



HAL
open science

Comparative study of the production of coumarins and furanocoumarins in three Ruteae species

Saida Bergheul, Mariana Limones-Mendez, Jeremy Grosjean, Alain Hehn, Alexandre Olry, Abdellah Berkani

► **To cite this version:**

Saida Bergheul, Mariana Limones-Mendez, Jeremy Grosjean, Alain Hehn, Alexandre Olry, et al.. Comparative study of the production of coumarins and furanocoumarins in three Ruteae species. Indian Journal of Natural Products and Resources, 2019, 10 (2), pp.137 - 142. hal-02917590

HAL Id: hal-02917590

<https://hal.univ-lorraine.fr/hal-02917590v1>

Submitted on 19 Aug 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Comparative study of the production of coumarins and furanocoumarins in three Ruteae species

Saida Bergheul¹, Mariana Limones-Méndez², Jérémy Grosjean², Alain Hehn², Alexandre Olry^{2*} and Abdellah Berkani¹

¹Plant Protection Laboratory, Faculty of Sciences and the Natural Sciences and Life, University of Mostaganem, BP300, 27000 Mostaganem, Algeria

²Université de Lorraine, INRA-LAE - F54000 Nancy, France

Received 19 April 2018; Revised 04 April 2019

Within specialized metabolites, coumarins and furanocoumarins represent a wide group of structurally diverse compounds and are specially produced in plants belonging to the Rutaceae family. Here we performed the furanocoumarin and coumarin-targeted chemical characterization of three Ruteae species collected from Algeria. Detection and quantification of 27 coumarins and furanocoumarins extracted from stems and leaves was carried out by UHPLC-MS. We highlighted significant chemical differences between these plants. *Ruta chalepensis* L. is the highest producer with 24.83 mmol/g dry material in stems and 15.70 mmol/g dry materials in leaves while *Haplophyllum tuberculatum* (Forsk.) is the lowest producer. We also showed a surprising chemical diversity between *R. chalepensis* L and *R. angustifolia* Pers. This chemical diversity might, therefore, be a helpful tool for phylogenetic identification of plants.

Keywords: Coumarins, Furanocoumarins, *Haplophyllum tuberculatum* (Forsk.) Juss, *Ruta angustifolia* Pers, *Ruta chalepensis* L., UHPLC-MS.

IPC Code; Int. cl. (2015.01)-A61K 36/00, A61K 36/75

Introduction

Rutaceae is a diverse and widely spread family of mainly tropical trees and shrubs¹. These plants gather almost 161 genera and 1815 species distributed throughout the temperate regions of the world². The most represented genera of the rutaceae family are *Citrus*, *Zanthoxylum*³, *Ruta*, *Ptelea*, *Murraya* and *Fortunella*. Both *Ruta* and *Haplophyllum* genera belong to the Ruteae tribe which gathered plants that share morphological traits such as the presence of actinomorphic, creamy-white to bright yellow flowers and specific classes of coumarins⁴. Nevertheless, *Ruta* and *Haplophyllum* show also morphological and phytochemical differences which have been described previously in literature^{5,6}. In Algeria, the aerial parts of these species are often used in traditional medicine as a herbal decoction to treat various pathologies: tumours, vomiting, digestive problems and carminative, against the bites of scorpions, diabetes and infertility⁷. In other countries these plants are also used for their laxative, anti-inflammatory, anti-seizure

analgesic, antispasmodic, abortifacient, antiepileptic, emmenagogue properties and for the treatment of skin diseases^{8,9}. Among all the secondary metabolites produced by these plants, many reports were dedicated to coumarins and furanocoumarins^{10,11}. These molecules are of greatest interest since they have a broad range of biological activities^{12,13}. In plants, coumarins display important allelochemical functions and play a major role as phytoalexins in response to biotic stresses^{14,15}. A recent report highlights also the involvement of coumarins in root iron homeostasis^{16,17}.

Many studies have focused on the beneficial effect on human health of furanocoumarins such as isoimperatorin, notopterol and bergapten¹⁸. These molecules possess anti-inflammatory, analgesic, anti-cancer and anti-coagulant activities^{19,21}. Furanocoumarins constitute a subclass of coumarins that are characterized by the presence of an additional furan ring branched at the C6/C7 or C7/C8 position of the coumarin core molecule²².

They have pharmacological activities as, anti-coagulant¹⁹, anti-inflammatory²³, antioxidant²⁴, antimicrobial²⁵. Xanthotoxin and bergapten possess photosensitising properties and are important drugs in

*Correspondent author
Email: alexandre.olry@univ-lorraine.fr
Tel: 0372744062

the therapy of leucodermy²⁶. Psoralen derivatives can inhibit DNA synthesis and may be valuable in promoting skin pigmentation and treat psoriasis^{27,28}. These molecules are also involved in the Grapefruit effect by inhibiting intestinal CYP3A4 enzymes²⁹. The production of these molecules is highly variable from Citrus species³⁰. In this report, we compare the production of coumarins and furocoumarins in the leaves and stems of *Ruta angustifolia* Pers, *R. chalepensis* L. and *Haplophyllum tuberculatum* (Forsk.) which are three species of the Ruteae. In the present research paper, we have described the extraction, identification and quantification of 27 coumarins and furocoumarins achieved by high pressure liquid chromatography coupled with mass spectrometry (UHPLC/MS) (Fig. 1). Furanocoumarins and coumarins are secondary metabolites produced in various concentration in Rutaceae plants¹⁰. In this report we assessed their production in three Rutaceae collected from Algeria.

Materials and Methods

Plant materials

The aerial parts of plants were collected at flowering stage, in March 2013, from three different

regions of Algeria: *R. chalepensis* L. was collected from Mostaganem (West region, 35°55'52" North, 0°05'21" East) at an altitude of 102 m; *R. angustifolia* Pers. From Bordj Bou Arreridj (East region, 36°4' 0" North, 4°46'0" East) at an altitude of 906 m and *H. tuberculatum* from Adrar (South region, 27°52'27" North, 0°17'37" West) at an altitude of 257m. Plants were identified by Dr. Sekkal, from the department of Biology, University of Mostaganem (Algeria). Specimens of the 3 species (*R. angustifolia* Pers.-1726-1, *R. chalepensis* L.- 1726-2 and *H. tuberculatum* (Forsk) Juss-1722) were deposited in the herbarium of the Laboratory of Plant Ecology, University Ahmed Ben Bella 1 Oran (Algeria).

The sample drying was carried out in the absence of light, at room temperature. They were weighed and then grounded in liquid nitrogen using a pestle and a mortar for subsequent phytochemical investigations.

Extraction of coumarins and furanocoumarins

The extraction was performed according to the protocol described by Dugrand and collaborators with some modifications such as the volume of the methanol:water extraction solution and the composition of this solution used to resuspend the

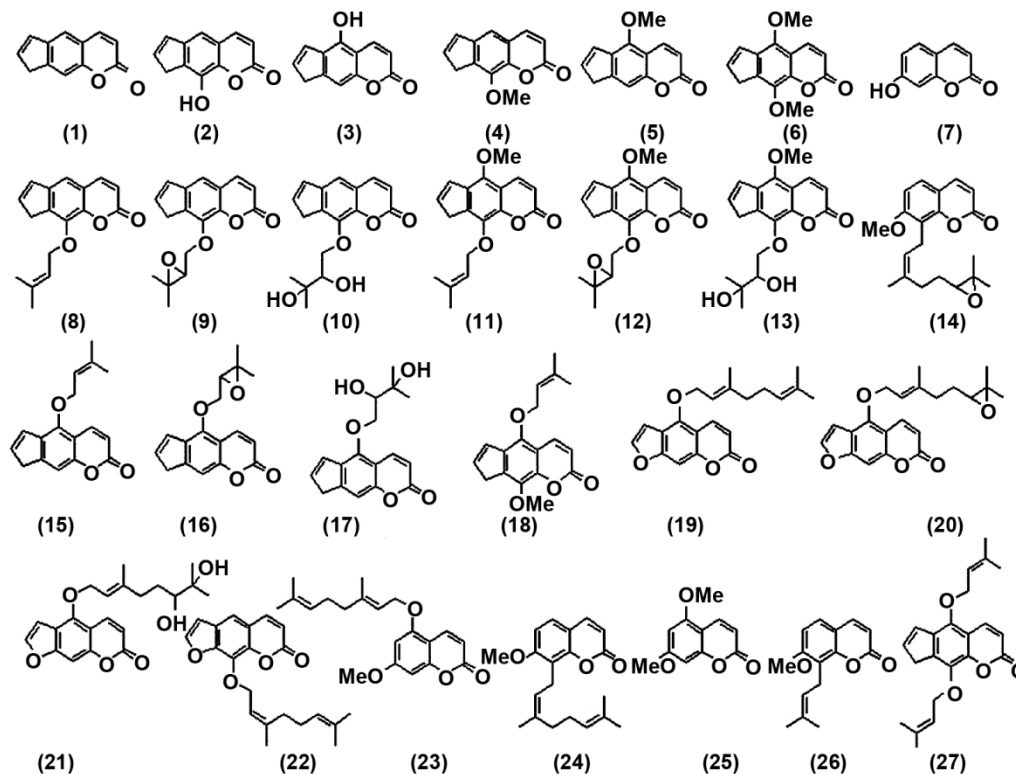


Fig. 1 — Chemical structure of the coumarins and furocoumarins analysed by UPLC/MS. The numbering corresponds to the numbering defined Table 2.

pellet after drying the methanolic extract³¹. Briefly, 20 mg of dried leaves or stems were crushed into powder in liquid nitrogen, and then 800 μ L of 80:20 methanol:water solvent was added in order to solubilize the polyphenolic derivatives. Grounded material was homogenized by vortexing vigorously for 30 seconds. The samples were subsequently incubated in an ultrasonic bath (Elma, Elmasonic S70) for 15 minutes in order to increase the extraction of polyphenolics. This methanolic solution was then centrifuged at 13200 g for 30 minutes. The supernatant was recovered and a second extraction was performed on the pellet in order to complete the extraction. The final solution was dried (Concentrator Plus, Eppendorf) overnight at room temperature. The pellet was resuspended in 100 μ L of 80:20 methanol:water solvent. Each extract was diluted 10 times in order to quantify molecules that were too concentrated in the stock solution³².

Identification of furanocoumarins

The chromatographic analysis was carried out using a NEXERA HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA), a detector (SPDM20A, Shimadzu) combined with a mass spectrometer (single quadrupole, LCMS2020, Shimadzu) according to Dugrand *et al.*³¹.

Quantification of coumarins and furanocoumarins

Quantification was performed using angelicin as an analytical internal standard. This molecule is an angular furanocoumarin which is not present in the Rutaceae family. Angelicin was added at the concentration of 5 μ M in the samples and in the calibration solutions. The calibration solutions contain all the molecules at the same concentrations ranging from 1 to 30 μ mol/L (1, 3, 6, 12, 18, 24 and 30). The calibration curves were generated for each compound by connecting the peak intensity to the theoretical concentration of the standard in the solution. A linear regression was then performed as described elsewhere^{31,32}.

Statistical analysis

All experimental measurements were performed in triplicates and were expressed as mean \pm SD of three analyzes (means \pm SD).

Results

Distribution of coumarins and furocoumarins in stems and leaves

The analysis of the total amount of these molecules made evidence that *H. tuberculatum* is a poor producer of such molecules and produced them mainly in leaves (Table 1). These results are consistent with those of Diar and collaborators who reported the presence of lower amounts of psoralen in *H. tuberculatum*³³. With 15 to 20 μ mol/g dry material, *R. chalepensis* accumulates the highest concentration of these molecules whereas *R. angustifolia* has an intermediate phenotype and produces 3-4 mmol/g dry material. Interestingly, the distribution of these molecules is different between the 3 Ruteae species. For *R. chalepensis* and *R. angustifolia*, equal amounts could be detected in stems and leaves. This distribution is unequal for *H. tuberculatum*: higher in stems in leaves for *H. tuberculatum* (5.7 times more) (Table 1).

To have a better overview of the production of these molecules in plants, the authors conducted a detailed analysis of stems and leaves of 3 plants. In the light of the overall analysis done on *H. tuberculatum*, it was not surprising to highlight a very poor diversity of these molecules in the extracts. Only 5 different molecules (iepsoralen, osthol, heraclenin, aurapten and cnidicin) could be detected although at very low concentration (Table 2). This might be explained by the absence of umbelliferon which has been described to be the precursor molecule of the furanocoumarin biosynthetic pathway³⁴. Consistently, umbelliferon was highlighted in *R. angustifolia* and *R. chalepensis* and both of them are producing a large diversity of downstream furanocoumarins (10 for *R. angustifolia* and 18 for *R. chalepensis*).

Phytochemical analysis

Bergaptol, xanthotoxol, byakangelicin, byakangelicol, imperatorin, phellopterin, cnidicin and 6,7-dihydroxybergamottin are present in *R. chalepensis* and not in *R. angustifolia*. The absence of umbelliferon in *H. tuberculatum* is however intriguing since its deriving prenylated derivatives, aurapten and osthol, could be detected in this plant. The major phototoxic

Table 1 — UHPLC/MS quantification of the 27 coumarins and furanocoumarins in the stems and leaves of the three Ruteae species.

Total coumarins and furanocoumarins (nmol/g dry matter)	<i>R. angustifolia</i>	<i>R. chalepensis</i>	<i>H. tuberculatum</i>
Stem	3685.2 \pm 1394.8	19731.7 \pm 6027.7	5.4 \pm 1.8
Leaves	3897.2 \pm 1282.4	15705.4 \pm 2666.6	30.9 \pm 17.7

Note: The results are the means of three independent replicates and error represent standard deviation

Table 2 — Quantitative analysis of 27 coumarins/furanocoumarins by UHPLC/MS in the 3 *Rutea* species

Cluster	Quantity coumarins and furocoumarins (nmol/g dry matter)	<i>R. angustifolia</i>		<i>R. chalepensis</i> L.		<i>H. tuberculatum</i>	
		Stem	Leaves	Stem	Leaves	Stem	Leaves
Coumarin	Umbelliferon (7)	127.4±121.9	60.2±26.6	432.7±159.2	211.2±78.5	N.D.	N.Q.
	Limettin (8)	14.5±9.4	15.6±4.6	79.0±58.8	59.3±19.1	N.D.	N.D.
	Cnidicin (19)	N.Q.	N.Q.	127.4±168.0	69.2±19.8	N.Q.	27.8±16.2
	Auraptin (22)	253.5±75.8	5.4±2.6	16.6±15.8	14.2±3.3	0.9±0.7	1.4±0.7
	Epoxyauraptin (25)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	osthol (27)	9.7±4.1	18.8±4.3	90.6±105.5	62.7±13.0	0.6±0.1	0.3±0.1
	5-Geranyloxy-7-methocoumarin (23)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Psoralen	Psoralen (1)	445.3±196.2	112.6±36.5	4034.9±1281.9	609.8±184.7	0.2±0.1	0.7±0.3
Xanthotoxin cluster	Xanthotoxol (2)	N.D.	N.D.	44.7±40.3	37.5±8.7	N.D.	N.D.
	Xanthotoxin (4)	851.2±251.7	491.1±308.1	3050.2±842.1	1156.1±451.4	N.D.	N.D.
	Heraclenol (11)	178.0±110.6	225.3±73.9	637.5±299.9	481.1±142.1	3.0±0.7	N.D.
	Heraclenin (10)	N.Q.	N.Q.	314.9±120.1	44.8±64.2	0.7±0.2	0.7±0.4
	Phellopterin (12)	N.D.	N.D.	2291.9±530.7	N.D.	N.D.	N.D.
	Byakangelicol (13)	N.D.	N.D.	31.5±26.6	12.3±3.9	N.D.	N.D.
	Imperatorin (9)	N.D.	N.D.	382.5±66.5	91.1±53.2	N.Q.	N.Q.
	Byakangelicin (24)	N.Q.	N.D.	1046.2±215.7	801.5±175.8	N.D.	N.D.
	8-Geranyloxypsoralen (14)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bergapten cluster	Bergaptol (3)	N.D.	N.D.	36.7±22.9	42.4±32.7	N.D.	N.D.
	Bergapten (5)	963.8±286.5	1332.7±326.7	4978.5±687.2	4262.2±55.1	N.Q.	N.Q.
	Cnidilin (15)	N.D.	N.D.	N.D.	N.D.	N.Q.	N.D.
	Bergamottin (20)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Epoxybergamottin (21)	285.6±104.9	563.3±160.1	1954.1±454.6	1976.7±300.1	N.D.	N.D.
	6,7-Dihydroxybergamottin (26)	N.Q.	N.Q.	876.1±98.9	1072.0±417.7	N.D.	N.D.
	Isoimperatorin (16)	191.6±75.6	471.2±143.5	1640.9±279.2	2208.9±246.4	N.D.	N.D.
	Oxypeucedanin (17)	253.5±75.8	452.8± 152.3	1889.4±227.5	1863.9±273.1	N.D.	N.D.
	Oxypeucedanin hydrate (18)	45.0±17.9	110.3±29.5	609.3±152.6	520.2±88.5	N.D.	N.Q.
Isopimpinellin cluster	Isopimpinellin (6)	66.1±64.4	37.9±13.7	265.2±173.7	109.2±35.3	N.D.	N.D.

Note: The numbering of the molecules corresponds to the numbering of the chemical structures. The results are the mean of three independent replicates and error represent standard deviation. N.D.= not detected, N.Q.= not quantifiable

furanocoumarins i.e. bergapten, xanthotoxin and psoralen are present in high quantities. The organ distribution of molecules is different between *R. chalepensis* and *R. angustifolia*. Regarding *R. chalepensis*, only two different groups of molecules can be observed: i) molecules which are detected nearly equal concentration in stems and leaves and ii) molecules which are present in higher amount in stems. None of them could be detected in significant higher concentration in leaves. All the molecules produced in higher concentration in stems (i.e., isopimpinellin, imperatorin, heraclenin, phellopterin, heraclenol and byakangelicol) are belonging to the xanthotoxin and the isopimpinellin clusters. Concerning *R. angustifolia*, this distribution is different. A first small group of molecules including the precursor psoralen, xanthotoxin, and auraptin are detected in higher concentration in stem. Both latest molecules also belong to the xanthotoxin cluster. The second group is composed by two

coumarins (limettin and osthol) and six furanocoumarins (bergapten, isoimperatorin, oxypeucedanin, heraclenol, oxypeucedanin hydrate, epoxybergamottin). Regarding the furanocoumarins they all belong to the bergapten and the isopimpinellin cluster.

Discussion

In previous studies, classification of *Ruta* and *Haplophyllum* genera based on morphological and phytochemical traits has been contradictory. Salvo *et al.* made additional molecular analysis based on three different cDNA sequences to establish the phylogenetic relationships⁴. Whereas morphological and phytochemical trees relate *Haplophyllum* and *Ruta* closely, molecular analyses confirm their distant relationship within the *Ruteae* tribe. This result is consistent with our results, since *Ruta* species are able to produce high levels of furanocoumarins while *Haplophyllum* did not. Since recent years, new precise, fast and reliable analytical methods helped

scientist to realize the chemotyping of plants and these methods became tools of choice to assess phylogenetic evolution of several plant genera. A report dedicated to the analysis of 23 eucalyptus and 64 Citrus species highlighted a distribution of total polyphenolics matching with their phylogenetic relationship^{30,35}. Such a genus specific distribution of phenolics for licorice for whom the synthesis of different kind of phenolics (for example: saponins such as glycyrrhizin and glabridin; chalcones such as lichochalcone and kanzonol C; flavonoids such as liquiritigenin and kanzonol D) has been reported to be species specific³⁶. For Astragalus the distribution of 131 flavones, flavonols, flavanones, flavan-4-ols, isoflavones, isoflavans, petrocarpans and miscellaneous over 60 species³⁷ helps to identify genus with higher or lower commercial value of the roots. This report dedicated to 3 different Ruteae collected in Algeria allowed us to highlight 3 different chemotype in the same plant species. Whereas one species produces only low concentration of furanocoumarins other plants are able to synthesize significant amounts of those defense related molecules. The detailed analysis of the phytochemical profile highlighted a difference of type of molecules which are synthesized in the 3 plants. These differences might reflect 3 different branches of evolution of this genus and confirms that these molecules could be used as biochemical markers for phylogenetic studies.

Conclusion

The present study showed diversity concentrations of coumarins and furanocoumarins in the three Ruteae species. *R. chalepensis* L. can be considered as a good source of natural compounds. Moreover, chemotyping of furanocoumarins/coumarins could help scientists to a better understanding of plant evolution.

Acknowledgment

This work was supported by the French Ministry of Sciences. It is part of cooperation between the ministry of Algeria and French.

References

- 1 Scott K D, McIntyre, C L and Playford J, Molecular analyses suggest a need for a significant rearrangement of Rutaceae subfamilies and a minor reassessment of species relationships within Flindersia, *Plant Syst Evol*, 2000, **223**(1-2), 15-27.
- 2 Stevens P, Angiosperm phylogeny Website, 2001, <http://www.mobot.org/MOBOT/research/APweb>.
- 3 Supabphol R and Tangjitjareonkun J, Chemical constituents and biological activities of *Zanthoxylum limonella* (Rutaceae): A Review, *Trop J Pharm Res*, 2014, **13**(12), 2119- 2130.
- 4 Salvo G, Bacchetta G, Ghahremaninejad F and Conti E, Phylogenetic relationships of Ruteae (Rutaceae): New evidence from the chloroplast genome and comparisons with non-molecular data, *Mol Phylogenet Evol*, 2008, **49**(3), 736–748.
- 5 Townsend C C, Taxonomic revision of the genus *Haplophyllum* (Rutaceae) (Hookers Icones Plantarum), 2018, <https://www.abebooks.com/9780950487656/Taxonomic-Revision-Genus-Haplophyllum-Rutaceae-0950487651/plp>.
- 6 Silva M F, Das G F, Gottlieb O R and Ehrendorfer F, Chemosystematics of the Rutaceae: Suggestions for a more natural taxonomy and evolutionary interpretation of the family, *Plant Syst Evol*, 1988, **161**, 97-134.
- 7 Haddouchi F, Chaouche T M, Zaouali Y, Ksouri R, Attou A, *et al.*, Chemical composition and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria, *Food Chem*, 2013, **141**(1), 253-258.
- 8 Al-Snafi A E, Pharmacological importance of *Haplophyllum* species grown in Iraq- A review, *IOSR J Pharm*, 2018, **8**(5), 54-62.
- 9 Raghav S K, Gupta B, Agrawal C, Goswami K and Das H R, Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells, *J Ethnopharmacol*, 2006, **104**(1-2), 234-239.
- 10 Milesi S, Massot B, Gontier E, Bourgaud F and Guckert A, *Ruta graveolens* L.: a promising species for the production of furanocoumarins, *Plant Sci*, 2001, **161**(1), 189-199.
- 11 Ivanova A, Mikhova B, Stambolijaska T and Kostova I, Lignan and coumarin glycosides from *Haplophyllum suaveolens*, *Z, Naturforschung C J Biosci*, 2001, **56**, 329-333.
- 12 Beier R C, Natural pesticides and bioactive components in foods, *Rev Environ Contam Toxicol*, 1990, **113**, 47-137.
- 13 Beier R C and Oertli E H, Psoralen and other linear furocoumarins as phytoalexins in celery, *Phytochem*, 1983, **22**(11), 2595-2597.
- 14 Niro E, Marzaioli R, De Crescenzo S, D’Abrosca B, Castaldi S, *et al.*, Effects of the allelochemical coumarin on plants and soil microbial community, *Soil Biol Biochem*, 2016, **95**, 30-39.
- 15 Yan Z, Wang D, Cui H, Zhang D, Sun Y, *et al.*, Phytotoxicity mechanisms of two coumarin allelochemicals from *Stellera chamaejasme* in lettuce seedlings, *Acta Physiol Plant*, 2016, **38**(10), 248.
- 16 Schmid N B, Giehl R F H, Doll S, Mock H P, Strehmel N, *et al.*, Feruloyl-CoA 6'-Hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in arabidopsis, *Plant Physiol*, 2014, **164**, 160–172.
- 17 Siwinska J, Siatkowska K, Olry A, Grosjean J, Hehn A, *et al.*, Scopoletin 8-hydroxylase: a novel enzyme involved in coumarin biosynthesis and iron-deficiency responses in Arabidopsis, *J Exp Bot*, 2018, **69**(7), 1735-1748.
- 18 Qian G S, Wang Q, Leung K S Y, Qin Y, Zhao Z, *et al.*, Quality assessment of Rhizoma et Radix Notopterygii by HPTLC and HPLC fingerprinting and HPLC quantitative analysis, *J Pharm Biomed Anal*, 2007, **44**(3), 812-817.

- 19 Jain P K and Joshi H, Coumarin: Chemical and pharmacological profile, *J Appl Pharm Sci*, 2012, **2**(6), 236-240.
- 20 Hoult J R S and Paya M, Pharmacological biochemical actions of simple coumarins: Natural products with therapeutic potential, *Gen Pharmacol*, 1996, **27**(4), 713-722.
- 21 Ge Z C, Qu X, Yu H F, Zhang H M, Wang Z H, *et al.*, Antitumor and apoptotic effects of bergaptol are mediated via mitochondrial death pathway and cell cycle arrest in human breast carcinoma cells, *Bangladesh J Pharmacol*, 2016, **11**(2), 489-494.
- 22 Bourgaud F, Olry A and Hehn A, Recent advances in molecular genetics of furanocoumarin synthesis in higher plants, *Recent Adv Redox Act Plant Microb Prod*, 2014, 363-375.
- 23 Uto T, Tung N H, Taniyama R, Miyanowaki T, Morinaga O, *et al.*, Anti-inflammatory activity of constituents isolated from aerial part of *Angelica acutiloba* kitagawa, *Phytother Res*, 2015, **29**(12), 1956-1963.
- 24 Zou Z, Xi W, Hu Y, Nie C and Zhou Z, Antioxidant activity of citrus fruits, *Food Chem*, 2016, **196**, 885-896.
- 25 Xie Q, Li S X, Liao D F, Wang W, Tekwani B, *et al.*, Bio-pesticidal and antimicrobial coumarins from *Angelica dahurica* (Fisch. Ex Hoffm), *Rec Nat Prod*, 2016, **10**(3), 294-306.
- 26 Robl M, Robl R, Marinoni L P, Abagge K T and Carvalho V O, Assemble the puzzle: Bizarre-looking lesions, *Arch Dis Child*, 2013, **98**(11), 915.
- 27 Paul M D, Medicinal Natural Products: A Biosynthetic Approach, <https://www.amazon.fr/Medicinal-Natural-Products-Biosynthetic-Approach/dp/0470741678>, 2018.
- 28 Conforti F, Marrelli M, Menichini F, Bonesi M, Statti G, *et al.*, Natural and synthetic furanocoumarins as treatment for vitiligo and psoriasis, *Curr Drug Ther*, 2009, **4**(1), 38-58.
- 29 Guo L Q and Yamazoe Y, Inhibition of cytochrome P450 by furanocoumarins in grapefruit juice and herbal medicines, *Acta Pharmacologica Sinica*, 2004, **25**(2), 129-136.
- 30 Dugrand-Judek A, Olry A, Hehn A, Costantino G, Ollitrault P, *et al.*, The distribution of coumarins and furanocoumarins in *citrus* species closely matches *citrus* phylogeny and reflects the organization of biosynthetic pathways, *Plos One*, 2015, **10**(11), e0142757.
- 31 Dugrand A, Olry A, Duval T, Hehn A, Froelicher Y, *et al.*, Coumarin and furanocoumarin quantitation in citrus peel via ultra performance liquid chromatography coupled with mass spectrometry (UPLC-MS), *J Agric Food Chem*, 2013, **61**, 10677-10684.
- 32 Royer M, Larbat R, Le Bot J, Adamowicz S and Robin C, Is the C: N ratio a reliable indicator of C allocation to primary and defence-related metabolisms in tomato?, *Phytochem*, 2013, **88**, 25-33.
- 33 Diar A, Enas J, Ekbal A K and Ali A S, Qualitative and quantitative investigations of furocoumarin derivatives (Psoralens) of *Haplophyllum tuberculatum* (Rutaceae), *Al-mustansiriyah J Pharm Sci*, 2005, **2**(2), 24-36.
- 34 Vialart G, Hehn A, Olry A, Ito K, Krieger C, *et al.*, A 2-oxoglutarate-dependent dioxygenase from *Ruta graveolens* L. exhibits p-coumaroyl CoA 2'-hydroxylase activity (C2' H): A missing step in the synthesis of umbelliferone in plants, *Plant J*, 2012, **70**(3), 460-470.
- 35 Senior J K, Potts B M, Davies N W, Wooliver R C, Schweitzer J A, *et al.*, Phylogeny explains variation in the root chemistry of eucalyptus species, *J Chem Ecol*, 2016, **42**(10), 1086-1097.
- 36 Rizzato G, Scalabrin E, Radaelli M, Capodaglio G and Piccolo O, A new exploration of licorice metabolome, *Food Chem*, 2017, **221**, 959-968.
- 37 Gorai D, Jash S K and Roy R, Flavonoids from *Astragalus* genus, *Int J Pharm Sci Res*, 2016, **7**(7), 2732-2747.