Supplementary material for:

Current approaches and challenges for the metabolite profiling of complex natural extracts

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Table S1. The databases used for MS-based dereplication. Some of these databases are interconnected; hence, a search conducted in one database can provide results from another database. However, to our knowledge, no exhaustive search engine exists.

Name	**Type of NP**	**Number of entries**	**Complementary information**					**Simulated data**	**SOAP or API for automated search**
			MS^2 spectra	**UV spectra**	**NMR spectra**	**Biological sources**	**Pathway**		
AntiBase [1]	Secondary metabolites	42,000	Yes	Yes	^{13}C	Yes	No	No	No
ChEBI [2,3]	Primary and secondary metabolites	32,000	No	No	No	Yes	No	No	Yes
Dictionary of Marine Natural Products [4]	Marine secondary metabolites	30,000	No	Yes	No	Yes	No	No	No
Dictionary of Natural Product [5]	Secondary metabolites	260,000	No	Yes	No	Yes	No	No	No
E. coli Metabolome Database (ECMDB) [6]	Primary and secondary metabolites	3,000	Yes	No	Yes	Yes	Yes	No	No
Golm Metabolome [7]	Primary and secondary metabolites	2,200	No	No	No	Yes	No	No	Yes
Human Metabolome database (HMDB) [8]	Primary metabolites and lipids	42,000	Yes	No	No	Yes	No	No	Yes
KNApSAcK [9]	Secondary metabolites	51,000	No	No	No	Yes	No	No	Yes
Kyoto Encyclopedia of Genes and Genomes (KEGG) [10]	Primary and secondary metabolites	17,000	No	No	No	Yes	Yes	No	Yes
Lipid Maps [11,12]	Lipids	38,000	Yes	No	No	No	Yes	No	Yes
LipidBlast [13]	Lipids	119,000	Yes	No	No	No	No	Yes	No
Madison Metabolomics Consortium Database [14]	Primary and secondary metabolites	20,000	No	No	Yes	Yes	No	No	No
MarinLit [15]	Secondary metabolites	5,000	No	Yes	^{1}H	Yes	No	No	No
MassBank [16]	Primary and secondary metabolites	15,000	Yes	No	No	No	No	No	Yes
MetaboLights [17,18]	Primary and secondary metabolites	11,000	Yes	No	Yes	Yes	Yes	No	No
MetaCyc [19]	Primary and secondary metabolites	11,000	No	No	No	Yes	Yes	No	Yes
MetLin [20]	Primary and secondary metabolites	240,000	Yes	No	No	No	No	No	No
MFSearcher [21]	Primary and secondary metabolites with a focus on flavonoids	-	No	No	No	No	No	No	Yes
MZedDB [22]	Primary and secondary metabolites	-	No	No	No	No	No	No	Yes
NIST12	Primary and secondary metabolites	7,000	Yes	No	No	No	No	No	No
Plant Metabolic Network (PMN) [23]	Primary and secondary metabolites	4,000	No	No	No	No	Yes	No	Yes
Seaweed Metabolite Database [24]	Secondary metabolites from seaweeds	1,000	No	No	No	Yes	No	No	No
SuperNatural II [25]	Primary and secondary metabolites	325,000	No	No	No	No	Yes	No	No
RIKEN [26]	Secondary metabolites	3,500	Yes	No	No	No	No	No	No
Yeast Metabolome database (YMDB) [27]	Primary and secondary metabolites	2,000	Yes	No	Yes	Yes	Yes	No	No

Table S2. A non-exhaustive list of activities evaluated by HPLC biological profiling

Assay	Post column evaluation		
	On-line by post-column reactions	**At-line by bioautography**	**At-line by microdilution assays**
Chemical assays			
radical scavenging properties	[28,29]	[30]	[31,32]
Enzymatic assays			
acetylcholine esterase inhibition	[33-35]	[36,37]	[37,38]
cathepsin B inhibition	[39]	-	-
xanthine oxidase inhibition	[40]	-	[41]
glucosidase inhibition	[42]	[43]	[32]
estrogen receptor binding activity	[44]	-	-
aldose reductase inhibition	-	-	[45]
protein kinase A inhibition	-	-	[46]
monoamine oxidase-A inhibition	-	-	[47]
$GABA_A$ receptor modulation	-	-	[48-51]
Anti-microbial assays			
anti-fungal	-	[52,53]	[54,55]
anti-bacterial	-	[56]	[57]
anti-parasitic	-	-	[58,59]
Cell based assay			
HIV inhibition	-	-	[60,61]
Ca^{2+} uptake inhibition	-	-	[62]
β_2 adrenergic agonist activation	-	-	[63]
NF-κB inhibition	-	-	[63]
quinone reductase induction	-	-	[64]
***In vivo* assays**			
Zebrafish assay	-	-	[65,66]

References:

[1] H. Laatsch, AntiBase 2013: The Natural Compound Identifier, Upgrade, Wiley-Vch, 2013.

[2] K. Degtyarenko, P. de Matos, M. Ennis, J. Hastings, M. Zbinden, A. McNaught, R. Alcántara, M. Darsow, M. Guedj, M. Ashburner, ChEBI: a database and ontology for chemical entities of biological interest, Nucleic Acids Res. 36 (2008) D344-D350, URL: http://www.ebi.ac.uk/chebi.

[3] J. Hastings, P. de Matos, A. Dekker, M. Ennis, B. Harsha, N. Kale, V. Muthukrishnan, G. Owen, S. Turner, M. Williams, C. Steinbeck, The ChEBI reference database and ontology for biologically relevant chemistry: enhancements for 2013, Nucleic Acids Res. 41 (2013) D456-D463.

[4] Dictionary of Marine Natural Products, in: J.W. Blunt, M.H.G. Munro (Eds.), CRC Press, URL: http://dmnp.chemnetbase.com/.

[5] Chapman, Hall, Dictionary of Natural Products on DVD (23:1), CRC Press, Taylor & Francis Group, URL: http://dnp.chemnetbase.com/, 2014.

[6] A.C. Guo, T. Jewison, M. Wilson, Y. Liu, C. Knox, Y. Djoumbou, P. Lo, R. Mandal, R. Krishnamurthy, D.S. Wishart, ECMDB: The *E. coli* Metabolome Database, Nucleic Acids Res. 41 (2013) D625-D630, URL: http://www.ecmdb.ca.

[7] J. Kopka, N. Schauer, S. Krueger, C. Birkemeyer, B. Usadel, E. Bergmüller, P. Dörmann, W. Weckwerth, Y. Gibon, M. Stitt, L. Willmitzer, A.R. Fernie, D. Steinhauser, GMD@CSB.DB: the Golm Metabolome Database, Bioinformatics 21 (2005) 1635-1638, URL http://gmd.mpimp-golm.mpg.de.

[8] D.S. Wishart, T. Jewison, A.C. Guo, M. Wilson, C. Knox, Y. Liu, Y. Djoumbou, R. Mandal, F. Aziat, E. Dong, S. Bouatra, I. Sinelnikov, D. Arndt, J. Xia, P. Liu, F. Yallou, T. Bjorndahl, R. Perez-Pineiro, R. Eisner, F. Allen, V. Neveu, R. Greiner, A. Scalbert, HMDB 3.0—The Human Metabolome Database in 2013, Nucleic Acids Res. 41 (2013) D801-D807, URL: http://www.hmdb.ca/.

[9] Y. Shinbo, Y. Nakamura, M. Altaf-Ul-Amin, H. Asahi, K. Kurokawa, M. Arita, K. Saito, D. Ohta, D. Shibata, S. Kanaya, KNApSAcK: A Comprehensive Species-Metabolite Relationship Database, in: K. Saito, R. Dixon, L. Willmitzer (Eds.), Plant Metabolomics, Springer Berlin Heidelberg, 2006, p. 165-181, URL: http://kanaya.naist.jp/KNApSAcK/KNApSAcK.php.

[10] M. Kanehisa, S. Goto, KEGG: Kyoto Encyclopedia of Genes and Genomes, Nucleic Acids Res. 28 (2000) 27-30, URL: http://www.genome.jp/kegg/.

[11] K. Schmelzer, E. Fahy, S. Subramaniam, E.A. Dennis, The Lipid Maps Initiative in Lipidomics, in: H.A. Brown (Ed.), Methods Enzymol., Academic Press, 2007, p. 171-183.

[12] E. Fahy, M. Sud, D. Cotter, S. Subramaniam, LIPID MAPS online tools for lipid research, Nucleic Acids Res. 35 (2007) W606-W612, URL: http://www.lipidmaps.org.

[13] T. Kind, K.-H. Liu, D.Y. Lee, B. DeFelice, J.K. Meissen, O. Fiehn, LipidBlast *in silico* tandem mass spectrometry database for lipid identification, Nat. Meth. 10 (2013) 755-758.

[14] Q. Cui, I.A. Lewis, A.D. Hegeman, M.E. Anderson, J. Li, C.F. Schulte, W.M. Westler, H.R. Eghbalnia, M.R. Sussman, J.L. Markley, Metabolite identification via the Madison Metabolomics Consortium Database, Nat. Biotechnol. 26 (2008) 162-164, URl: http://mmcd.nmrfam.wisc.edu/.

[15] MarinLit, a database of the marine natural products literature, in: S. Dabb, H. Potter (Eds.), Royal Society of Chemistry 2014.

[16] H. Horai, M. Arita, S. Kanaya, Y. Nihei, T. Ikeda, K. Suwa, Y. Ojima, K. Tanaka, S. Tanaka, K. Aoshima, Y. Oda, Y. Kakazu, M. Kusano, T. Tohge, F. Matsuda, Y. Sawada, M.Y. Hirai, H. Nakanishi, K. Ikeda, N. Akimoto, T. Maoka, H. Takahashi, T. Ara, N. Sakurai, H. Suzuki, D. Shibata, S. Neumann, T. Iida, K. Tanaka, K. Funatsu, F. Matsuura, T. Soga, R. Taguchi, K. Saito, T. Nishioka, MassBank: a public repository for sharing mass spectral data for life sciences, J. Mass Spectrom. 45 (2010) 703-714, URL: http://www.massbank.jp/.

[17] C. Steinbeck, P. Conesa, K. Haug, T. Mahendraker, M. Williams, E. Maguire, P. Rocca-Serra, S.-A. Sansone, R. Salek, J. Griffin, MetaboLights: towards a new COSMOS of metabolomics data management, Metabolomics 8 (2012) 757-760, URL: http://www.ebi.ac.uk/metabolights.

[18] K. Haug, R.M. Salek, P. Conesa, J. Hastings, P. de Matos, M. Rijnbeek, T. Mahendraker, M. Williams, S. Neumann, P. Rocca-Serra, E. Maguire, A. González-Beltrán, S.-A. Sansone, J.L. Griffin, C. Steinbeck, MetaboLights—an open-access general-purpose repository for metabolomics studies and associated meta-data, Nucleic Acids Res. 41 (2013) D781-D786.

[19] P.D. Karp, M. Riley, S.M. Paley, A. Pellegrini-Toole, The MetaCyc Database, Nucleic Acids Res. 30 (2002) 59-61, URL: http://www.metacyc.org.

[20] C.A. Smith, G. O'Maille, E.J. Want, C. Qin, S.A. Trauger, T.R. Brandon, D.E. Custodio, R. Abagyan, G. Siuzdak, METLIN: a metabolite mass spectral database, Ther. Drug Monit. 27 (2005) 747-751, URL: http://metlin.scripps.edu/index.php.

[21] N. Sakurai, T. Ara, S. Kanaya, Y. Nakamura, Y. Iijima, M. Enomoto, T. Motegi, K. Aoki, H. Suzuki, D. Shibata, An application of a relational database system for high-throughput prediction of elemental compositions from accurate mass values, Bioinformatics 29 (2013) 290-291, URL: http://webs2.kazusa.or.jp/mfsearcher/.

[22] J. Draper, D. Enot, D. Parker, M. Beckmann, S. Snowdon, W. Lin, H. Zubair, Metabolite signal identification in accurate mass metabolomics data with MZedDB, an interactive m/z annotation tool utilising predicted ionisation behaviour 'rules', BMC Bioinf. 10 (2009) 227, URL: http://maltese.dbs.aber.ac.uk:8888/hrmet/index.html.

[23] 2008, p. http://www.plantcyc.org/tools/tools_overview.faces on www.plantcyc.org.

[24] G.D.J. Davis, A.H.R. Vasanthi, Seaweed metabolite database (SWMD): A database of natural compounds from marine algae, Bioinformation 5 (2011) 361-364, URL: http://www.swmd.co.in/home.html.

[25] R. Preissner, M. Dunkel, P. Banerjee, J. Erehman, B.O. Gohlke, Super Natural II - a database of natural products URL: http://bioinf-applied.charite.de/supernatural_new/index.php?site=home.

[26] Y. Sawada, R. Nakabayashi, Y. Yamada, M. Suzuki, M. Sato, A. Sakata, K. Akiyama, T. Sakurai, F. Matsuda, T. Aoki, M.Y. Hirai, K. Saito, RIKEN tandem mass spectral database (ReSpect) for phytochemicals: A plant-specific MS/MS-based data resource and database, Phytochemistry 82 (2012) 38-45, URL: http://spectra.psc.riken.jp/.

[27] T. Jewison, C. Knox, V. Neveu, Y. Djoumbou, A.C. Guo, J. Lee, P. Liu, R. Mandal, R. Krishnamurthy, I. Sinelnikov, M. Wilson, D.S. Wishart, YMDB: the Yeast Metabolome Database, Nucleic Acids Res. 40 (2012) D815-D820, URL: http://www.ymdb.ca.

[28] T. van Beek, K.R. Tetala, I. Koleva, A. Dapkevicius, V. Exarchou, S.F. Jeurissen, F. Claassen, E.C. van der Klift, Recent developments in the rapid analysis of plants and tracking their bioactive constituents, Phytochem. Rev. 8 (2009) 387-399.

[29] M. Mnatsakanyan, T.A. Goodie, X.A. Conlan, P.S. Francis, G.P. McDermott, N.W. Barnett, D. Shock, F. Gritti, G. Guiochon, R.A. Shalliker, High performance liquid chromatography with two

simultaneous on-line antioxidant assays: Evaluation and comparison of espresso coffees, Talanta 81 (2010) 837-842.

[30] C.A. Simões-Pires, E.F. Queiroz, A.T. Henriques, K. Hostettmann, Isolation and on-line identification of anti-oxidant compounds from three *Baccharis* species by HPLC-UV-MS/MS with post-column derivatisation, Phytochem. Anal. 16 (2005) 307-314.

[31] S.G. Wubshet, N.T. Nyberg, M.V. Tejesvi, A.M. Pirttilä, M. Kajula, S. Mattila, D. Staerk, Targeting high-performance liquid chromatography−high-resolution mass spectrometry−solid-phase extraction−nuclear magnetic resonance analysis with high-resolution radical scavenging profiles−bioactive secondary metabolites from the endophytic fungus Penicillium namyslowskii, J. Chromatogr. A 1302 (2013) 34-9.

[32] S.G. Wubshet, J.S. Schmidt, S. Wiese, D. Staerk, High-Resolution Screening Combined with HPLC–HRMS–SPE–NMR for Identification of Potential Health-Promoting Constituents in Sea Aster and Searocket - New Nordic Food Ingredients, J. Agric. Food Chem. 61 (2013) 8616-23.

[33] I.K. Rhee, N. Appels, T. Luijendijk, H. Irth, R. Verpoorte, Determining acetylcholinesterase inhibitory activity in plant extracts using a fluorimetric flow assay, Phytochem. Anal. 14 (2003) 145-149.

[34] C.F. de Jong, R.J.E. Derks, B. Bruyneel, W. Niessen, H. Irth, High-performance liquid chromatography–mass spectrometry-based acetylcholinesterase assay for the screening of inhibitors in natural extracts, J. Chromatogr. A 1112 (2006) 303-310.

[35] K. Ingkaninan, C.M. de Best, R. van der Heijden, A.J.P. Hofte, B. Karabatak, H. Irth, U.R. Tjaden, J. van der Greef, R. Verpoorte, High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products, J. Chromatogr. A 872 (2000) 61-73.

[36] E.F. Queiroz, J.R. Ioset, K. Ndjoko, A. Guntern, C.M. Foggin, K. Hostettmann, On-line identification of the bioactive compounds from *Blumea gariepina* by HPLC-UV-MS and HPLC-UV-NMR, combined with HPLC-micro-fractionation, Phytochem. Anal. 16 (2005) 166-174.

[37] A. Landreau, S. Bertrand, C. Simoes-Pires, L. Marcourt, T. Dan Bach, M. Litaudon, D. Guilet, P. Richomme, P.-A. Carrupt, J.-L. Wolfender, Guttiferones as acetylcholinesterase inhibitors retrieved by normal phase HPLC profiling of apolar plant extract., Nat. Prod. Commun. Submitted Manuscript.

[38] S.-F. Tsai, S.-S. Lee, Characterization of Acetylcholinesterase Inhibitory Constituents from Annona glabra Assisted by HPLC Microfractionation, J. Nat. Prod. 73 (2010) 1632-1635.

[39] A.R. de Boer, J.M. Alcaide-Hidalgo, J.G. Krabbe, J. Kolkman, C.N. van Emde Boas, W.M.A. Niessen, H. Lingeman, H. Irth, High-Temperature Liquid Chromatography Coupled On-Line to a Continuous-Flow Biochemical Screening Assay with Electrospray Ionization Mass Spectrometric Detection, Anal. Chem. 77 (2005) 7894-7900.

[40] D.Q. Li, J. Zhao, S.P. Li, High-performance liquid chromatography coupled with post-column dual-bioactivity assay for simultaneous screening of xanthine oxidase inhibitors and free radical scavengers from complex mixture, J. Chromatogr. A 1345 (2014) 50-6.

[41] S.-F. Tsai, S.-S. Lee, Neolignans as xanthine oxidase inhibitors from Hyptis rhomboides, Phytochemistry 101 (2014) 121-7.

[42] D.-Q. Li, Z.-M. Qian, S.-P. Li, Inhibition of Three Selected Beverage Extracts on α-Glucosidase and Rapid Identification of Their Active Compounds Using HPLC-DAD-MS/MS and Biochemical Detection, J. Agric. Food Chem. 58 (2010) 6608-6613.

[43] C.A. Simões-Pires, B. Hmicha, A. Marston, K. Hostettmann, A TLC bioautographic method for the detection of α- and β-glucosidase inhibitors in plant extracts, Phytochem. Anal. 20 (2009) 511-515.

[44] U. Schobel, M. Frenay, D.A. Van Elswijk, J.M. McAndrews, K.R. Long, L.M. Olson, S.C. Bobzin, H. Irth, High Resolution Screening of Plant Natural Product Extracts for Estrogen Receptor a and f3 Binding Activity Using an Online HPLC-MS Biochemical Detection System, J. Biomol. Screen. 6 (2001) 291-303.

[45] J.H. Paek, K.H. Shin, Y.-H. Kang, J.-Y. Lee, S.S. Lim, Rapid Identification of Aldose Reductase Inhibitory Compounds from Perilla frutescens, BioMed Res. Int. 2013 (2013) 679463.

[46] M. Giera, F. Heus, L. Janssen, J. Kool, H. Lingeman, H. Irth, Microfractionation Revisited: A 1536 Well High Resolution Screening Assay, Anal. Chem. 81 (2009) 5460-5466.

[47] C. Grosso, A.K. Jäger, D. Staerk, Coupling of a high-resolution monoamine oxidase-A inhibitor assay and HPLC–SPE–NMR for advanced bioactivity profiling of plant extracts, Phytochem. Anal. 24 (2012) 141-147.

[48] D.C. Rueda, A. Schöffmann, M.D. Mieri, M. Raith, E.A. Jähne, S. Hering, M. Hamburger, Identification of dihydrostilbenes in Pholidota chinensis as a new scaffold for GABAA receptor modulators, Bioorg. Med. Chem. 22 (2014) 1276-84.

[49] A. Schramm, S.N. Ebrahimi, M. Raith, J. Zaugg, D.C. Rueda, S. Hering, M. Hamburger, Phytochemical profiling of Curcuma kwangsiensis rhizome extract, and identification of labdane diterpenoids as positive GABAA receptor modulators, Phytochemistry 96 (2013) 318-29.

[50] J. Zaugg, I. Baburin, B. Strommer, H.-J. Kim, S. Hering, M. Hamburger, HPLC-Based Activity Profiling: Discovery of Piperine as a Positive GABAA Receptor Modulator Targeting a Benzodiazepine-Independent Binding Site, J. Nat. Prod. 73 (2010) 185-191.

[51] D.C. Rueda, M. De Mieri, S. Hering, M. Hamburger, HPLC-Based Activity Profiling for GABAA Receptor Modulators in Adenocarpus cincinnatus, J. Nat. Prod. 77 (2014) 640-9.

[52] Q. Favre-Godal, E. Ferreira Queiroz, J.-L. Wolfender, Latest developments in assessing antifungal activity using TLC- bioautography: A review, J. AOAC Int. 96 (2013) 1175-1188.

[53] I. Cesari, E.F. Queiroz, Q. Favre-Godal, L. Marcourt, G. Caccialanza, P.F. Moundipa, G. Brusotti, J.-L. Wolfender, Extensive phytochemical investigation of the polar constituents of Diospyros bipindensis Gürke traditionally used by Baka pygmies, Phytochemistry 96 (2013) 279-87.

[54] K.T. Kongstad, S.G. Wubshet, A. Johannesen, L. Kjellerup, A.-M.L. Winther, A.K. Jäger, D. Staerk, High-Resolution Screening Combined with HPLC–HRMS–SPE–NMR for Identification of Fungal Plasma Membrane H-ATPase Inhibitors from Plants, J. Agric. Food Chem. (2014) DOI: 10.1021/jf501605z.

[55] S. Bertrand, C. Petit, L. Marcourt, R. Ho, K. Gindro, M. Monod, J.-L. Wolfender, HPLC profiling with at-line microdilution assay for the early identification of antifungal compounds in plants from French Polynesia, Phytochem. Anal. 25 (2014) 106-112.

[56] S. Takashi Saito, D. da Silva Trentin, A. José Macedo, C. Pungartnik, G. Gosmann, J. de Deos Silveira, T. Nikolova Guecheva, J.A. Pêgas Henriques, M. Brendel, Bioguided Fractionation Shows *Cassia alata* Extract to Inhibit *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* Growth and Biofilm Formation, Evid.-based Comp. Alt. Med. 2012 (2012) 867103.

[57] Y. Liu, M. Nielsen, D. Staerk, A.K. Jäger, High-resolution bacterial growth inhibition profiling combined with HPLC–HRMS–SPE–NMR for identification of antibacterial constituents in Chinese plants used to treat snakebites, J. Ethnopharmacol. (2014) DOI: 10.1016/j.jep.2014.07.019.

[58] Y. Hata, M. De Mieri, S.N. Ebrahimi, T. Mokoka, G. Fouche, M. Kaiser, R. Brun, O. Potterat, M. Hamburger, Identification of two new phenathrenones and a saponin as antiprotozoal constituents of Drypetes gerrardii, Phytochem. Lett. (2014) DOI: 10.1016/j.phytol.2014.05.005.

[59] T. Julianti, M. De Mieri, S. Zimmermann, S.N. Ebrahimi, M. Kaiser, M. Neuburger, M. Raith, R. Brun, M. Hamburger, HPLC-based activity profiling for antiplasmodial activity in the traditional Indonesian medicinal plant Carica papaya L, J. Ethnopharmacol. (2014) DOI: 10.1016/j.jep.2014.05.050.

[60] V. Vidal, O. Potterat, S. Louvel, F. Hamy, M. Mojarrab, J.-J. Sanglier, T. Klimkait, M. Hamburger, Library-Based Discovery and Characterization of Daphnane Diterpenes as Potent and Selective HIV Inhibitors in Daphne gnidium, J. Nat. Prod. 75 (2011) 414-419.

[61] S. Louvel, N. Moodley, I. Seibert, P. Steenkamp, R. Nthambeleni, V. Vidal, V. Maharaj, T. Klimkait, Identification of compounds from the plant species *Alepidea amatymbica* active against HIV, South Afr. J. Botany 86 (2013) 9-14.

[62] P. Tammela, T. Wennberg, H. Vuorela, P. Vuorela, HPLC micro-fractionation coupled to a cell-based assay for automated on-line primary screening of calcium antagonistic components in plant extracts, Anal. Bioanal. Chem. 380 (2004) 614-618.

[63] Y. Hou, X. Cao, L. Wang, B. Cheng, L. Dong, X. Luo, G. Bai, W. Gao, Microfractionation bioactivity-based ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry for the identification of Nuclear Factor-κB inhibitors and β2 adrenergic receptor agonists in an alkaloidal extract of the folk herb Alstonia scholaris, J. Chromatogr. B 908 (2012) 98-104.

[64] A. Azzollini, S. Bertrand, A. Nievergelt, J. Boccard, S. Rudaz, M. Cuendet, J.-L. Wolfender, Integrative approach in natural product research: at-line coupling of micro-fractionation with NMR, LC-MS and bioassays, CHIMIA 66 (2012) 487.

[65] S. Challal, N. Bohni, O.E. Buenafe, C.V. Esguerra, P.A.M. de Witte, J.-L. Wolfender, A.D. Crawford, Zebrafish bioassay-guided microfractionation for the rapid *in vivo* identification of pharmacologically active natural products, CHIMIA 66 (2012) 229-232.

[66] N. Bohni, M.L. Cordero-Maldonado, J. Maes, D. Siverio-Mota, L. Marcourt, S. Munck, A.R. Kamuhabwa, M.J. Moshi, C.V. Esguerra, P.A.M. de Witte, A.D. Crawford, J.-L. Wolfender, Integration of Microfractionation, qNMR and Zebrafish Screening for the *In Vivo* Bioassay-Guided Isolation and Quantitative Bioactivity Analysis of Natural Products, PLoS One 8 (2013) e64006.