragmentation trace for compound 3.15min, 397.0933m/z



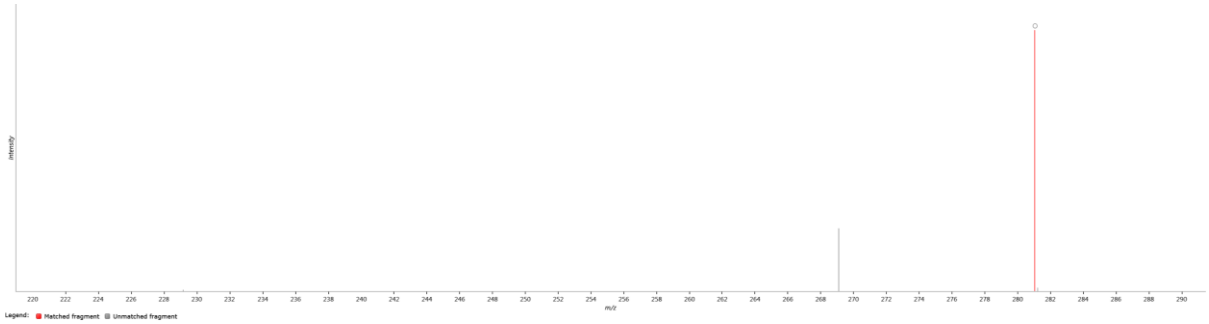
Fragmentation trace for compound 2.47min, 707.1802m/z



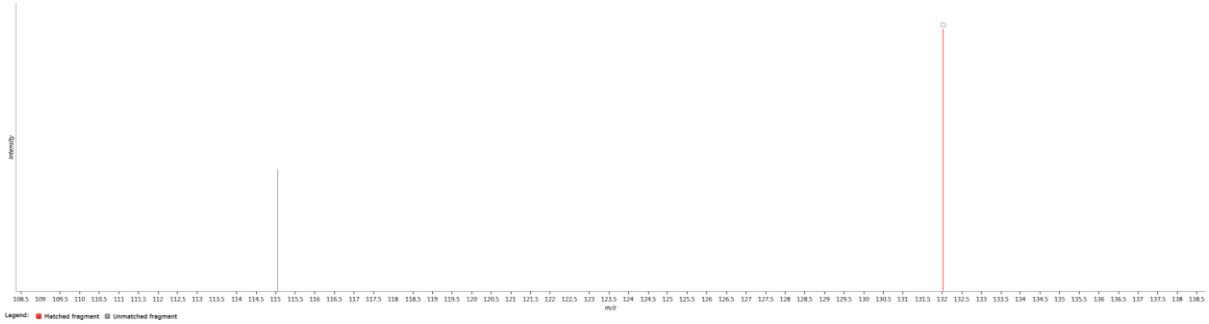
Fragmentation trace for compound 2.44min, 385.0926m/z



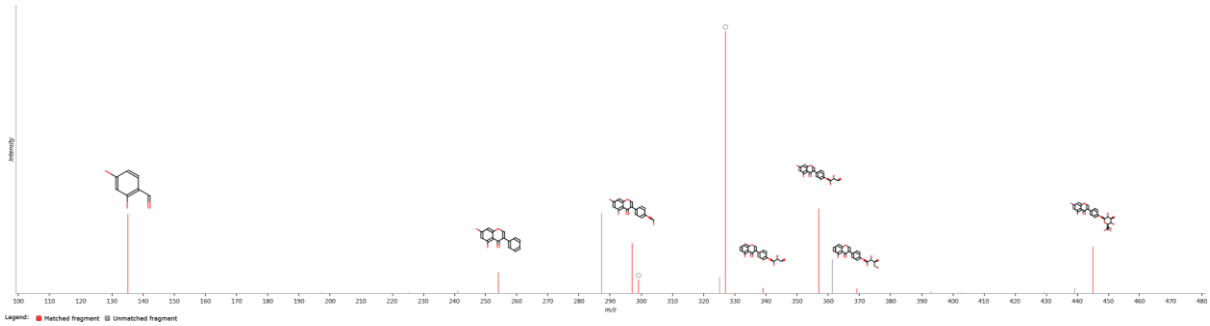
Fragmentation trace for compound 1.30min, 577.1370m/z



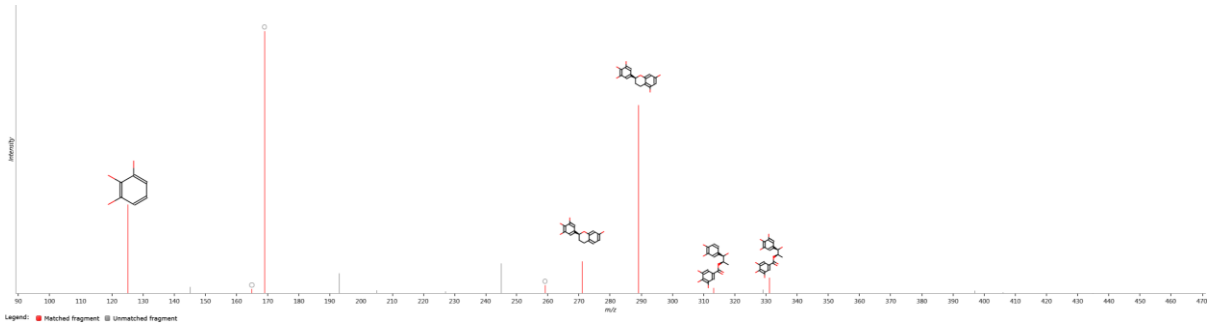
Fragmentation trace for compound 2.69min, 314.0786n



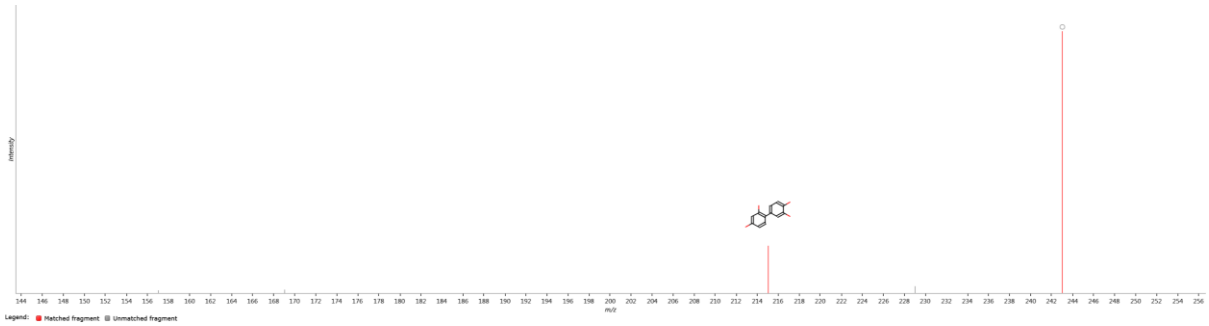
Fragmentation trace for compound 1.18min, 167.0344m/z



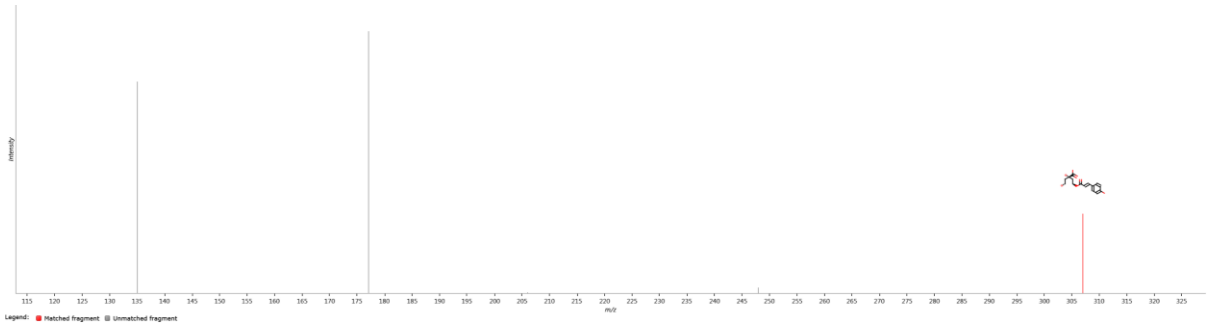
Fragmentation trace for compound 1.87min, 445.0779m/z



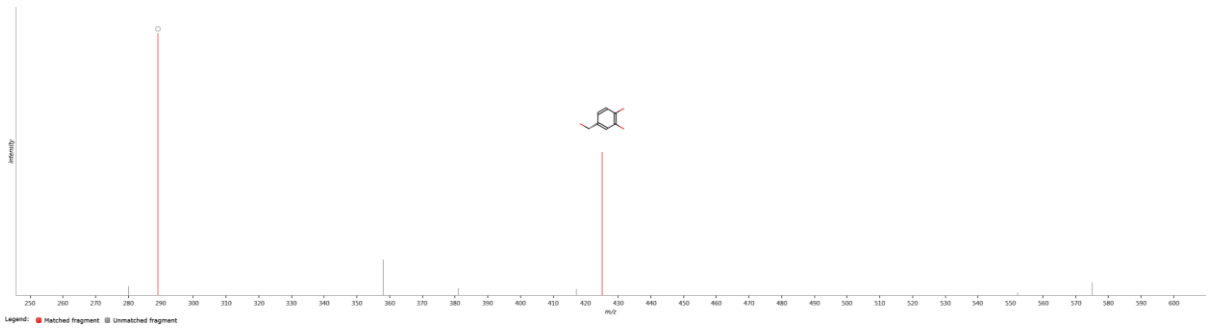
Fragmentation trace for compound 1.76min, 439.0679m/z



Fragmentation trace for compound 2.15min, 243.0288m/z



Fragmentation trace for compound 1.54min, 337.0923m/z



Fragmentation trace for compound 1.20min, 575.1184m/z

Figure S8. Example mass spectra of identifications ordered from high to low score value.

Figure S9. Abundance profile of (−)-epicatechin (CSID65230), *m/z* 289.0732, score 38.1

Figure S10. Alexanders, *Smyrnium olusatrum.*

Tato grasso [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0/)]

Figure S11. Stinging Nettle, *Urtica dioica.*

Skalle-Per Hedenhös [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)]

Figure S12. Wild garlic, *Allium ursinum.*

Kurt Stüber [1] [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0/)]

Figure S13. Gorse, *Ulex europaeus.*

PaleCloudedWhite [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)]