Chemistry of Natural Compounds, Vol. 47, No. 6, January, 2012 [Russian original No. 6, November–December, 2011]

CHEMICAL COMPOSITION AND PHYSICOCHEMICAL CHARACTERISTICS OF FIXED OILS FROM ALGERIAN *Nigella sativa* SEEDS

Farid Benkaci-Ali,[1*] Aoumeur Baaliouamer,[1,2] Jean Paul Wathelet,[3] and Michel Marlier[3]

UDC 547.915 + 547.918

The fatty acids, sterols, and polyphenols from the fixed oils of Nigella sativa *seeds originating from four locations were determined. The seeds contained respectively 30.63–34.27% and 25.66–32.77% of fixed oils using hexane and isopropyl alcohol in solvent extraction. Linoleic, oleic, and palmitic acids formed the main proportion using the two solvents, respectively: hexane 54.47–61.28%, isopropanol 56.98–67.30%; hexane 19.62–22.94%, isopropanol 18.85–21.96%, and hexane 11.17–13.60%, isopropanol 9.20–14.18%. Other minor unsaturated fatty acids were identified. Eight phytosterols were isolated and identified in the fixed oils by GC and GC/MS analysis, wherein* β*-sitosterol was the dominating compound that inhibits the absorption of dietary cholesterol, followed by stigmasterol, campesterol, and* Δ^5*-avenasterol.*

Keywords: *Nigella sativa* Linn., chemical composition, fixed oil, fatty acids, sterols, phenols.

Nigella sativa L. is an erect, herbaceous, and annual species of the Ranunculaceae family, investigated recently for the oil, essential oil, and other biologically active components of their seeds [1, 2]. They are important sources of oils of nutritional, industrial, and pharmaceutical value [3, 4]. Because the traditional and folkloric uses of black seeds are supported by a long history of human experience, this plant may be a valuable source of potential drugs. It has been employed for thousands of years as a spice, food preservative, and curative remedy for numerous disorders [5]. In Algerian folk medicine, *Nigella sativa* seeds and their oils are used as carminatives, diuretics, and for bronchial asthma and lactation.

A wide spectrum of medicinal uses can also be mentioned, such as analgesic and anti-inflammatory, antibacterial [6], antiasthmatic [7], anticarcinogenic [8], and antiviral [9].

The aim of this study was to extend our knowledge on seed oils from *Nigella sativa* grown in the Algerian Sahara [Adrar (A), Timimoun (T), and Ouargla (O)] and in North Algeria [Medea (M)] extracted by organic solvents.

Agronomic experiments of *Nigella sativa* seed yield have been carried out for the first time in the Algerian Sahara and the North areas. Besides, no published study has reported on the chemical composition of the fatty acids (FA) and sterols (ST) in the fixed oil of the plants grown in these regions.

To investigate the fixed oil of *Nigella sativa*, Soxhlet extraction was employed with hexane (H) and isopropanol (IP) as solvent. Isopropyl alcohol is one of the most favorable alternatives to hexane for oil extraction. Compared to hexane and other solvents such as ethanol, IP is less flammable, less toxic, and is free of restrictive governmental regulations [10]. It therefore provides an environmentally attractive and "green" approach to extraction by eliminating the use of organic solvents.

1) Universite des Sciences et de la Technologie Houari Boumediene U.S.T.H.B, Faculte de Chimie, Laboratoire d′Analyse Organique Fonctionnelle BP. 32 El Alia, Bab Ezzouar, Algerie, e-mail: benkaciaf@yahoo.fr; 2) Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques C.R.A.P.C, B. P. 248 RP 16004, Alger, Algerie; 3) Faculte Universitaire des Sciences Agronomiques de Gembloux, Laboratoire de Chimie Generale, Passage des Deportes 2 B-5030, Gembloux, Belgique. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 811–815, November–December 2011. Original article submitted June 6, 2010.

0009-3130/12/4706-0925

TABLE 1. Origin and Yields of Fixed Oils from Algerian *Nigella sativa* Seeds, % of Seed Weight

Solvent	Ouargla	Timimoun	Adrar	Medea
Hexane	33.61 ± 0.75*	32.15 ± 0.65	30.63 ± 0.58	34.27 ± 0.90
Isopropanol	28.05 ± 0.61	25.66 ± 0.55	32.77 ± 0.72	29.38 ± 69

*M ± SD (Mean ± standard deviation).

In this paper we study the extraction yields and chemical composition of fatty acids and sterols according to solvents and regions. Considering the reported presence of phenolic products in other part (shoots and roots) of the same species and the biological activity of seeds, we decided to carry out the isolation and identification of compounds of this class in the plant. These compounds may be responsible for the biological activity of the plant. We also intended to develop an HPLC method for the detection and quantification of the flavonoids eventually isolated and to confirm the presence of some phenolic compounds not previously reported.

Extraction Yields and Color. As shown in Table 1, the H solvent provides yields of fixed oils that are relatively higher than those obtained by means of IP solvent (25.66–32.77%). In the literature, the yields of *Nigella sativa* seed oil have been found to be 28–40% [11].

The fixed oils were more yellow than other vegetable oils and they can protect against UV light. This coloration is probably related to the presence of greater amounts of thymoquinone (yellow dark), which could be responsible for the color in addition to what was suggested by S. Cheikh-Rouhou et al. [11]; ''the coloration is due to the presence of more yellow pigments of carotenoids and linoleic acid''. Indeed, at –4°C during 72 h, the thymoquinone isolated from the volatile oils [12, 13] showed that this compound is black, and when it is dissolved in hexane we note the dark yellow coloration of the solution formed.

Regarding the UV absorption, on the whole, IA oils absorbed in the UV-C (100–290 nm), UV-B (290–320 nm), and UV-A (320–400 nm) ranges, whereas H oils present absorbance only in the UV-C and UV-B ranges. Therefore, the oils extracted by H or IA can be used to protect against UV radiation with relatively high shielding power and protection factor scores. The optical transmission of *Nigella* seed oil, especially in the UV range (290–400 nm), was comparable to those of date seed oil, raspberry seed oil, and titanium dioxide preparations, which can be used as sun protection factors for UV-B (SPF) and protection factors for UV-A (PFA) [14, 15].

The strong absorptivity of IA oils at the average value of 440 nm, a wavelength which is approximatively at the lower limit of detectability for the human eye, corresponded to high levels of yellow pigments and thymoquinone in this oil [12, 13]. IA oils are more yellow than H oils, as indicated by the absorbance (0.25 against 0.05) at 400–490 nm.

Fatty Acid Composition. The average composition of *Nigella sativa* fatty acids given in Table 2 with a total of 11 different methyl ester acids were identified by GC and GC-MS in the *Nigella sativa* seed oils.

The major saturated fatty acids were palmitic (C16:0), stearic (C18:0), and heneicosanoic acid (C21:0); however linoleic (C18:2n6) and oleic (C18:1n9) acids represent on the whole the main unsaturated fatty acids, which account for 78.94% at 87.42% in IA oils and 74.09% at 81.95% in H oils (Table 2). The ratio of unsaturated to saturated fatty acids (U/S) was higher in IA (IA: 5.41 to 8.66) compared to H (5.04 to 5.8). These ratios were higher than those reported by Ramadan et al. [16] and Cheikh-Rouhou et al. [11], which vary from 3.41 to 3.8. It is becoming increasingly clear that UFAs are beneficial to human health, and new research highlights further potential benefits on cognitive function and behavior, and inflammatory conditions such as arthritis, Crohn′s disease, and asthma [17]. Thus, the IA extracts confer the most benefits for human health compared to other solvents.

It is interesting to note that the total saturated fatty acid (TSFA) content was higher with H than with IA extraction, whereas the IA extract contained more total unsaturated fatty acids (TUFA).

In the same way, measurable amounts of χ-linolenic acid C18:3n6 (tr – 0.3%), palmitoleic acid C16:1 (0–0.47%), and linolenic acid C18:3n3 (0 – tr) as saturated acids were detected in H and IA oils.

FA such as lignoceric, behenic, eicosenoic, arachidic, margaroleic, margaric, and myristoleic acids reported in previously work [11] were not detected in this study for all regions and solvents investigated.

The source of qualitative and quantitative variability may be genetics (variety grown), seed quality (diversity in maturity of seed and agricultural conditions of cultivated area), extraction method, or analysis technique [18]. The tridecanoic and pentadecanoic acids detected in negligible amounts were rarely reported in previously published data.

TABLE 2. Percentage of Fatty Acids (g/100 g of total fatty acids) Identified by GC and GC-MS in the Algerian Fixed Oils

Fatty acid	O_H	O_{IA}	T_H	T_{IA}	A_H	A_{IA}	M_H	M_{IA}
13:0	0.17 ± 0.02	Tr.	0.14 ± 0.02	Tr.	0.16 ± 0.03	Tr.	0.24 ± 0.04	0.14 ± 0.01
15:0	Tr.	Nd.	Tr.	Nd.	Tr.	Nd.	Tr.	0.09 ± 0.01
16:0	11.17 ± 0.60	9.20 ± 0.45	11.72 ± 0.80	10.37 ± 0.92	11.75 ± 0.75	11.72 ± 0.55	13.60 ± 0.81	14.18 ± 1.10
16:1	0.19 ± 0.03	Nd.	0.16 ± 0.02	Nd.	Tr.	0.23 ± 0.03	0.26 ± 0.03	0.47 ± 0.05
18:0	2.22 ± 0.5	1.16 ± 0.04	2.45 ± 0.44	1.34 ± 0.14	2.81 ± 0.56	2.31 ± 0.25	2.72 ± 0.22	1.09 ± 0.08
18:1n9	22.94 ± 1.11	20.12 ± 1.24	20.67 ± 1.45	18.85 ± 1.57	21.73 ± 1.38	20.88 ± 1.75	19.62 ± 1.01	21.96 ± 1.80
18:2n6	55.70 ± 2.01	67.30 ±1.98	61.28 ± 1.88	66.05 ± 1.59	56.73 ± 1.09	59.78 ± 1.47	54.47 ± 1.66	56.98 ± 0.97
18:3n6	0.23 ± 0.04	Tr.	0.19 ± 0.04	Nd.	0.30 ± 0.03	Tr.	0.13 ± 0.02	0.29 ± 0.03
18:3n3	Tr.	Tr.	Tr.	Nd.	Tr.	Tr.	Tr.	Tr.
21:0	2.13 ± 0.15	Tr.	0.38 ± 0.04	Nd.	1.48 ± 0.21	1.46 ± 0.11	Tr.	Tr.
20:2	5.15 ± 0.57	2.23 ± 0.35	2.89 ± 0.33	2.81 ± 0.20	4.35 ± 0.31	2.94 ± 0.41	8.94 ± 0.82	4.23 ± 0.43
TSFA	15.69	10.36	14.69	11.71	16.20	15.49	16.56	15.50
TUFA	84.21	89.69	85.19	87.71	83.11	83.83	83.42	83.93
U/S	5.36	8.66	5.80	7.50	5.13	5.41	5.04	5.41

The values given are means of three determinations. Tr.: traces, less than 0.01%. Nd.: not detected. TSFA: total saturated fatty acids. TUFA: total unsaturated fatty acids. U/S: ratio of unsaturated to saturated fatty acids.

TABLE 3. Percentage of the Main Sterols Identified by GC and GC-MS in the Algerian Fixed Oil

Sterol	O_H	O_{IA}	T_H	T_{IA}	A_H	A_{IA}	M_H	M_{IA}
Cholesterol	1.01 ± 0.10	1.45 ± 0.15	1.08 ± 0.08	1.41 ± 0.15	1.68 ± 0.12	1.94 ± 0.21	1.44 ± 0.13	1.96 ± 0.22
Campesterol*	12.51 ± 1.12	12.17 ± 1.10	15.65 ± 1.35	14.93 ± 1.23	13.62 ± 1.47	12.98 ± 1.73	13.00 ± 1.05	12.28 ± 1.41
Desmosterol*	1.01 ± 0.09	0.98 ± 0.10	Nd.	Nd.	0.48 ± 0.37	0.49 ± 0.42	1.61 ± 0.11	0.55 ± 0.07
Stigmasterol*	18.22 ± 1.87	17.73 ± 1.76	21.48 ± 2.22	20.88 ± 1.97	18.79 ± 1.56	15.21 ± 1.21	16.18 ± 1.48	18.28 ± 1.50
γ-Sitosterol*	0.59 ± 0.05	0.57 ± 0.04	Nd.	Nd.	1.09 ± 0.05	1.12 ± 0.08	1.55 ± 0.09	1.06 ± 0.05
β-Sitosterol*	53.00 ± 2.51	54.87 ± 3.10	44.41 ± 1.97	47.31 ± 2.21	52.12 ± 2.09	56.70 ± 2.41	53.16 ± 1.88	53.62 ± 2.25
Δ^5-Avenasterol*	12.22 ± 1.10	10.92 ± 1.50	15.71 ± 1.33	13.98 ± 1.12	11.57 ± 0.97	10.87 ± 0.85	11.26 ± 1.12	10.28 ± 0.88
3β-Hydroxy-cholest-5-en-24-one (24-oxocholesterol)	0.54 ± 0.03	0.53 ± 0.04	0.81 ± 0.06	0.73 ± 0.05	Tr.	Nd.	0.57 ± 0.04	0.65 ± 0.07
							1.23 ± 0.05	1.31 ± 0.07
Δ^7-Cholesterol	0.81 ± 0.07	0.79 ± 0.07	0.86 ± 0.09	0.76 ± 0.09	0.65 ± 0.04	0.67 ± 0.07		
IIPA	4.22	4.83	2.84	3.17	2.84	4.37	4.10	4.36
TS, mg/g oil	0.983	1.066	0.812	0.898	0.810	0.907	1.053	1.114
TSO, mg/g oil	0.988	1.067	0.819	0.905	0.812	0.907	1.059	1.122

The values given are means of three determinations. Tr.: traces, less than 0.01%. *Phytosterols. IIPA: index of identification of purity and authentification [19]. Nd.: not detected. TS: total sterols. TSO: total sterols and oxysterols.

They are generally localized in animal lipids, but recent investigations reported the presence of these fatty acids in some vegetable plants [20]. Moreover, it is necessary to underline that the pentadecanoic acid was only detected in fixed oils extracted by hexane.

Sterol Composition. This study is the first one on the sterol composition and content of the examined species of *Nigella sativa* cultivated in Algeria.

As a whole, the average composition of sterols from the regions studied (Table 3) were close to one another and constituted mainly of β-sitosterol (H: 44.41–53.16%, IA: 47.31–56.70%), stigmasterol (H: 16.18–21.48%, IA: 15.21–20.88%), campesterol (H: 12.51–15.65%, IA: 12.17–14.93%), and Δ^5-avenasterol (H: 11.26–15.71%; IA:10.28–13.98%). Information on sterols of black cumin (*Nigella sativa*) is important for characterization of seed species and phytosterols in human nutrition [19].

TABLE 4. Composition of Polyphenolic Compounds in the Oils, mg/kg

Polyphenol identified	t_R	Name, (synonym)	Ouargla	Timimoun	Adrar	Medea
Peleargonin chloride	4.50	Anthocyanin	Nd.	Nd.	Nd.	Nd.
(+)-Catechin	5.59	Flavan-3-ol (catechol)	124.63 ± 4.8	58.72 ± 2.84	118.90 ± 4.23	56.56 ± 2.04
Cyanidin chloride	6.50	Anthocyanin	Nd.	Nd.	Nd.	Nd.
Epicathechin	6.89	Flavan-3-ol	98.14 ± 3.57	39.67 ± 2.10	44.87 ± 2.07	Tr.
Malvin	9.00	Anthocyanin	Nd.	Nd.	Nd.	Nd.
Rutin	13.54	Flavonol glycoside (3-*O*-rutinosyl-quercetol)	47.84 ± 2.36	37.03 ± 1.97	117.74 ± 3.80	14.05 ± 0.37
Quercitrin dihydro	20.50	Flavanone (catechin hydrate)	14.72 ± 0.85	Nd.	31.45 ± 1.15	4.05 ± 0.26
Paeonin chloride	20.80	Anthocyanin	Nd.	Nd.	Nd.	Nd.
Naringine	24.53	Flavanone (4′,5,7-trihydroxyflavanone-7-rutinoside naringoside)	38.03 ± 1.97	14.15 ± 0.91	58.68 ± 2.71	2.16 ± 0.15
Quercimeritrin	27.28	Flavonol glycoside (quercetin 7-*O*-β-D glucoside)	20.61 ± 1.43	6.90 ± 0.55	39.89 ± 1.79	5.89 ± 0.34
Quercetin	32.62	Flavonol	14.92 ± 0.72	5.03 ± 0.43	35.67 ± 1.52	11.29 ± 0.48
Callistephin	33.17	Flavonol glycoside (pelargonidin 3-glucoside)	33.61 ± 1.48	Tr.	Tr.	15.99 ± 0.78
Total polyphenols			392.50	161.5	447.2	109.99

Nd.: not detected. Tr.: traces, less than 0.01%.

The presence of β-sitosterol in *Nigella sativa* oils in appreciable concentrations makes it an excellent agent in the absorption of dietary cholesterol and constitutes an important factor when evaluating the quality of the oil because this compound is of capital importance for its health benefits [21].

The ratio β-sitosterol/campesterol (IIPA) [22] varies from 2.84 (T) to 4.22 (O). In fact, the composition of T is different compared to the other samples. The principal factor responsible for this value can be attributed to agricultural conditions of the cultivated garden (salinity degrees of water irrigation and nature of the ground).

It is important to note that plant sterols, including β-sitosterol, have been established as plasma cholesterol lowering agents in mammals [19].

The results suggest that dietary phytosterol content covaries with changes in TUFA and TSFA. Globally, total phytosterol content decreases with increasing TSFA and is notably elevated by increasing TUFA. The high quantity of phytosterols in fixed oils could be responsible of the important percentage of TUSA and the lower TSFA content.

These results support previous published data which indicate that dietary sources of saturated fatty acids have far lower phytosterol content than most commonly used unsaturated vegetable oils [23].

The GC and GC-MS analysis revealed a non-negligible amount of oxysterol in the unsaponifiable fraction as 3β-hydroxy-cholest-5-en-24-one (24-oxocholesterol). However, some oxysterols can be destroyed during analysis under too drastic conditions [21].

Phenol Content. The amount of total phenolics varied in different accessions and ranged from 202 ± 15.50 to 560 ± 23.11 mg gallic acid/kg of seeds. The highest total phenolic levels were detected in A (648 ± 25.12) and O (260 ± 11.38 mg/kg), and the lowest in M and T.

Thus, *Nigella sativa* seeds are considered to be a rich source of phenolic compounds that are able to minimize oil oxidation during handling and storage and may have a beneficial effect in the prevention of cancer and coronary disease; this may explain the fact that *Nigella sativa* oil presents high oxidation induction times comparable to virgin olive oil [11].

In addition, according to these authors, the oxidation stability could be attributed to the higher natural antioxidant content in the oils, which explains the greater resistance to oxidation.

On the other hand, A and O showed a higher total phenol level than other samples and constitute the best oils. Thus, for seeds rich in polyphenols, it is recommended to cultivate the *Nigella sativa* species in Adrar or Ouargla. Timimonun and Medea soils and other geographic factors can be responsible for these differences in polyphenol yields compared to those of A and O.

Polyphenol Composition. RP-HPLC coupled with a UV-Vis multiwavelength detector was employed to separate and quantify the phenolic compounds. Eight phenolic compounds were successfully identified in the seeds samples. These compounds have been identified according to their retention times and the spectral characteristics of their peaks compared to

those of standards, as well as by spiking the sample with standards. Results demonstrated that differences in phenolic composition were significantly more quantitative than qualitative.

The amounts of the different identified flavonoids are shown in Table 4. (+)-Catechin and rutin were detected as the major phenolic components in the four samples studied, contributing about 34.61 to 59.29% to the total amount and showing levels of 56.56 to 124.63 mg/kg seeds and 14.05 to 117.74 mg/kg seeds, respectively.

Epicatechin was also predominant but higher in O (98.14 mg/kg seeds) than in A and T (44.87 vs 39.67 mg/kg seeds), and found in trace amounts in *M. naringine*. Quercimeritrin and quercetin were also found in appreciable levels in all samples.

The levels of total phenolic compounds in *N. sativa* determined by HPLC were 392.50, 161.5, 447.2, and 109.99 mg/kg seeds for A, T, A, and M, respectively, and thus less than the ones obtained by the Folin-Ciocalteu method. Indeed, this result is probably due to the weak selectivity of the Folin-Ciocalteu reagent, since it reacts positively with antioxidant phenolic and non-phenolic compounds.

EXPERIMENTAL

Plant Material. Mature black cumin (*Nigella sativa* L.) seeds were gathered during July and stored at 4°C until extraction. They were authenticated by the Botanical Department of the National Agronomic Institute, Algiers, Algeria. The initial average moisture of these seeds was 9%.

Four varieties of mature black seed were collected in the Algerian regions: North (Medea) and South (arid areas Ouargla, Adrar, and Timimoun).

Seed material (150 g) was finely ground to 180–250 μm size using an electric grinder at a speed of 20 000 rpm for 30 sec and subjected immediately to oil isolation.

Solvent Extraction Apparatus and Procedure. Extraction was carried out in a Soxhlet apparatus (250 mL) for 90 min [24]. The extraction procedure was repeated twice, and the collected solvent was removed under vacuum using a rotary evaporator at 40 and 50°C. The oils were stored at –8°C until analyzed.

Reagents, Solvents, and Standard Phenolics. Polyphenols standards (+)-catechin, epicathechin, naringin, dihydroquercitrin, quercetin, quercetin 3-rutinoside (rutin), quercetin 7-*O*-β-D-glucoside (quercimeritrin), pelargonidin 3-glucoside (callistephin), cyanidin chloride, malvin, and paeonin chloride were from Sigma-Aldrich.

Reversed-Phase HPLC Analysis. Chromatographic analysis was performed on a Hewlett–Packard (model 1100, USA) liquid chromatograph equipped with a vacuum degasser G1322A, a quaternary pump G1311A, an autosampler G1329A ALS, a thermostatted column compartment, and a DAD detector G1315B, and controlled by Chemstation software. A reversed phase (RP) Varian C18 (250 × 4.6 mm i.d.) column (Chromspec, PA, USA) and guard column (Agilent Technology, USA) were used. Solvents that constituted the mobile phase were A (water) and B (acetonitrile). The elution conditions were: 0–5 min, 0% B isocratic; 5–10 min, linear gradient from 0% to 20% B; 10–20 min, 20% B isocratic; 20–30 min, linear gradient from 20% to 40% B; 30–40 min, 40% B isocratic; 40–50 min, linear gradient from 40% to 50% B; 90–136 min. The flow rate was 1 mL/min, and the injection volume was 50 μL. The column was operated at 30°C. Flavan-3-ols, flavanones, and anthocyanins were monitored and quantified at 280 nm, and flavonols at 330 nm.

Determination of Fatty Acid Composition. GC analysis was performed on a HP 6890 standard model using the following conditions: polar RTX-2330 fused-silica capillary column (30 m × 0.25 mm ID, 0.25 μm, cyanopropyl 50%, dimethylpolysiloxane 50%); volume injection V_{inj} = 0.5 μL, detector FID, carrier gas helium (0.03 MPa, flow rate 0.5 mL min^{-1}), injector and detector temperature regulated at 260 and 280°C, respectively. The splitless injection mode was used; injection volume for all samples 0.5 μL; oven temperature increased from 40 to 100°C at 4°C min^{-1} and held at 100°C for 10 min, then increased to 240°C at 8°C/min and held at 240°C for 20 min.

The fatty acid methyl esters samples were injected into an HP 6890 chromatograph connected to a Hewlett–Packard 5973 mass-selective detector.

Oven temperature progression, column operating conditions, volume and injection mode, carrier gas conditions, and injector temperature were similar to GC ones. The temperature interface of the mass spectrometer was fixed at 280°C, the solvent delay time was 4 min. The source temperature was 230°C, and the quadrupole temperature 150°C. The instrument was operated in the electron impact mode, with an electron energy of 70 eV, and scanned in the 30–550 *m/z* range.

Identification and quantitative analysis of fatty acid methyl esters was based on comparison of retention times, mass spectra, and percentage of unknown peaks with authentic fatty acid methyl esters (standard mixture: 37 methyl esters of fatty acids C4-C22, Sigma-Aldrich).

Determination of Sterols. Characterization of the sterols (ST) in the oils [25] was performed after saponification of 1 g of oil sample with 50 mL of methanolic potassium hydroxide solution (2 mol L^{-1}, 1 h at 75–80 °C). To the solution obtained, 50 mL of distilled water was added. Extraction of the unsaponifiable matter was made with 3 × 100 mL diethyl ether followed by the washing of the ethereal extract with 3 × 50 mL of water. TLC was performed on a plate (Merck-G-60, thickness 0.25 mm) eluted with the solvent mixture (hexane–diethyl ether–NH_3 90:10:0.5 (v/v/v)). After detection with fluorescence in UV (254 nm), the sterols were extracted using $CHCl_3$ solvent (5, 3, 3 mL solvent for 30, 10, 10 min). The derivatization was carried out with 100 μL of BSTFA–TCMC, 99:1(v/v) and 100 μL pyridine. The temperature of silylation was fixed at 90°C during 30 min.

Sterols were analyzed by GC on an HP6890 standard model using the following conditions: fused-silica capillary column with an apolar stationary phase HP5-MS (Hewlett Packard, 30 m × 0.25 mm × 0.25 μm film thickness, 5% biphenyl, 95% dimethylpolysiloxane); column temperature 55°C (2 min) to 280°C (30 min) at 40°C/min; injection mode splitless; detector, FID; injector and detector temperature, 310°C and 320°C respectively; carrier gas, N_2; flow rate 0.3 mL/min; injected volume, 0.5 μL. The sterols samples were then injected into an HP 6890 chromatograph connected to a Hewlett–Packard 5973 mass-selective detector under the same condition as the GC analysis. The temperature interface of the mass spectrometer, the solvent delay time, the source temperature, and the quadrupole temperature were similar to those used in the fatty acid analysis.

The internal standard sample used was betuline (triterpenoid $C_{30}H_{50}O_2$: lup-20(29)-ene-3b,28-diol) from Sigma-Aldrich.

Component identification was verified by comparison of the mass spectra of the components using a computerized MS data bank (commercial library-NIST, Wiley7).

All analyses were performed at least three times, and values were expressed as means ± standard deviation.

Phenol Content Analysis. The amount of total phenolic extracts was determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad [26] as modified by Lister and Wilson [27]. To 50 mL of each sample, 2.5 mL 1/10 dilution of the Folin-Ciocalteau reagent and 2 mL of Na_2CO_3 (7.5%, w/v) were added and the whole incubated at 45°C for 30 min. The absorbance of samples was measured at 755 nm using a UV/VIS/IR spectrophotometer (JASCO, Tokyo). Results were expressed as mg of gallic acid equivalent per kilogram of seeds.

Identification and Quantitation of Phenolic Compounds. The identification of phenolic compounds was carried out by comparison of their retention times and their UV-visible spectra with those obtained by injecting standards under the same conditions. Quantitation was performed by introducing the measured integration areas in the calibration equation of standards more similar to the polyphenol being quantified. Thus, flavan-3-ols were quantified as (+)-catechin and epicatechin; flavanones as naringin and dihydroquercitrin; flavonol as quercetin, flavanone as dihydroquercitrin, and flavonol glycosides as quercetin 3-rutinoside (rutin), quercetin 7-*O*-*β*-D-glucoside (quercimeritrin), and pelargonidin 3-glucoside (callistephin).

UV-spectrometry Analysis. Absorbances of oils diluted in hexane (1:400) were measured with a V-530 UV/VIS/IR spectrophotometer (JASCO, Tokyo).

ACKNOWLEDGMENT

This work was supported by Research Centre of Physico-chemical Analysis CRAPC, the Functional Organic Analysis Laboratory (Algeria), and the General Chemistry Laboratory of Agronomic Sciences University, Gembloux (Belgium).

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