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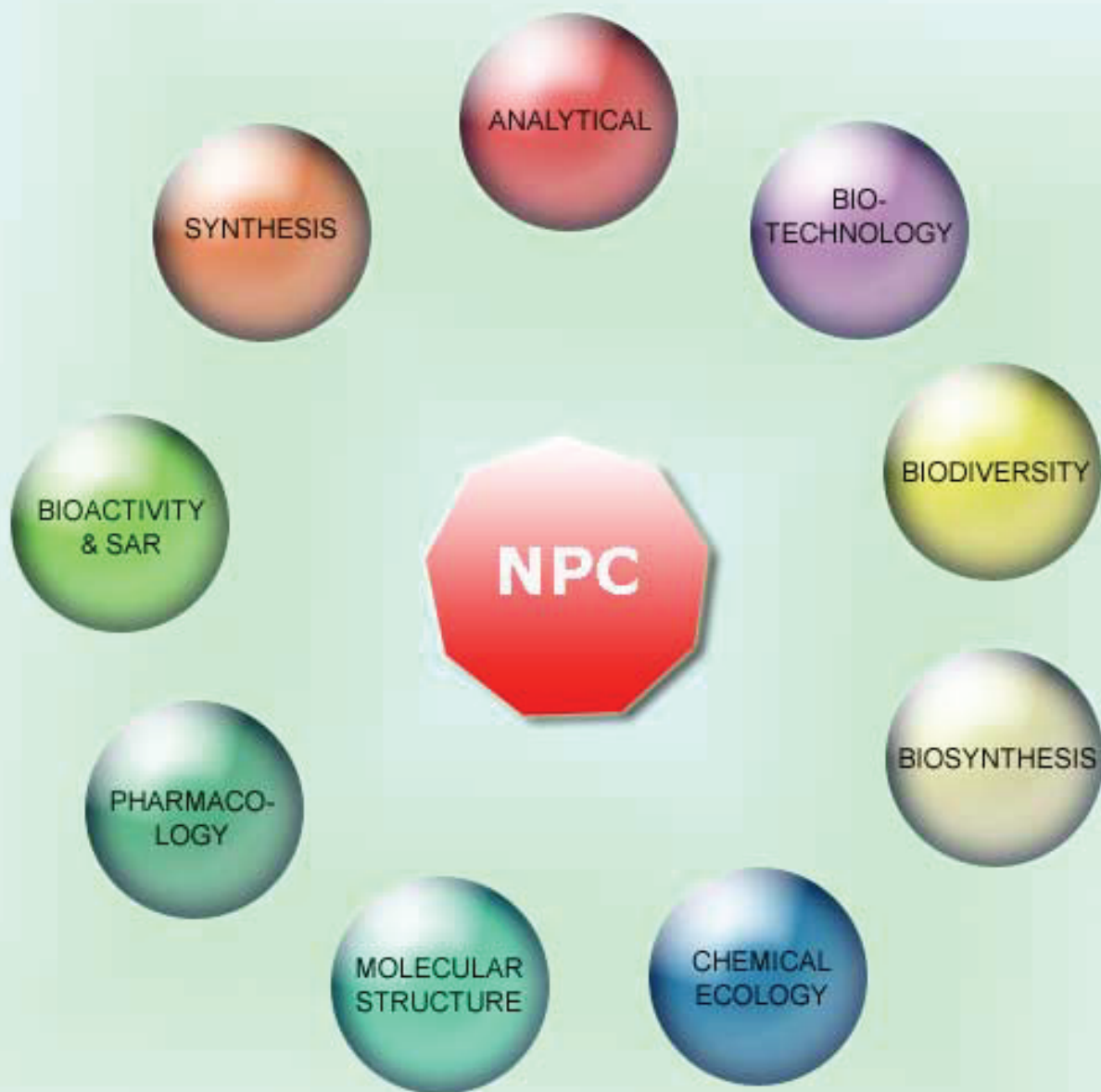
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Pteridaceae Fragrant Resource and Bioactive Potential: a Mini-review of Aroma Compounds

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Seven ferns of *Pteridaceae*, grown in a botanical garden or wild, harvested in France were investigated for their Volatile Organic Compounds (VOC) profile using GC-MS: *Adiantum pedatum* L., *Adiantum peruvianum* Klotzsch, *Anogramma leptophylla* (L.) Link, *Cheilanthes maderensis* Lowe, *Cryptogramma crista* (L.) R. Br., *Pteris cretica* L. and *Pteris vittata* L. Fifty-three VOC biosynthesized from lipidic, shikimic, terpenic and carotenoid pathways were identified. The two *Adiantum* species show different VOC composition. The main linalool (10.8%) in *A. pedatum* has several biological activities of great interest. This Maidenhair fern contains the highest proportion (57.9%) of isoprenoid flavor precursors, i.e., ionone derivatives with various scent notes. The two major odorant unsaturated hexenoic acids derivatives of *A. peruvianum* are used as flavouring agents. *Anogramma leptophylla* concentrates 6-methoxymellein (71.5%), a bitter phytoalexin which contributes to stress or pathogen resistance. *Cheilanthes maderensis* produces mainly coumarin (89%) and vanillin (3.5%) with a low odor detection threshold, both used in perfumery and cosmetic industry or as flavouring agent and drug additives. *Cryptogramma crista* accumulates a broad-spectrum of carotenoid derivatives (52.1%) and three major shikimic derivatives: the spicy 4-vinylguaiaicol (flavouring agent), the floral phenylethanal and benzyl alcohol with floral, balsamic scent. *Pteris cretica* accumulates mostly furan derivatives, i.e., 5-hydroxymethylfurfural (33.2%) and 3-hydroxy-2,3-dihydromaltol (18.3%) used as food and beverage additives with caramel or roasty flavour and also found in fortified wines, toasty or heat-treated foods. *Pteris vittata* produces predominantly shikimic derivatives applied in perfumery and food industries as benzaldehyde (26%, with almond scent), benzyl alcohol (22%, floral fruity balsamic scent), nonanal (19.8% cucumber note) and phenylethanal (11%; floral note). *Pteridaceae* resources are of great interest as a reservoir of odorous and bioactive compounds.

Keywords: Benzaldehyde, Coumarin derivatives, Furan derivatives, Linalool, 6-Methoxymellein, Nonanal, 4-Vinylguaiaicol.

Pteridaceae E. D. M. Kirchner is a heterogeneous family of ferns including approximately nine hundred species worldwide distributed. Only twelve species belonging to seven genera (*Adiantum* L., *Anogramma* Link, *Cheilanthes* Swartz, *Cosentinia* Tod., *Cryptogramma* R. Br. ex Richardson, *Notholaena* R. Br. = *Paragymnopteris* K.H. Shing and *Pteris* L.) are reported in France [1a,b].

Pteridaceae includes several species well-known in traditional medicines and non-pharmacological interventions, i.e., Native American people medicine (Navajo Indian Tribe), Ayurvedic medicine, homeopathy linked with various diseases or disorders as follows. Leaves of *Adiantum pedatum* L. are employed for pectoral affections, *Adiantum poiretii* Wickstr. against fever and diabetes, *Notholaena eckloniana* Kunze (L.) as ointment on the scalp and *Pteris wallichiana* J. Agardh applied to stop bleeding. Fronds of *Pellaea calomelanos* (Sw.) Link are used against asthma; those of *Pteris multifida* Poir. and *Pteris cretica* L. are applied against dysentery and wounds, respectively [1c-f]. Leaves of *Adiantum capillus-veneris* L. (also known as Venus-hair fern) are used against throat affections and, as purgative and demulcent. This fern is also the main ingredient of the renowned "Sirop de Capillaire" supposed to cure a large number of diseases [1f]. On the other hand, Venus-hair fern produces a pleasant tonic flavor and syrups which are used as a flavor modifier [1f]. Rhizomes of *Cheilanthes tenuifolia*

(Burm. f.) Sw., are administered as general tonic while those of *Pteris ensiformis* Burm. f. and *Pteris quadriaurita* Retz. are applied on swollen glands in the neck or healing of boils, respectively. It should be mentioned *Adiantum lunulatum* Burm. to be used against fever due to elephantiasis. Finally root of *Cheilanthes farinosa* (Forsk.) Kaulf. treats eczema and stomachache [1c-f].

During the last decades several studies have been carried out regarding the biological properties of *Pteridaceae*: an antioxidant activity was detected in *Adiantum trapezifolium* L. and *C. tenuifolia* [2a,b] and antimicrobial compounds were characterized in *Pteris vittata* L. and *Pteris biaurita* L. [2c,d]. Biological activities involved in metabolic syndrome and anti-tumor activity were investigated for *P. vittata* [2e,f]. The impact of heavy metals on antioxidant polyphenols of this hyperaccumulator fern was also analysed [2g]. In addition, an anti-tumor activity was detected in *Pteris semipinnata* L. and *Pteris multifida* [2h,i] while an aqueous extract of *Pteris ensiformis* demonstrates an immunomodulatory activity [2j].

Very few *Pteridaceae* are known having an odor [3a]: only *Adiantum pantadactylon* Langsd. & Fisch. and *Pteris tremula* R. Br. are reported to smell tom cat urine. *Pteris multifida* has an acrid and biting flavor whereas *Adiantum pedatum* has a slightly aromatic odor.

Table 1: Percentage of volatile organic compounds^a in fresh aerial part of seven species of *Pteridaceae*.

Compounds	RI ^b	<i>Adiantum pedatum</i>	<i>Adiantum peruvianum</i>	<i>Anogramma leptophylla</i>	<i>Cheilanthes maderensis</i>	<i>Cryptogramma crispa</i>	<i>Pteris cretica</i>	<i>Pteris vittata</i>
Lipidic derivatives								
Furfural	840						3.0	
Furfuryl alcohol	910						1.2	
5-Methylfurfural	965						2.1	
1-Octen-3-ol	976	5.0	7.1			5.6	3.9	9.5
2,3-Octanedione	979		2.0					
3-Octanol	990		1.6				1.1	
(<i>E</i>)-3-Hexenoic acid	1043		23.3					
(<i>E</i>)-Oct-2-en-1-ol	1067		0.8					
(<i>E</i>)-2-Hexenoic acid	1068		19.0					
Furanol	1083						3.8	
Nonanal	1107	0.8	0.8	0.8	1.0	0.7	1.6	19.8
5-Hydroxymethylfurfural	1273						33.2	
6-Methoxymellein	1893			71.5				
NI	1907			1.7				
6-Hydroxymellein	1947			1.8				
NI ^c	1980			1.0				
Shikimic derivatives								
Benzaldehyde	962	2.4		7.7		3.3	1.2	26.0
Benzyl alcohol	1034	5.6	4.6			6.1	1.2	22.0
Phenylethanal	1045	1.9		1.0		8.8	0.8	11.0
Acetophenone	1065							4.0
2-Phenylethanol	1118		2.9			3.2		
3,5-Dihydroxy-6-methyl-2,3-dihydro-4 <i>H</i> -pyran-4-one	1169						18.3	
4-Ethenyl-2-methoxyphenol	1316		0.6			10.0	2.7	
NI	1320	2.4						
2,3-Dihydrocoumarin	1389		1.1	0.3	3.4			
4-Hydroxy-3-methoxybenzaldehyde (vanillin)	1402	1.6	1.7		3.5	3.5	1.9	6.1
Coumarin	1443		1.2	7.8	89.0			
4-Hydroxybenzoic acid	1575					3.2		
4-Hydroxy-3-methoxybenzoic acid (vanillic acid)	1603					2.1		0.3
4-Hydroxy-3-methoxyacetophenone	1743			3.5				
6,7-Dimethoxycoumarin (scoparone)	1775			0.8				
7-Methoxy-6-prenylcoumarin (suberosin)	1970			1.0				
Terpenic derivatives								
Linalool	1102	10.8	0.6					
(<i>Z</i>)-2,6-Dihydroxy-2,6-dimethyl-3,7-octadiene	1194	4.6	1.4					
2,6-Dimethyl-3,5,7-octatriene-2-ol (<i>E,E</i> or <i>E,Z</i>)	1212	3.9						
8-Hydroxylinalool	1369	2.6	1.4					
Carotenoid derivatives								
3,4-Dehydro-7,8-dihydro- β -ionone	1413	2.4				1.6	0.4	
α -Ionone	1428		0.6					
NI	1434					1.2	2.4	
NI	1457						0.9	
3,4-Dehydro- β -ionone	1475						1.1	
β -Ionone	1477		1.2				0.5	
NI	1486						0.7	
10-Methyl- α -ionone	1492						0.7	
NI	1520					4.8		
Dihydroactinidiolide	1527		0.4			0.2		
8-Methyl- α -ionone	1548					8.8		
NI	1580	0.4						
(<i>E,E</i>)-Pseudoionone	1584	11.0					0.5	
NI	1605		6.1				0.5	
NI	1615	3.0						
3-Hydroxy- β -damascone	1616		1.2				0.5	
3-Hydroxy- β -ionol	1630	3.0						
3-Hydroxy-7,8-dehydro- α -ionol	1633		1.2				0.4	
9-Methyl- α -ionol	1637	5.0	0.6			9.2	2.5	0.2
3-Oxo- α -ionol	1650	4.0	1.2		0.3	5.2	1.5	
4-Hydroxy-7,8-dihydro- β -ionone	1651	3.6	3.1			6.8	1.5	
3-Hydroxy- β -ionone	1664	3.6					2.6	
4-Hydroxy-5,6-epoxy-ionol	1675	13.4	3.2		1.6	1.1	4.5	
3-Hydroxy-7,8-dihydro- β -ionol	1678	2.0	2.6					
3-Hydroxy-5,6-epoxy-ionone	1691	6.5	4.4			12.2	1.6	0.3
2-Hydroxy- β -ionone	1693		1.9			1.0		
3-Oxo-6-hydroxy- α -ionone	1796		0.6					

^a Relative percentage of the VOC based on the GC-MS chromatographic area; ^b RI = Retention Indices on SLBTM-5MS column (Supelco); ^c NI = Non Identified

With a view to continue our study of Volatile Organic Compounds (VOC) with bioactive potential, fresh aerial parts of seven ferns of *Pteridaceae* harvested in France were investigated for their VOC profile using GC-MS: *Adiantum pedatum* L., *Adiantum peruvianum* Klotzsch, *Anogramma leptophylla* (L.) Link, *Cheilanthes maderensis* Lowe, *Cryptogramma crispa* (L.) R. Br., *Pteris cretica* L. and *Pteris vittata* L.

In the concentrated diethyl ether extracts of the seven species, fifty-three components biosynthesized from lipidic, shikimic, terpenic and carotenoid pathways were identified (Table 1). The volatile fraction of the ferns represents about 0.01% of the fresh aerial materials.

Twenty volatile compounds were identified in *Adiantum pedatum*. Lipidic derivatives are mainly represented by 1-octen-3-ol (5%) responsible for the mushroom-like odor and flavor [3b-d] but also found in many plants [3e-g]. This fatty alcohol is valuable to perfume and food industries [3h, 4a,b] and more recently proposed for mosquito control as an insect attractant [4c,d]. Benzyl alcohol (5.6%), the major compound of the shikimic pathway with floral odor also described as phenolic or balsamic [4a] and the main terpenic derivative linalool (10.8%) with floral, fruity scent [4c] or woody note (depending on the enantiomer) were also reported in table 1. Linalool is a well-known terpenic alcohol of essential oil from various plant families (*Lauraceae*, *Rutaceae*, *Lamiaceae*...); it gives insect repellent property as well as anxiolytic, anti-

inflammatory, antioxidant, antifungal, antibacterial, antiparasitic, antitumoral activities [4e-i]. This fern contained the highest proportion of isoprenoid flavor precursors (57.9%), i.e., mainly (*E,E*)-pseudoionone (11%) with odor descriptors as sweet, waxy, citrus, floral balsamic, spicy [4a,j], 4-hydroxy-5,6-epoxy-ionol (13.4%), 3-hydroxy-5,6-epoxy- β -ionone (6.5%) and 9-methyl- α -ionol (5%).

Adiantum peruvianum showed a VOC profile based on twenty-nine compounds, radically different from the previous *Adiantum* species: lipidic derivatives (54.6%) were mainly represented by (*E*)-3-hexenoic acid (23.3%) with honey odor and waxy, fruity or herbal notes [4a], (*E*)-2-hexenoic acid (19%) with fruity odor and 1-octen-3-ol (7.1%) with mushroom-like scent. The two major odorant hexenoic acids, used as flavouring agents, were previously found in other ferns such as *Athyrium filix-femina*, *Gymnocarpium dryopteris*, *Polystichum setiferum*, *Pteridium aquilinum* [5a,b] and plant allies (*Equisetum palustre*) [5c] but not in *Adiantum capillus-veneris*. In Venus-hair fern, (*E*)-2-decenal, lauric amide or (*E*)-2-heptenal were found in high quantities with a plastic or oxidized mutton fat odor [5a], also responsible for the unpleasant scent of “stink bug”. The VOC profiles of the three species of *Adiantum* are therefore different.

Carotenoid derivatives of *A. peruvianum* (28.3%) were composed by small amounts of α -ionone, β -ionone and ionone derivatives, i.e., 3-hydroxy-5,6-epoxy- β -ionone. The VOCs from the shikimic pathway (12.1%) were represented by few compounds including benzyl alcohol (4.6%) also described in *A. pedatum* (Table 1) as well as 2-phenylethanol, vanillin or coumarin previously found in *A. pedatum*, *A. peruvianum* and *A. trapeziforme* [5d]. Three minor terpenic compounds including linalool were also identified in *A. peruvianum* (3.4%). This second *Adiantum* species, as well as the previously analysed Venus-hair fern, produced small amounts of terpenic derivatives. The five other ferns analysed in this work and belonging to four other genera of *Pteridaceae* did not produce any terpenic derivatives.

Ten VOCs were detected from *Anogramma leptophylla*. The volatile pattern was mainly based on lipidic derivatives (76%), i.e., the major 6-methoxymellein (71.5%) and the minor 6-hydroxymellein (1.8%) which are 3,4-dihydroisocoumarins. The former is a polyketide-derived phytoalexin well-known in the carrot and would contribute to pathogen or stress resistance. It is the first compound related to the bitterness of the carrot and its content varies in the commercial products with storage and processing conditions [6a-c]. Dihydroisocoumarins have been isolated from other plants species and also from macrofungi [6d,e]. The others VOC isolated from *A. leptophylla* were shikimic derivatives (22.1%), i.e., benzaldehyde (7.7%) widespread in plants and mushrooms with bitter almond odor and coumarin (7.8%) with pleasant scent. These VOCs are two aroma agents commonly used in perfume, cosmetic and food industries.

The volatile content of *Cheilanthes maderensis* was mainly dominated by shikimic derivatives (95.9%) essentially coumarin (89%: hay and dried herb odor), 3,4-dihydrocoumarin (3.4%; hay-like, herbal, coconut note) and vanillin (3.5%; vanilla, sweetish smell) usually used in perfume and food industries [3h; 4a,b]. Such high content of coumarin and coexistence of its dihydro derivative as natural products are very rare. Recently, a Japanese group reported the similar data from the bryophyte, *Takakia lepidozoioides* [3i], as those reported in the present paper. At the same time, chemophylogenetic relationship between both phyla (Pteridophytes and Bryophytes) has been fully discussed [3j].

The broad spectrum of volatile components identified in *Cryptogramma crispera* showed a VOC profile including nineteen identified compounds. Table 1 lists major carotenoid derivatives (52.1%), i.e., 3-hydroxy-5,6-epoxy-ionone, 9-methyl- α -ionol, 8-methyl- α -ionone, 4-hydroxy-7,8-dihydro- β -ionone and 3-oxo- α -ionol. Shikimic derivatives (40.2%) are mainly represented by three VOCs. 4-Ethenyl-2-methoxyphenol also called 2-methoxy-4-vinylphenol or 4-vinylguaiaicol (10%) with powerful, clove-like, spicy, smoky odor is also a flavouring agent and a pheromone for insects [4b,j; 7a,b]. It was previously found in a horsetail, *Equisetum telmateia* [5c]. Phenylethanal (8.8%) with floral odor (lilac, hyacinth, geranium: [3b, 4b]) was also identified in other ferns and plant allies (*Athyrium filix-femina*, *Blechnum spicant*, *Phegopteris connectilis*, *Equisetum scirpioides*) [5a-c]. The third shikimic derivative was benzyl alcohol (6.1%) with floral or balsamic odor. Only 1-octen-3-ol from lipidic pathway was identified in a significant amount (Table 1).

Table 1 lists a broad spectrum of VOCs for *Pteris cretica* based on twenty-seven identified components mainly lipidic derivatives (49.9%), shikimic derivatives (26.1%) and carotenoid derivatives (22.8%) in low amounts. Only two VOCs were abundant as 5-hydroxymethylfurfural (33.2%) and 3,5-dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (18.3%). The major 5-hydroxymethylfurfural is described with odor of chamomile flowers (or butter, caramel, musty) while the four others furan derivatives (10%) have various descriptors [8a-d] as furfural (almond, woody, sweet, toasty), furfuryl alcohol (faint burning odor), furaneol (sweet, caramel, pineapple, strawberry) and 5-methylfurfural (caramel, almond, spicy, sweet, roasty). These compounds usually found in fortified wines, in roasted, toasted or heat-treated foods and drinks, are produced, in particular, by sugar alteration (Maillard reaction). Suspected but not proved to be carcinogenic, they contribute to caramel aroma and colour in food additives [8e-h]. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4*H*-pyran-4-one (or 3-hydroxy-2,3-dihydromaltol 18.3%, Table 1), the second major VOC exhales an odor with toasty character and fruity-caramel overtones [7a].

Pteris cretica was the single species of the seven analysed ferns with a high level of furaneol and furfural derivatives (43.2%, Table 1) whereas the others (in particular *P. vittata*) contain none at all. Furan derivatives were not found in *A. capillus-veneris*, another species from *Pteridaceae* previously studied [5a]. However, ferns species from other families may also produce furan derivatives in small amounts, i.e., *Pteridium aquilinum*, *Asplenium trichomanes*, and the twelve species of the *Asplenoideae* family [5b,9a]. Other authors found furan derivatives in coalified *Trigonocarpus grandis* [9b] or in aerial parts and rhizomes of current species of ferns: *Angiopteris esculenta*, *Cibotium barometz*, *Coniogramme japonica*, *P. aquilinum*, and five species of *Polypodiaceae* [9c-g].

Pteris vittata revealed a VOC fraction very different from that of *P. cretica* with a low diversity (ten volatile compounds), barely any carotenoid derivatives and a majority of shikimic derivatives (69.4%) with the three major odorant benzaldehyde (26%; bitter almond scent), benzyl alcohol (22%; floral notes) and phenylethanal (11%; sweet odor of hyacinth-type). These three VOCs were also detected in five others ferns of the same family analysed in this work but in lower amounts. Lipidic derivatives were represented by nonanal (19.8%; floral-waxy note, [7a]) and 1-octen-3-ol (9.5%; fungal aroma, [3b-d]) recently reported as antifungal agent [10a] and attractant for *Anopheles* and *Aedes* mosquitoes, repellent to *Culex quinquefasciatus* [10b], respectively.

This paper demonstrates that *Peridaceae* can generate a broad spectrum of VOCs for both odorous and bioactive ingredients. Within the former, lipidic derivatives, terpenic compounds and ionone derivatives with fruity odor, herbal scent or floral notes, are the main fragrant components required for cosmetic and hygiene products industries as well as aroma applications: it should be noted that surprising high amount of furan derivatives with caramel or roasty flavor was detected to be used as food additives. Within the last, coumarin derivatives are of various biological interests for pharmaceutical industry and in plant protection products. *Pteridaceae* species resources are potential candidates for bioactive aroma ingredients and for the discovery of new drugs with various therapeutic applications due to their potential anti-inflammatory [10c] and antitumor [10d] promoting properties.

Experimental

Plant material: Fresh aerial parts of ferns were collected in France, as follows: *Pteris cretica*, *P. vittata* and *Adiantum peruvianum*: 31/08/2010, Botanical Garden of Strasbourg; *Cryptogramma crispa*: 01/09/2010, Botanical Garden of Nancy; *Adiantum pedatum*: 01/09/2010, Botanical Garden of Col de Saverne; *Anogramma leptophylla*: 14/04/2010, Le Lavandou (Var);

Cheilanthes maderensis: 13/04/2010, Rayol-Canadel-sur-Mer (Var); Voucher specimens are deposited at the Laboratory of Botany (Faculty of Pharmacy, Limoges, France).

Plant part and GC-MS analyses: Fresh aerial parts of ferns were cubed and extracted with diethyl ether (Carlo Erba, 6 ppm BHT). After one week of maceration at room temperature, the concentrated organic extracts were used for Gas Chromatography Mass Spectrometry (GC-MS) analyses as reported in the literature [5a-c]. The main volatile components of *Pteridaceae* were identified by comparison with National Institute of Standards and Technology Mass Spectral Library [11a-b]. Internal standards (*n*-alkanes) were used as reference points in the calculation of relative retention indices. GC-MS analyses were performed at the « Plateforme d'Analyses Chimiques en Ecologie », technical facilities of the LabEx CeMEB (Centre Méditerranéen pour l'Environnement et la Biodiversité).

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Element Content is a Highly Reliable Marker for Niche Vegetable Oils Faez Mohammed, Dom Guillaume, Nada Abdulwali, Rahma Bchitou, Souad El Hajjaji and Ahmed Bouhaouss	609
Bentonite as a Refining Agent in Waste Cooking Oils Recycling: Flash Point, Density and Color Evaluation Alberto Mannu, Gina Vlahopoulou, Veronica Sireus, Giacomo Luigi Petretto, Gabriele Mulas and Sebastiano Garroni	613
Chemical Composition of the Essential Oils of <i>Pogostemon auricularius</i>, a Vietnamese Medicinal Plant Prabodh Satyal, Nguyen Thi Hong Chuong, Van The Pham, Nguyen Huy Hung, Vu Thi Hien and William N. Setzer	617
Comparative Chemical Profiles of Essential Oil Constituents of Eight Wild <i>Cinnamomum</i> Species from the Western Ghats of India Ramamoorthy Ananthakrishnan, Ettickal. S. SanthoshKumar and Koranappallil B. Rameshkumar	621
Constituents of Essential Oils from <i>Dasymaschalon bachmaensis</i> and <i>Phaeanthus vietnamensis</i> Le T. Huong, Dao T.M. Chau, Ly N. Sam, Tran D. Thang, Do N. Dai and Isiaka A. Ogunwande	627
Antileishmanial Potentialities of <i>Croton linearis</i> Leaf Essential Oil Jesús García Díaz, Julio César Escalona Arranz, Denise da Gama Jaén Batista, Lianet Monzote Fidalgo, Jorge de la Vega Acosta, Maira Bidar de Macedo and Paul Cos	629
Circadian Rhythm, and Antimicrobial and <i>Anticholinesterase</i> Activities of Essential Oils from <i>Vitex gardneriana</i> Evaristo Jose Pires Pereira, Jean Parcelli Costa do Vale, Priscila Teixeira da Silva, Joyce dos Reis Lima, Daniela Ribeiro Alves, Patricia Silva Costa, Tigressa Helena Soares Rodrigues, Jane Eire Silva Alencar de Menezes, Selene Maia de Moraes, Paulo Nogueira Bandeira, Raquel O.S. Fontenelle and Hécio Silva Santos	635
Antiacne-causing Bacteria, Antioxidant, Anti-Tyrosinase, Anti-Elastase and Anti-Collagenase Activities of Blend Essential Oil comprising Rose, Bergamot and Patchouli Oils Nuntapol Wongsukkasem, Orawan Soynark, Montira Suthakitmanus, Emprang Chongdiloe, Chidchanok Chairattanapituk, Peamjit Vattanikitsiri, Tapanee Hongratanaworakit and Sarin Tadtong	639
<u>Accounts/Reviews</u>	
Tubeimoside-1, Triterpenoid Saponin, as a Potential Natural Cancer Killer Muhammad Zafar, Iqra Sarfraz, Azhar Rasul, Faiza Jabeen, Khizar Samiullah, Ghulam Hussain, Ammara Riaz and Muhammad Ali	643
<i>Pteridaceae</i> Fragrant Resource and Bioactive Potential: a Mini-review of Aroma Compounds Françoise Fons, Didier Froissard, Sylvie Morel, Jean-Marie Bessière, Bruno Buatois, Vincent Sol, Alain Fruchier and Sylvie Rapior	651

Natural Product Communications

2018

Volume 13, Number 5

Contents

Gerald Blunden Award (2017)

Page

Molecular Insights of Hyaluronic Acid as Potential Source of Polymer-Drug Conjugate in the Target-Mediated Treatment of Cancer

Gnanendra Shanmugam, Rajesh Salem Varadharajan, Desika Prabakar, Syed Mohammed, Sathiyapriya Renganathan, Murano Erminio and Vincent Aroulmoji

513

Original Paper

Sesquiterpene Lactones and Phenols from Polyfollicles of *Magnolia vovidesi* and their Antimicrobial Activity

Thalia Ramírez-Reyes, Juan L. Monribot-Villanueva, Oscar D. Jiménez-Martínez, Ángel S. Aguilar-Colorado, Israel Bonilla-Landa, Norma Flores-Estévez, Mauricio Luna-Rodríguez and José A. Guerrero-Analco

521

Chemical Composition and Antinociceptive Potential of *Plinia edulis* Fruits Peels

Luciane Angela Nottar Nesello, Adriana Campos, Karla Capistrano, Fátima de Campos Buzzi and Valdir Cechinel Filho

527

Two New Antidepressant Steroidal Aglycones from *Stephanotis mucronata*

Shu-juan Hao, Li-juan Gao, Shi-fang Xu, Yi-ping Ye and Xiao-yu Li

531

Strychnuxinal, A New Alkaloid from *Strychnos nux-blanda* Fruits

Jirapast Sichaem, Santi Tip-pyang, Kiattisak Lugsanangarm and Lien Do Thi My

533

Chemical Constituents of the Different Parts of *Colchicum micranthum* and *C. chalcedonicum* and their Cytotoxic Activities

Gizem Gulsoy-Toplan, Fatih Goger, Ayca Yildiz-Pekoz, Simon Gibbons, Gunay Sariyar and Afife Mat

535

Hairy Root Cultures of *Eurycoma longifolia* and Production of Anti-inflammatory 9-Methoxycanthin-6-one

Trang Thu Tran, Nam Trung Nguyen, Ngoc Bich Pham, Huy Nhat Chu, Trong Dinh Nguyen, Tadamitsu Kishimoto, Minh Van Chau and Ha Hoang Chu

539

Eliciting Effect of Catharanthine on the Biosynthesis of Vallesiachotamine and Isovallesiachotamine in *Catharanthus roseus* Cambial Meristematic Cells

Jianhua Zhu, Shuijie He, Pengfei Zhou, Jiachen Zi, Jincui Liang, Liyan Song and Rongmin Yu

543

Anti-inflammatory Effect of Pradol in LPS-stimulated RAW 264.7 Cells via NF- κ B Signaling Pathways

You Chul Chung, Sung-Min Park, Jin Hwa Kim, Geun Soo Lee, Jung No Lee and Chang-Gu Hyun

547

Flavonoid Aglycones and Glycosides from the Leaves of some Japanese *Artemisia* Species

Ayumi Uehara, Kazuhide Shimoda, Yoshinori Murai and Tsukasa Iwashina

551

LC-MS Identification of Proanthocyanidins in Bark and Fruit of six *Terminalia* species

Awantika Singh, Sunil Kumar and Brijesh Kumar

555

Protective Effects of Compounds in *Bombax ceiba* flower on Benzo[a]pyrene-Induced Cytotoxicity

Souichi Nakashima, Yoshimi Oda, Yuki Ogawa, Seikou Nakamura, Miyako Uno, Mariko Kishimoto, Masayuki Yoshikawa and Hisashi Matsuda

561

Antioxidant and Cosmeceutical Activities of *Agarum cribrosum* Phlorotannin Extracted by Ultrasound Treatment

Kasira Phasanasophon and Sang Moo Kim

565

Bioactive Metabolites from a Hydrothermal Vent Fungus *Aspergillus* sp. YQ-13

Qiannan Tao, Chihong Ding, Bibi Nazia Auckloo and Bin Wu

571

***Osmanthus fragrans* Flower Aqueous Extract and its Enriched Acteoside inhibit Melanogenesis and Ultraviolet-induced Pigmentation**

Shuo Liu, Zhen Zhao, Zhijun Huo, Zhiru Xu, Yan Zhong, Xiaoling Wang, Yiting Yang and Zhiyong Wang

575

Synthesis of new A-conjugated Quinolone and Spiroindole Dammaranes by the Ozonolysis of 2,3-Indolodipterocarpol

Irina E. Smirnova, Elmira F. Khusnutdinova, Alexander N. Lobov and Oxana B. Kazakova

581

A New Cytotoxic Tetrahydroxanthene-1,3(2H)-dione Derivative from *Uvaria cordata* and Structure Revision of Valderramenol A

Duc Viet Ho, Hung Quoc Vo, Tho Huu Nguyen, Thao Thi Do and Hoai Thi Nguyen

585

Synthesis of Novel 2-Thioxothiazolidin-4-one and Thiazolidine-2,4-dione Derivatives as Potential Anticancer Agents

Alleni Suman Kumar, Rathod Aravind Kumar, Elala Pravardhan Reddy, Vavilapalli Satyanarayana, Jajula Kashanna, Boggu Jagan Mohan Reddy, Basireddy Venkata Subba Reddy and Jhillu Singh Yadav

589

A Short Step Conversion of Alkynyl Propargyl Sulfones into Six-Membered Cyclic β -Ketosulfones via an Amine-Induced Novel Ring Closure

Md. Ashraful Alam, Kazuaki Shimada, Hironobu Kamoto, Kasumi Shingo, Toshinobu Korenaga and Chizuko Kabuto

593

Synthesis of Sex Pheromones of the Citrus Leafminer (CLM) (*Phyllocnistis citrella*)

Alleni Suman Kumar, Vavilapalli Satyanarayana, Ahmad Alkhamzi Alghamdi and Jhillu Singh Yadav

599

Composition, Anti-inflammatory Activity, and Bioaccessibility of Green Seaweeds from Fish Pond Aquaculture

Andrea Ripol, Carlos Cardoso, Cláudia Afonso, João Varela, Hugo Quental-Ferreira, Pedro Pousão-Ferreira and Narcisca M. Bandarra

603

Continued inside backcover