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Phenolic Constituents of *Erigeron floribundus* (Asteraceae), a Cameroonian Medicinal Plant

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HPLC-MS^n analysis of extracts of the Cameroonian medicinal plant *Erigeron floribundus* (Kunth) Sch. Bip. (Asteraceae) led to the identification of 40 different phenolic constituents. Four triterpene derivatives were isolated using semi-preparative HPLC and structures were elucidated on the basis of 1D and 2D NMR measurements. Compound **1**, olean-3-oleil-12,18 diene, was a new natural product. Quali-quantitative measurements on the infusion obtained from dried aerial parts were also performed by HPLC-MS^n and HPLC-DAD analysis showing that a cup of *E. floribundus* tea contains about 2.7 mg/mL of phenolics, with the caffeoyl quinic derivatives being the most abundant constituents.

Keywords: *Erigeron floribundus*, Asteraceae, Triterpenes, Phenolic acids, Olean-3-oleil-12,18 diene, Triglycerides, Tea.

Erigeron floribundus (Kunth) Sch. Bip. (Asteraceae) is a herb of 1.5 m in height, with pubescent, lanceolate leaves and flowers in pale-yellow panicles. In Cameroon it is commonly found as a weed along roadsides, and it is used in traditional medicine [1–4] for the treatment of angina [5], female infertility [6], dental pain and headache [3]. In Ivory Cost the plant is used for the treatment of skin disorders [7], dyspepsia [2], abdominal pains [8], various diseases of microbial and non-microbial origin [9] and in AIDS therapy [10]. Previous publications demonstrated several pharmacological properties for different plant extracts. Peripheral and central analgesic effects, as well as anti-inflammatory activity were reported for the leaf aqueous extract [1]. Antifungal activity against dermatophytes was described for the dichloromethane extract [7], while ethanol and pentane leaf extracts revealed antiplasmodial activity, comparable with *Azadirachta indica* and *Artemisia annua* extracts [11]. The use of the plant as a herbal treatment of AIDS suggested the potential immunomodulatory properties of the extract. Recently, such properties were reported for the aqueous extract that stimulated the increase of neutrophils, total lymphocytes and TCD4+ in rabbits [10].

Despite the interest in its bioactivity, the plant has received little phytochemical investigation. Only C_{10}-polyacetylenes with allelopathic properties [12] and essential oils were previously characterized [13]. In the present work a comprehensive phytochemical analysis was performed on *E. floribundus* from Cameroon. The compounds were identified using HPLC-MS on the basis of their mass spectral fragmentation, as well as by comparison with reference compounds. Moreover, phytochemical investigation allowed us to isolate and characterize a new triterpene ester, olean-3-oleil-12,18 diene (**1**) (Figure 1), as well as β-amyrin, isoaleuritolic acid and β-amyrenone. The investigation showed the presence of numerous phenolic and terpene derivatives, thus giving new information about the possible bioactive constituents of the plant.

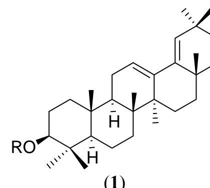

Figure 1: Structure of olean-3-oleil-12,18-diene; R= oleic acid (**1**).

HPLC-MS^n in negative mode allowed us to identify forty different constituents on the basis of their spectral data (Figure 2). Table 2 summarizes the results and the identified constituents both in roots (R) and aerial parts (AP). The plant contains mainly caffeoyl and feruloyl esters with quinic acid, quercetin glucoside and glucuronide, as well as salicylic acid and erigeside. Comparing the chemical composition of epigeal and hypogeal parts, differences were observed due to the absence of coumaroyl esters, and salicylic and syringic acid derivatives in the roots, compared with the aerial parts. Furthermore, fulvic acid and leucotoxins were also revealed by HPLC-MS measurements (see Table 2 compounds 41-44) and such metabolites may be related to the presence of microorganisms in the plant material.

No phenolics were identified in the lipophylic extracts that were mainly composed of triglycerides. HPLC- MS^n with APCI ion source allowed the identification of a triacylglycerol with a molecular ion $[M+H]^+$ at *m/z* 856. Fragmentation of such a species revealed loss of palmitic (256 Da) and linoleic acids (280 Da) suggesting that the triacylglycerol was PLL. A second species with a different fragmentation pathway was also observed, showing loss of palmitic, oleic (282 Da) and linolenic acids (278 Da), thus being POLn triglyceride.

It is well known that Asteraceae are characterized by the production of triterpenes and that such compounds are of great interest for their different medicinal properties. For this reason we performed

Table 1: NMR spectral data of olean-3-oleil-12,18-diene (compound **1**).

Position	δ_H	δ_C
1	1.14	37.3
2	1.76	23.4
3	3.99 dd	82.0
4	-	38.9
5	1.85	50.0
6	1.35	22.1
7	1.93	30.8
8	-	41.0
9	2.74	18.6
10	-	37.2
11	2.69	25.0
12	5.39 brs	129.7
13	-	138.1
14	1.32	47.3
15	1.94	30.9
16	1.39	37.8
17	-	40.3
18	-	148.1
19	5.37 brs	130.1
20	-	31.1
21	2.05	39.6
22	1.34 s	37.0
23	1.03 s	28.5
24	0.90 s	18.6
25	0.92 s	21.6
26	1.07 s	20.9
27	1.32 m	26.9
28	0.93m	18.6
29	0.94 s	19.9
30	0.94 s	12.8
Oleic acid-3		
1'	-	176
2'-8'	2.34 t	27.8
10'-17'	1.32m	23.4–25.5
18'	0.93 t	18.5

chromatographic separation on the dichloromethane extract by means of flash chromatography and semipreparative HPLC. Four different triterpenes were isolated and characterized on the basis of their MS and NMR data. The most abundant derivatives were β-amyrin and β-amyrenone. A minor compound with unusual structure was also isolated (**1**).

Compound **1** (Figure 1) was isolated as a clear oil. The MS in positive ion mode showed a molecular ion at *m/z* 687. The ESI-MS^n measurements allowed the detection of the molecular fragments at *m/z* 423 and 405. The ^{1}H NMR spectrum (Table 1) was characterized by the presence of eight quaternary methyl groups at δ 1.03, 0.90, 0.92, 1.07, 1.32 0.94 , 0.95 and 0.93 ppm integrating for three protons each. Signals due to a fatty acid were also present, namely a triplet ascribable to the terminal methyl group, a broad multiplet at 1.32 ppm assigned to the fatty acid CH_2 chain and a double bond at 4.80 ppm. Further deshielded signals at 5.39 and 5.37 were observed and a multiplet integrating for one proton at 4.00 ppm was also present. The HSQC-DEPT spectrum revealed the presence of five methyl groups, two ascribable to sp^2 carbons (5.39–130.1 and 5.37–129.6), one to a deshielded aliphatic position (4.00-82.0) and two to further aliphatics (C-5 and C-9). HMBC correlations allowed us to establish the nature of the triterpene. Esterification with oleic acid was deduced from the HMBC correlation observed from H-3 with the carbonyl of the fatty acid residue (168.0). Thus the structure of compound **1** was deduced as olean-3-oleil-12,18-diene.

Dried aerial parts were used to prepare a tea and this preparation was subjected to HPLC-MS and HPLC-DAD analysis in order to assess its quali-quantitative composition (Table 3). The main extracted constituents were the caffeoyl quinic derivatives and it was estimated that 100 mL of tea would contain 210.8 mg, expressed as chlorogenic acid equivalents. Flavonoids, expressed as rutin equivalents, were about 59.6 mg for 100 mL of tea. Thus, the total phenolic content amounted to 270.3 mg for 100 mL of tea.

Experimental

Plant materials: Leaves of *E. floribundus* were harvested in Dschang, West Province of Cameroon (1450 m a.s.l.), in February 2014. The plant was identified at the Cameroon National Herbarium (Yaoundé), where a voucher specimen was deposited (5619SRF/Cam). Plant material was dried at room temperature in the shade for one week before undergoing total extraction.

Extraction of compounds: Dried aerial parts were ground and 300 g was extracted in an ultrasound bath using solvents of increasing polarity, namely light petroleum, dichloromethane and methanol. Each step was repeated 4 times with 450 mL of solvent in flasks extracting for 15 min each time. Liquid was decanted, filtered and solvents removed under vacuum to obtain 3 different extracts. Extraction yields, on the basis of dried weight of extracts on dried plant materials, were the following: light petroleum 2.5% (APE), dichloromethane 3.6% (ADM), and methanol 6% (AME). The same extraction procedure was applied to dried roots (100 g). On the basis of dried weight of extracts on dried plant materials extraction yields were the following: light petroleum 0.3% (RPE), dichloromethane 0.44% (RDM), and methanol 1% (RME). Due to the limited amount of material and high presence of lipids, only APE, ADM, RPE and RDM extracts were used for analytical purposes. Methanol extracts from aerial parts (AME) and roots (RME) were both used for analytical and preparative purposes.

Preparation of the infusion: For tea preparation, 2 g of dried plant material was treated with boiling water (50 mL) and infused for 15 min. The obtained liquid was then filtered and cooled. The volume was adjusted to 50 mL in a volumetric flask. An aliquot of the infusion was then filtered through a 0.45 µm membrane and used for HPLC analysis.

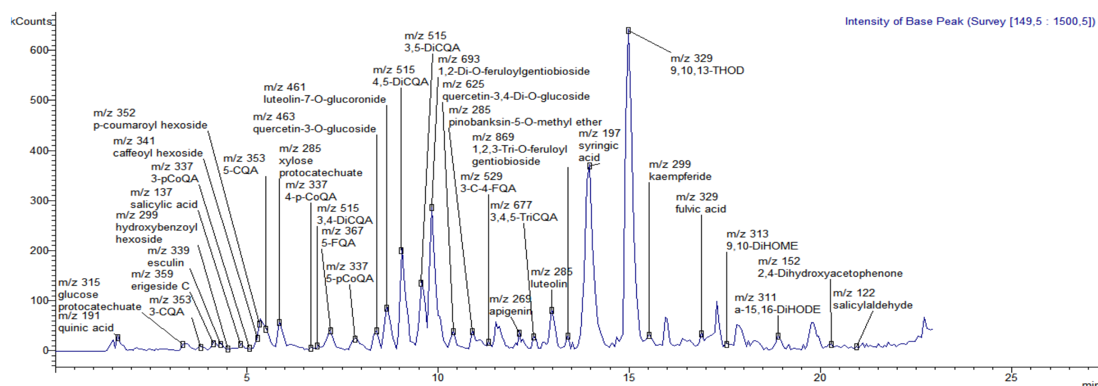

Figure 2: HPLC-MS^n chromatographic profile of the aerial part extract.

Table 2: HPLC-MS^n identified compounds with relative fragmentations present in aerial parts (AP) and roots (R) of *E. floribundus*. *Compared with standard compound.

	Compound name	$[M-H]^-$	MS^2	MS^3	MS^4	AP	R
1	Quinic acid (QA) *	191	127 173 111	85		x	x
2	Glucose protochatecuate [14,15]	315	153 162	109		x	x
3	3-Caffeoyl QA [16,17]	353	191 179	85		x	x
4	Erigeside C [18]	359	197	153 182	138 121	x	
5	Esculin* [19]	339	177 133			x	
6	Hydroxy benzyl hexoside [15]	299	137	93		x	
7	Salicylic acid*	137	93			x	
8	3-*O*-*p*-Cumaroyl QA [20]	337	163	119	93	x	
9	Caffeoyl hexoside [16,17]	341	179	135		x	
10	*p*-Cumaroyl hexoside [20]	325	163	119	93	x	
11	5-*O*-Caffeoyl QA [16,17]	353	191	85		x	x
12	Xylose protocatecuate [15,21]	285	153 133	109		x	x
13	4-*O*-*p*-Cumaroyl QA [16,17]	337	173	93		x	
14	3,4-Dicaffeoyl QA [16,17]	515	353 335	191 179		x	x
15	Quercetin-7-O-glucuronide*	477				x	
16	5-*O*-Feruloyl QA [16,17]	367	191	85		x	
17	Erigeside I [18]	435				x	
18	5-*O*-*p*-Cumaroyl QA [16,17]	337	191	127		x	
19	Caffeoyl hexoside	345				x	
20	Quercetin-3-*O*-glucoside*[21]	463	301	151 179 257		x	
21	Luteolin-7-*O*-glucuronide* [21]	461	285	175 243 199		x	
22	Eriodictyol-7-*O*-glucoside [21]	463	265			x	
23	4,5-Dicaffeoyl QA [16,17]	515	353	173		x	x
24	3,5-Dicaffeoyl QA [16,17]	515	353	191		x	x
25	1,3-diCQA glucoside [16,17]	677	515 497 353			x	
26	1,2-di-*O*-Feruloyl gentiobioside [22]	693	517 337 193			x	x
27	Scutellarin [21]	461	295			x	
28	Quercetin-3,4'-*O*-diglucoside [21]	625	463	301	151 271	x	
29	5-Methoxy pinobanskin [23]	285	267	239	211	x	
30	3-Caffeoyl-5-feruloil QA [16,17]	529	353 367			x	
31	3,4,5-Tricaffeoyl QA [16]	529	5353 367			x	x
32	Apigenin* [21]	269	225 149 107			x	
33	Luteolin*[21]	285	175 199 241			x	
34	1,2,3,-tri-Feruloyl gentiobioside [22]	869	693			x	
35	Syringic acid* [20]	197	182 153 138			x	
36	Kemferide [21]	299	284	256		x	
37	Salicylaldehyde*	122	124 137 109			x	
38	2,4-Diidrossiacetofenone [19]	154	125 137 109			x	
39	Pinobanskin*[23]	271	225 197			x	x
40	Protocathecuic acid*	153	97 79			x	x
	Other compounds	**$[M-H]^-$**	**MS^2**	**MS^3**	**MS^4**	**AP**	**R**
41	Fulvic acid [25]	329	311 293			x	x
42	9,10,13-Trihydroxy-11-octadienoic	329	311 293 275	229 293 275		x	x
43	15,16-Dihydroxy-9,12-octadecadienoic	311	267	223 253		x	x
44	9,10-Dihydroxy-12-octadecenoic	313	295 201 171				

Table 3: Quali-quantitative determination of phenolics in the *E. floribundus* tea.

Compound name	μg/mL
Quinic acid (QA)	25.0± 0.1
Glucose protochatecuate	17.0± 0.1
3-Caffeoyl QA	55.3± 0.1
Erigeside C	26.6± 0.1
Esculin	275.9± 0.1
Hydroxy benzyl hexose	21.0± 0.1
Salicylic acid	20.8± 0.2
3-*O*-*p*-Cumaroyl QA	9.5± 0.1
Caffeoyl Hexoside	38.1± 0.3
p-Cumaroyl hexoside	56.8± 0.1
5-*O*-Caffeoyl QA	49.3± 0.3
Xylose protocatecuate	46.3± 0.2
4-*O*-*p*-Cumaroyl QA	20.0± 0.1
3,4-Dicaffeoyl QA	22.0± 0.4
Quercetin-7-*O*-glucuronide	54.5± 0.2
5-*O*-Feruloyl QA	122.0±0.1
Erigeside I	90.9± 0.1
5-*O*-*p*-CumaroylQA	27.2±0.1
Caffeoyl glucose	8.0±0.2
Quercetin-3-*O*-glucoside	23.3± 0.1
Luteolin-7-*O*-Glucuronide	112.5± 0.1
Eriodictyol-7-O-glucoside	15.5± 0.1
4,5-Dicaffeoyl QA	541.8±0.5
3,5-Dicaffeoyl QA	67.7± 0.1
1,3-diCQA glucoside	46.4± 0.2
1,2-di-*O*-Feruloyl gentiobioside	15.6± 0.1
Scutellarin	28.8± 0.2
Quercetin-3,4'-*O*-diglucoside	12.0± 0.1
5-Methoxy pinobanskin	16.0± 0.2
3-Caffeoyl-5-feruloyl QA	384.6±0.2
3,4,5-Tricaffeoyl QA	200.0± 0.3
Apigenin	21.0± 0.1
Luteolin	11.0±0.03
1,2,3-tri-Feruloyl gentiobioside	45.0± 0.06
Syringic acid	107.0±0.07
Kaemferide	15.1± 0.3
Salicylic aldehyde	12.0± 0.3
2,4-Diidrossiacetofenone	20.0± 0.4
Pinobanskin	9.9± 0.2
Protocathecuic acid	12.0± 0.2
Total amount μg/mL	**2703.5**

HPLC/MS analysis: HPLC-MS^n were obtained on a Varian 212 chromatograph equipped with a Prostar 430 (Varian) autosampler and Ion trap Mass detector MS500 using both Electrospray (ESI) and Atmospheric Pressure Chemical Ionization (APCI). Separations were obtained on an Agilent Eclipse plus C-18 2.1 x 150 mm 3.5 μm. For the analysis of polar constituents the mobile phases were acetonitrile (A) and water with 0.1% of formic acid (B). The gradient started with 10% (A) and in 20 min reached 54% of (A), then in 23 min 100% (A). Re-equilibration time was 8 min. Flow rate was 200 μL/min. ESI parameters were: capillary voltage 80 V, needle voltage 5000 V, RF loading 100%, nebulizing gas pressure 35 psi, drying gas pressure 10 psi, drying gas temperature 350°C. Mass range was 50-200 Da. Fragmentation patterns of eluted compounds were obtained using the turbo detection data scanning (TDDS®) function of the instrument.

For lipid analysis, eluents were a mixture of methylterbutyl ether and methanol (90:10) (A) and acetonitrile (B). Gradient elution started with 5% of A and reached 95% in 15 min. Re-equilibration time was 10 min. APCI parameters were: drying gas temperature 300°C, mass range 50–2000 Da, ionization in positive ion mode. Fragmentation patterns of eluted compounds were obtained using the turbo detection data scanning (TDDS) function of the instrument.

HPLC analysis: The quantitative analysis of tea was performed on an Agilent 1260 series HPLC system equipped with autosampler and diode array detector (DAD). Chromatographic separation was performed on an Agilent Eclipse plus C18 column 2.1 x 150 mm, 3.5 μm. The mobile phase consisted of acetonitrile (A) and aqueous formic acid 0.1% (B). The solvent flow rate was 1 mL/min. The gradient used was as follows: from 1% of (A) to 99% of (A) in 35 min and isocratic until 50 min. Re-equilibration time to initial

conditions was from 51 to 55 min. The column was thermostated at 25°C. The selected wavelengths were 330 and 350 nm for caffeoyl quinic derivatives and flavonoids, respectively. As reference compounds, chlorogenic acid and rutin were used at 4 levels of concentrations each to build the calibration curves. For chlorogenic acid the concentration were 2.7 µg/mL, 5.4 µg/mL, 27 µg/mL and 270 µg/mL; for rutin 6.7 µg/mL, 13.5 µg/mL, 27 µg/mL, 270 µg/mL. Curves were y= 55.32 x + 6.41 for chlorogenic acid and y= 13.37 x + 0.8932 for rutin.

NMR analysis: NMR spectra were obtained on a Bruker Avance III spectrometer (400 MHz) dissolving the samples in deuterated chloroform. 2D experiments namely COSY, HSQC-DEPT, HMBC, TOCSY and NOESY were used for structure elucidation.

Optical rotation: Measurements were obtained on a Yasco 2000 digital polarimeter in a 1 dm tube at 25°C.

Compound 1
$[\alpha]^{25}_{D}$: – 3.0 (*c* 0.25, MeOH).
^{1}H and ^{13}C NMR ($CDCl_3$): Table 1.

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