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1 **SUPPLEMENTARY MATERIAL**

2 # Chemical characterization of unconventional palm oils from *Hyophorbe*
3 *indica* and two other endemic Arecaceae species from Reunion Island.

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5 Yanis Caro[a,b,*], Thomas Petit[a,b], Isabelle Grondin[a], Patricia Clerc[a], Hermann
6 Thomas[c], Daniele Giuffrida[d], Barbara Giocastro[e], Peter Q. Tranchida[e], Ivan
7 Aloisi[e], Daniella Murador[f], Luigi Mondello[e,g], Laurent Dufossé[a]
8
9 [a]*Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA),*
10 *Université de La Réunion, Sainte-Clotilde, Réunion ;*
11 [b]*Département Hygiène Sécurité Environnement (HSE), IUT de La Réunion, Université de La*
12 *Réunion, Saint-Pierre, Réunion ;*
13 [c]*Parc National de La Réunion, Saint-Denis, Réunion;*
14 [d]*B.I.O.M.O.R.F. Department, University of Messina, Messina, Italy;*
15 [e]*Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of*
16 *Messina, Messina, Italy;*
17 [f]*Departamento de Biosciências, Universidade Federal de São Paulo, Santos, Brazil;*
18 [g]*Chromaleont s.r.l., c/o "Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed*
19 *Ambientali", University of Messina, Messina, Italy.*
20 *Corresponding author e-mail: yanis.caro@univ-reunion.fr
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22 **Abstract**

23 Chemical characteristics of novel seed oils, yet not investigated, from three endemic Arecaceae (palm) species
24 from Reunion Island are described. Fatty acid profiles are performed using two-dimensional gas
25 chromatography-mass spectrometry. Carotenoid contents are determined by high performance liquid
26 chromatography-mass spectrometry. The results of the investigations emphasize the particular composition of
27 the unconventional red seed oil from *Hyophorbe indica*. Characteristic features of this oil reveal a high degree of
28 unsaturation (50% of polyunsaturated fatty acids, with a high content (17%) of omega-3), which is possibly a
29 unique fatty acid composition in the Arecaceae family. The two other palm oils from *Dictyosperma album* and
30 *Latania lontaroides* contain high level of saturated fatty acids very similar to that of the edible palm oil. *H.*
31 *indica* oil is also very rich in valuable carotenoids; in particular, lutein, β-carotene and lycopene are detected in a
32 high content (respectively 45, 23 and 35 mg.kg^{-1} in oil).

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34 **Keywords:** *Hyophorbe indica*; *Dictyosperma album; Latania lontaroides*; unconventional
35 oil; omega-3; Arecaceae; carotenoid.

Experimental section

1. Samples collection

The ripe fruits from the three endemic plant species were wild collected in the forest area of the Reunion Island (see **Table S1**). The endemic species were authenticated by the botanist Hermann Thomas of the National Park of La Réunion.

2. Oil extraction from seeds

The ripe fruits were cleaned and oven-dried at 60 °C during 24 to 48 hours. The shell was cracked to remove the seeds (kernels) and dried at 60 °C for 2 hours. The seeds (1.9 – 3.8 kg) were first milled in a laboratory grinder for 30 seconds in order to obtain a fine powder. Oil extraction was then carried out using cyclohexane (99.8% purity, Carlo Erba) from 50-60g of dried seed powder by pressurized liquid extraction under nitrogen at 90 °C under 100 bar with a Dionex ASE^{TM}300 apparatus (Dionex, USA). The following experimental conditions were applied for the pressurized liquid extraction: temperature 90 °C, cells were preheated 6 min, 17 min static time, 5 min dynamic time. The cycle was repeated 5 times. This program ensures complete extraction of the neutral lipids from the sample. A flush volume of 100% of the cell was used and finally the cell was purged for 120 sec with nitrogen to collect the extract in the collection vial. The solvent was finally driven off at 40 °C using a rotary evaporator (RC 600, KNF Neuberger, France) to obtain the extracted lipids. All quantitative determinations were performed in triplicate.

3. Fatty acids (FAs) analysis by cryogenic-modulation two-dimensional gas chromatography-quadrupole mass spectrometry (CM GC×GC-QMS)

Derivatization of fatty acid methyl esters (FAMEs) was performed as follows: 100 µL of a solution of KOH/MeOH 2N was added to 10 mg of oil sample, dissolved in 1 mL of n-hexane and was left to react for 5 min at room temperature. The reaction mixture was shaken for 2 min using a vortex mixing. The upper hexanic phase, containing FAMEs, was subjected to CM GC×GC-QMS analysis. All CM GC×GC-QMS analyses were carried out using a system consisting of two independent Shimadzu GC-2010 gas chromatographs, and a QP2020 quadrupole mass spectrometer (Kyoto, Japan). The first gas chromatograph (GC1) was equipped with an AOC-20i auto-injector, and a split-splitless injector (310°C). The first column was an SLB-5ms [(silphenylene polymer, which can be considered equivalent in polarity to poly (5%diphenyl/95% dimethylsiloxane)] with dimensions 30 m × 0.25 mm id × 0.25 µm d_f. The second column was an SLB-35ms [(polymer which can be considered equivalent in polarity to poly (35%diphenyl/65% dimethylsiloxane)] with dimensions 3 m × 0.25 mm id × 0.25 µm d_f. A 1.5 m segment of the column was used to create the modulator

72 loop, leaving a 1.5 m segment for the analytical separation. All the columns used were kindly
73 provided by Merck Life Science (Merck KGaA, Darmstadt, Germany). The connections
74 between first and second columns, were made by using two SilTite mini unions (Trajan,
75 Ringwood, Victoria, Australia). Carrier gas (helium) was supplied at a pressure of 60.3 kPa.
76 Sample (1 μL) was injected in the split mode (30:1). Temperature programs were set at 110-
77 320°C, and 3°C/min. Modulation was performed by using a cryogenic fluid-free modulator
78 (under license from Zoex Corporation); modulation period was 2.5 sec (the heating step was
79 performed at 340°C, for 0.5 s). For quadrupole MS analysis, the sample was analyzed in the
80 full scan mode with a scan speed of 20,000 amu/sec, a mass range of 45-500 *m/z* and a
81 sampling frequency of 33 spectra/sec. The temperature of the interface was set at 310°C and
82 the ion source temperature was set at 200°C, with analyte fragmentation induced by electron
83 ionization (70 eV). Data were acquired by using the GC-MS solution software (Shimadzu).
84 The MS database used was the Lipids GC-MS library v.1.0 (Shimadzu). Bidimensional
85 chromatograms were generated using the ChromSquare software v. 2.3 (Shimadzu). FAMEs
86 were identified by comparison of their retention times with those of pure reference standards.
87 The fatty acid profile of the oil was obtained by relative quantification from CM GCxGC-
88 QMS peak areas.
89
90 **4. Extraction of the unsaponifiable fraction of the oils**

91 Carotenoid analyses were performed on the unsaponifiable fractions of the oils. Firstly, the
92 unsaponifiable fraction was extracted from oils (4 g) using diethyl ether (3 x 100 mL) after
93 saponification of the oil by using 50 mL KOH/EtOH 1M under reflux for 1 hour according to
94 ISO standard 3596-1. Organic layer was washed with water (2 x 40 mL), aqueous solution of
95 KOH 0.5M (1 x 40 mL), water, KOH/H_2O, and water (4 x 40 mL) up to neutral. The organic
96 phase was dried over anhydrous sodium sulfate, filtered and the solvent was removed on a
97 rotary evaporator at 40 °C to obtain the unsaponifiable fraction of the oil. All quantitative
98 determinations were achieved in triplicate.
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100 **5. Carotenoids determination by High Performance Liquid Chromatography-**
101 **Photodiode Array detector-Mass Spectrometry (HPLC-PDA-MS)**
102 The oil samples were fully diluted in 1 mL of a methanol (MeOH)/ Methyl-tert-butyl-ether
103 (MTBE) (1:1) mixture, and if necessary, they were further diluted with the same solvent
104 mixture, passed through a 0.2 um nylon filter before injection into the HPLC-PDA-MS
105 system. The carotenoid analyses were carried out using a Nexera X2 liquid chromatography
106 system (Shimadzu, Milan, Italy), consisting of a CBM-20A controller, four HPLC-30AD

107 dual-plunger parallel-flow pumps, a DGU-20 A5R degasser, a CTO-20AC column oven and a
108 SIL-30AC autosampler. A 0.1 mm I.D. stainless steel tubing (zero dead volume) was
109 employed for column connection and a SPD-M30A photodiode array detector (PDA). The
110 HPLC system was coupled to an LC-qMS-2020 mass spectrometer through an APCI source
111 (Shimadzu, Kyoto, Japan). Data acquisition was performed by means of the LabSolutions
112 software (Version 5.91, Shimadzu Corporation). Separations were carried out on a YMC C30
113 column (250 mm × 4.6 mm i.d., 5 μm particle size). The mobile phases were A
114 (MeOH/MTBE/H_2O, 81:15:4) and phase B (MeOH/MTBE/H_2O, 16:80.4:3.6); a linear
115 gradient was used changing from 99 to 66% A in 30 min, maintaining this condition for 5
116 min, changing from 66 to 44% A in 15 min, keeping this condition for 5 min, changing from
117 44 to 22% A in 15 min and from 22 to 0% A in 5 min, returning to the initial conditions (99%
118 A) in 5 min, and keeping this condition for 5 min. The flow rate was set at 0.8 mL/min, the
119 column temperature was maintained at 35 °C, the UV/vis spectra were acquired between 220
120 and 700 nm and the chromatograms were processed at 450 nm. The LC-qMS-2020 detection
121 was achieved through an APCI interface operated in positive and negative mode; detector
122 voltage, 1.05 kV; interface temperature: 350 °C; DL temperature, 300 °C; heat block
123 temperature, 300 °C; nebulizing gas flow (N2), 2.0 L/min; drying gas flow (N2), 5.0 L/min;
124 full scan range (positive and negative mode), 300-1200 m/z; event time, 0.2 s. The different
125 carotenoids were characterized using their UV-vis and mass spectra, their available standards
126 and their elution order. Quantitative data were obtained by HPLC-DAD using external
127 calibration curves from carotenoids standards, in the concentration range from 1 to 100
128 μg/mL at five concentrations levels. The results were obtained from the average of three
129 determinations and the CV% was below 7% in all the LC measurements. Standard purity was
130 above 98% and the R coefficient for the calibration curves was always above 0.9992, with
131 LOD and LOQ values of respectively for β-carotene 0.07 and 0.1, for lutein of 0.06 and 0.18,
132 for lycopene of 0.08 and 0.3, μg/mL. The carotenoid concentrations are expressed in ppm
133 (mg/kg^{-1} of oil).

134 # Tables

135 **Table S1.** Botanical identification of the three endemic *Arecaceae* species from Reunion
136 Island.

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Species	Voucher number / harvest date and location - geographical coordinates
Hyophorbe indica	*Reference voucher number : LCSNSA012* *Harvest date : April, 2016* *Ripe fruits harvested in full season of fructification (advanced reproductive phase)* *Location : Saint Denis, La Bretagne, Réunion* *GPS : 343304/7685267 ou (-20°55'35")/55°29'35"* *Population of 1 individual.* *Ripe fruits (1.9 kg) are harvested on one individual* *Color of ripe fruit : dark orange ; Fruit 2.5-3.5 cm long / 3.0-4.0 cm diameter*
Dictyosperma album	*Reference voucher number : LCSNSA010* *Harvest date : July, 2015* *Ripe fruits harvested in full season of fructification (advanced reproductive phase)* *Location : Saint Denis, La Bretagne, Réunion* *GPS : 343304 / 7685267 ou (-20°55'35") / 55°29'35"* *Population of 2 individuals.* *Ripe fruits (3.8 kg) are harvested on one individual* *Color of ripe fruit : dark purple ; Fruit 1,6-1,8 cm long / 0,8-1 cm diameter*
Latania lontaroides	*Reference voucher number : LCSNSA011* *Harvest date : March, 2016* *Ripe fruits harvested in full season of fructification (advanced reproductive phase)* *Location : La Possession- Moulin Joli-Ravine Balthazar* *GPS : 326411/7681604 ou (-20°57'28")/55°19'49"* *Population of 17 individuals.* *Ripe fruits (2.2 kg) are harvested on one female individual* *Color of ripe fruit: dark green ; Fruit 4.0-5.0 cm long / 4.0-5.0 cm diameter*

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170 **Table S2.** Compositional characteristics of the oils extracted from the three endemic
171 *Arecaceae* species from Reunion Island.

Plant species	Seed oil content (g/100g seed DM)[a]	Color of the extracted oil	Unsaponifiable content in oil (g/100g oil DM)[a]
Hyophorbe indica Gaertn.	*3.09 ± 0.19*	*dark orange-red*	*13.41 ± 1.50*
Dictyosperma album (Bory) Scheff.	*8.81 ± 0.08*	*dark green*	*2.76 ± 0.22*
Latania lontaroides (Gaertn.) H.E. Moore	*8.68 ± 0.29*	*pale green*	*0.74 ± 0.31*

172 [a]*Mean value and standard deviation of triplicate samples. DM: Dry material.*

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174 **Table S3.** Fatty acids identification and composition (%) of the three seed oils obtained from
175 endemic *Arecaceae* species from Reunion Island.

Peak number (Fig.S2)	**Fatty acid methyl ester (FAMEs) identification**	***H. indica***	***D. album***	***L. lontaroides***
		(%)	*(%)*	*(%)*
n.i	C6:0, Caproate	*n.d*	*n.d*	*n.d*
n.i	C8:0, Caprylate	*n.d*	*n.d*	*1.77*
1	C10:0, Caprate	*0.14*	*0.93*	*1.05*
2	C12:0, Laurate	*1.89*	*20.93*	*34.14*
3	C14:0, Myristate	*1.18*	*16.14*	*22.96*
4	C15:0, Pentadecanoate	*0.39*	*0.08*	*n.d*
7	C16:0; Palmitate	*15.80*	*13.62*	*9.90*
8	C17:0: Heptadecanoate	*0.51*	*0.10*	*0.04*
13	C18:0; Stearate	*4.49*	*5.4*	*4.20*
14	C19:0; Nonadecanoate	*0.17*	*n.d*	*n.d*
19	C20:0; Arachidate	*0.61*	*0.21*	*0.09*
20	C21:0; Heneicosanoate	*0.10*	*n.d*	*n.d*
21	C22:0; Behenate	*0.74*	*0.10*	*0.03*
22	C23:0; Tricosanoate	*0.26*	*0.007*	*n.d*
23	C24:0; Lignocerate	*0.52*	*0.35*	*0.09*
24	C25:0; Pentacosanoate	*0.08*	*n.d*	*n.d*
25	C26:0; Cerotate	*0.04*	*0.18*	*n.d*
	∑ SFAs	**26.9 %**	**58.0 %**	**74.3 %**
	- MUFA Omega-9:			
5	C16:1ω9; cis-7 hexadecenoate	*0.07*	*0.06*	*n.d*
10	C18:1ω9; Oleate	*20.29*	*22.49*	*21.01*
17	C20:1ω9; Gondoate	*0.08*	*0.32*	*0.09*
	- MUFA Omega-7:			
12	C18:1ω7; cis-Vaccenate	*1.78*	*0.38*	*0.04*
18	C20:1ω7; Paulinate	*0.05*	*0.24*	*0.10*
	- MUFA Omega-5 & Omega-11			
6	C16:1ω5; 11(Z)-hexadecenoate	*0.43*	*0.38*	*n.d*
	∑ MUFAs	**22.7 %**	**23.9 %**	**21.2 %**
	- PUFA Omega-6:			
9	C18:2ω6; Linoleate	*33.70*	*16.91*	*4.46*
16	C20:2ω6; Eicosa-(11Z,14Z)-dienoate	*0.11*	*0.05*	*n.d*
	- PUFA Omega-3:			
11	C18:3ω3; alpha-Linolenate	*16.46*	*1.13*	*0.03*
	- Other PUFA :			
15	C20:2ω4; Eicosa (13,16)-dienoate	*0.12*	*n.d*	*n.d*
	∑ PUFAs	**50.4 %**	**18.1 %**	**4.5 %**
	ω6/ω3-PUFA ratio	**2 : 1**	**15 : 1**	**n.d**
	Edible oil equivalent	Oil rich in ω3	Oil rich in SFA	Oil rich in SFA

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177 n.i.: not identified; n.d: not quantified/not detected; Peak numbers only refer to the identifications reported in **Figure S2**
178 relative to the bidimensional plot of *H. indica* seed oil. SFAs: saturated fatty acids. MUFAs: monounsaturated fatty acids.
179 PUFAs: polyunsaturated fatty acids (unsaturation ≥2). ω6/ω3-PUFA ratio: ratio between omega-6 and omega-3 PUFA fatty
180 acids.
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183 **Table S4.** Characteristics (retention time, ʎmax and mass spectrometry data) of the not
184 identified carotenoids from *Hyophorbe indica* and *Latania lontaroides* seed oils.
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Plant source	Carotenoid not identified	t_R (min)	λ max (nm)	$[M + H]^+$ (*m/z*)	$[M]^{.-}$ (*m/z*)
Hyophorbe indica seed oil	unknown compound (**17**)	52.2	372, 397, 423, 445, 468	537	536
Latania lontaroides seed oil	unknown compound (**1**)	7.3	450	413	411

186 Peak numbers refer to the identifications reported in **Figure 1.**
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Figures

Figure S1. Photograph of the plant, ripe fruit, seeds and extracted seed oil of the three endemic *Arecaceae* species of Reunion island: a) *Hyophorbe indica* Gaertn.; b) *Dictyosperma album* (Bory) Scheff.; c) *Latania lontaroides* (Gaertn.) H.E. Moore.

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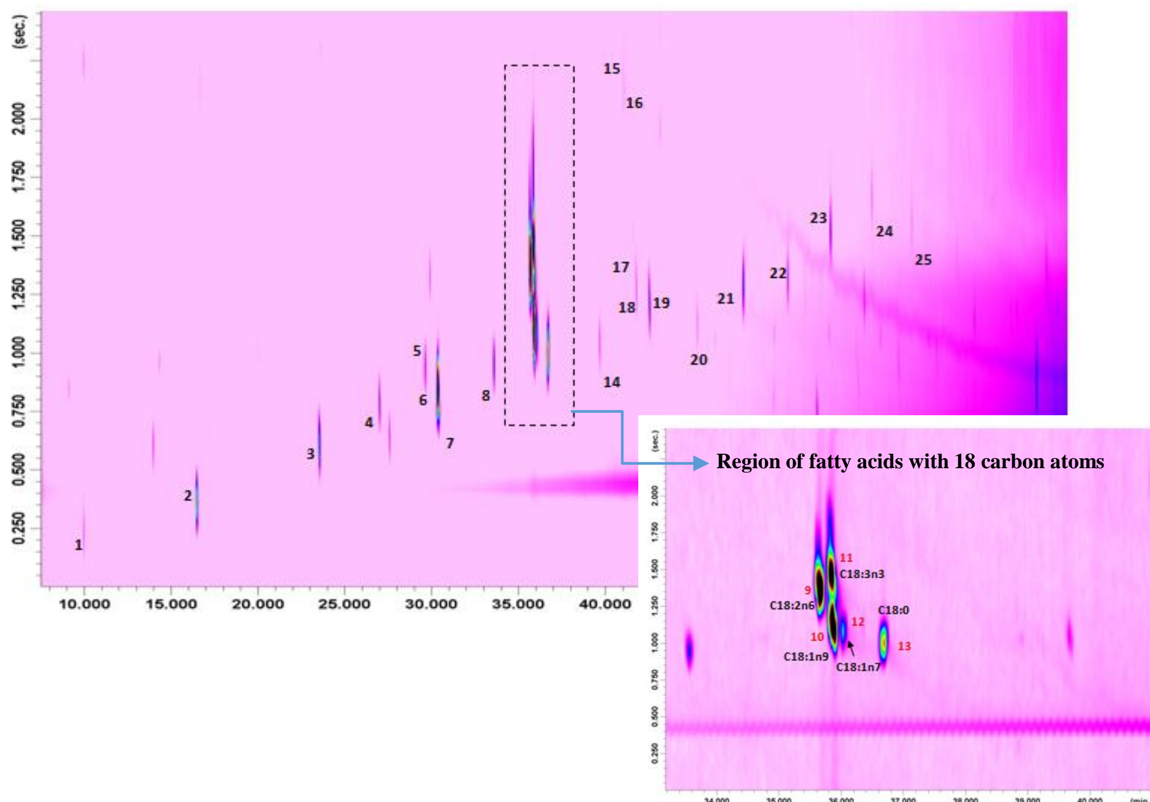

Hyophorbe indica Gaertn. seed oil					
Peak	**Compound (fatty acid methyl esters)**	**Sim. %**	**LRIexp**	**LRIlibr**	**ΔLRI**
1	Me. C10:0; Caprate	93	1321	1322	-1
2	Me. C12:0; Laurate	94	1523	1523	0
3	Me. C14:0, Myristate	95	1723	1724	-1
4	Me. C15:0; Pentadecanoate	94	1824	1825	-1
5	Me. C16:1n9; (7Z)-hexadecenoate	90	1898	1897	1
6	Me. C16:1n5; 11(Z)-hexadecenoate	93	1902	1912	-10
7	Me. C16:0; Palmitate	92	1925	1925	0
8	Me. C17:0; Heptadecanoate	92	2025	2026	-1
9	Me. C18:2n6; Linoleate	96	2093	2096	-3
10	Me. C18:1n9; Oleate	95	2099	2098	1
11	Me. C18:3n3; alpha-Linoleate	90	2099	2107	-8
12	Me. C18:1n7; (E)-Vaccenate	91	2105	2107	-2
13	Me. C18:0; Stearate	93	2127	2126	1
14	Me. C19:0; Nonadecanoate	90	2226	2227	-1
15	Me. C20:2n4; Eicosa-(13,16)-dienoate	87	2273	2280	-7
16	Me. C20:2n6; Eicosa-(11,14)-dienoate	85	2295	2293	2
17	Me. C20:1n9; Eicos-(11Z)-enoate	91	2299	2298	1
18	Me. C20:1n7; Eicos-(13)-enoate	90	2300	2300	0
19	Me. C20:0; Arachidate	90	2327	2326	1
20	Me. C21:0; Heneicosanoate	90	2427	2429	-2
21	Me. C22:0; Behenate	95	2529	2528	1
22	Me. C23:0; Tricosanoate	90	2629	2629	0
23	Me. C24:0; Lignocerate	93	2730	2729	1
24	Me. C25:0; Pentacosanoate	90	2831	2830	1
25	Me. C26:0; Cerotate	85	2932	2931	1

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218 **Figure S2:** Bidimensional plot of the fatty acids detected in the endemic *Hyophorbe indica*
219 Gaertn. seed oil and peak identification.

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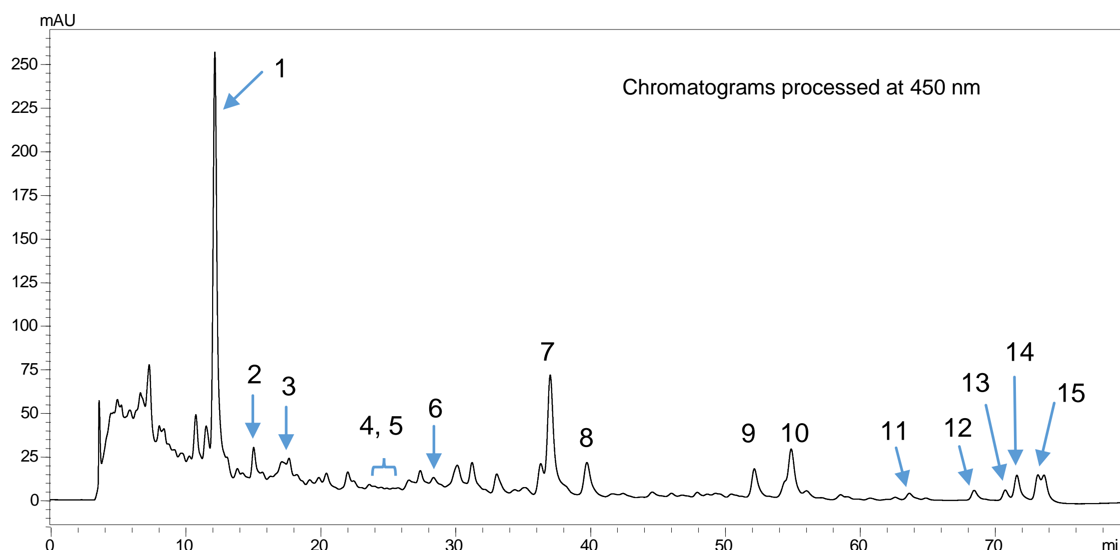

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peak	carotenoid	t_R (min)	λ_{max} (nm)	$[M + H]^+$ (*m/z*)	$[M]^{-\cdot}$ (*m/z*)
1	all-*trans*-lutein	12.2	420, 445, 473	551	568
2	n.i.	15.1	328, 403, 425, 467	n.d.	n.d.
3	Phytofluene isomer	17.2	331, 347, 367	543	542
4	Phytofluene isomer	25.4	331, 348, 367	543	542
5	Phytofluene isomer	25.8	331, 348, 367	543	542
6	Phytofluene isomer	28.4	332, 348, 367	543	542
7	all-*trans*-β-carotene	37.1	424, 452, 477	537	536
8	9-cis-β-carotene	39.8	339, 423, 447, 471	537	536
9	n.i.	52.2	372, 397, 423, 445, 497	537	536
10	*cis*-lycopene isomer	54.9	294, 361, 445, 468, 498	537	536
11	*cis*-lycopene isomer	63.7	295, 362, 440, 467, 498	537	536
12	*cis*-lycopene isomer	68.5	294, 362, 444, 468, 496	537	536
13	*cis*-lycopene isomer	70.8	295, 362, 443, 471, 500	537	536
14	*cis*-lycopene isomer	71.7	295, 364, 445, 471, 500	537	536
15	*cis*-lycopene mixture	73.2-73.7	295, 363, 447, 473, 504	537	536

224 n.i.: not identified; n.q.: not quantified

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226 **Figure S3:** Chromatograms of the carotenoids in the endemic *Hyophorbe indica* Gaertn. seed
227 oil and peak identification.

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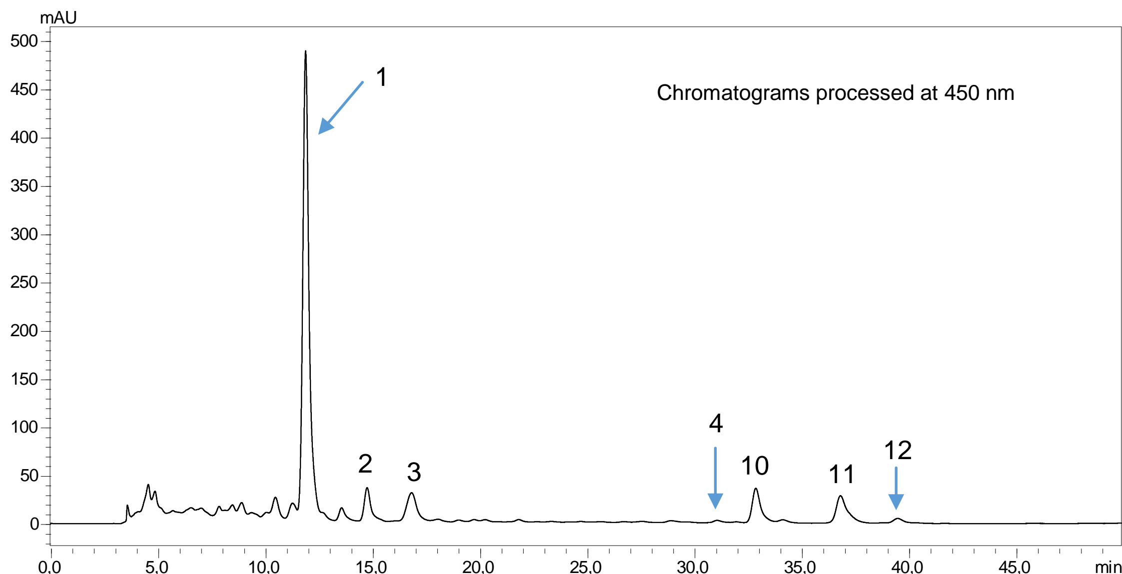

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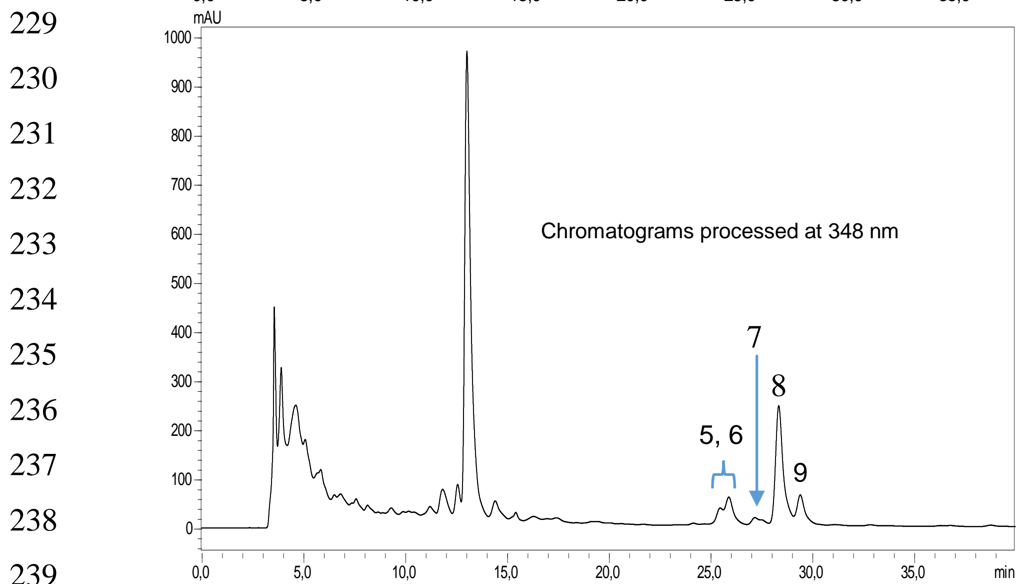

Peak	Carotenoid	t_R (min)	λ_{max} (nm)	$[M + H]^+$ (*m/z*)	$[M]^{\cdot -}$ (*m/z*)
1	all-*trans*-lutein	11.9	422, 445, 473	551	568
2	9-*cis* or 9'-*cis*-lutein	14.8	417, 439, 468	551	568
3	9-*cis* or 9'-*cis*-lutein	16.8	419, 441, 468	551	568
4	phytoene	24.9	276, 286, 298	545	n.d.
5	Phytofluene-1	25.5	330, 345, 364	543	n.d.
6	Phytofluene-2	25.9	332, 348, 367	543	n.d.
7	Phytofluene-3	27.2	332, 348, 367	543	n.d.
8	Phytofluene-4	28.4	332, 348, 367	543	541
9	Phytofluene-5	29.4	332, 348, 367	543	541
10	all-*trans*-α-carotene	32.9	422, 446, 474	537	536
11	all-*trans*-β-carotene	36.8	424, 451, 478	537	536
12	9-*cis*-β-carotene	39.5	425, 448, 474	537	536

240 n.d.: not detected
241
242 **Figure S4**: Chromatograms HPLC-PDA of the carotenoids in the endemic *Dictyosperma*
243 *album* (Bory) Scheff. seed oil and peak identification.

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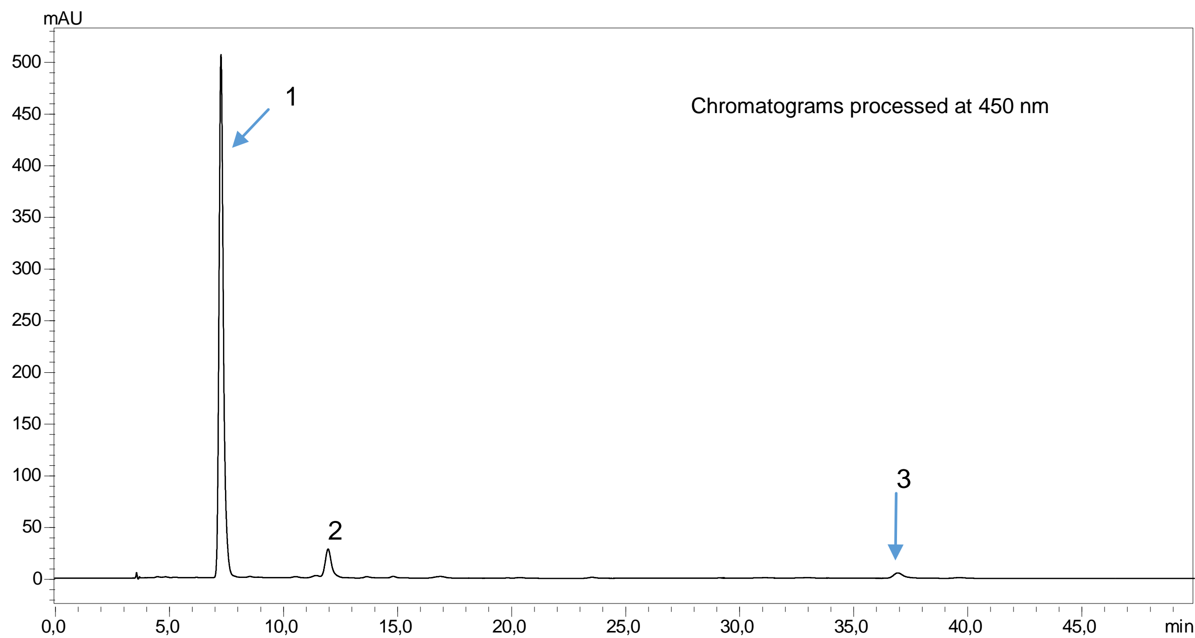

Peak	Carotenoid	t_R (min)	λ_{max} (nm)	$[M + H]^+$ (m/z)	$[M]^{\cdot -}$ (m/z)
1	n.i.	7.3	450	413	411
2	all-*trans*-lutein	12	421, 445, 473	551	568
3	all-*trans*-β-carotene	37	421, 452, 480	537	536

265 n.i.: not identified

266
267 **Figure S5**: Chromatograms HPLC-PDA of the carotenoids in the endemic *Latania*
268 *lontaroides* seed oil and peak identification.
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