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Antioxidant activity of various extracts and fractions of Chenopodium quinoa and Amaranthus spp. seeds

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Abstract

The antioxidant potency of various extracts and fractions from *Chenopodium quinoa* and *Amaranthus* sp. was evaluated using three established methods, specifically the DPPH scavenging activity, FRAP, and β-carotene bleaching assays. Satisfying results were obtained, which lead to expect the use of these seeds as health-promoting ingredients. The antioxidant activity was less correlated to the phenolics content suggesting that non-phenolic compounds might play major free radicals scavenging activity in studied plant materials.

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Keywords: Chenopodium quinoa; Amaranthus sp.; DPPH; FRAP; Oxidative stress; Total phenolics content; Antioxidant activity

1. Introduction

The consumption of many grains has been associated with a lower risk of degenerative diseases, which depends on oxidative stress namely atherosclerosis, cancers, diabetes, Alzheimer's disease ([Rimm et al., 1996; Steinmetz &](#page-6-0) [Potter, 1996](#page-6-0)). Recently, a great interest has been given to naturally occurring antioxidants, which may play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes ([Carini](#page-6-0) [et al., 1990](#page-6-0)). Therefore, screening plant materials on the basis of their antioxidant potency seems to be of central importance in order to identify extracts or fractions possessing the ability either in scavenging both free radicals

and chain-reactions initiation or in binding with catalysts of the oxidative reactions, such as some metal ions [\(Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003\)](#page-6-0). From the viewpoint of their high antioxidant potency, the consumption at high scale of many plants have been recommended ([Kitts, Yuan, Wijewickreme, & Hu, 2000\)](#page-6-0), therefore, the evaluation of antioxidant activities of extracts and fractions is considered as an important step prior to the isolation of antioxidant phytochemicals they contain.

Amaranthus is a fast-growing crop mainly cultivated in Latin America, Africa, and Asia [\(Becker et al., 1981;](#page-6-0) [Teutonico & Knorr, 1985\)](#page-6-0). Because this pseudocereal is resistant to drought, hot climate and pests, and have little requirements on cultivation inputs [\(Hauptli, 1977; Paredes](#page-6-0) [Lopez, Schevenin, Hernandez Lopez, & Carabez Trejo,](#page-6-0) [1989\)](#page-6-0), it has attracted much attention to the world's food supply. Quinoa (Chenopodium quinoa), another pseudocereal from the Andean origin, is extensively cultivated at mountain altitudes in Peru and Bolivia. Based on both their higher protein content than conventional used cereal grains and their higher protein nutritional quality

Abbreviations: AC, Amaranthus cruentus; AH, Amaranthus hypochondriacus; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing ability of plasma, or ferric reducing antioxidant power; TAE, tannic acid equivalent; TPTZ, 2,4,6-tris(2-pyridyl)-1,3,5-triazine; UV–VIS, ultraviolet–visible.

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([Lambert & Yarwood, 1992\)](#page-6-0), they have been gained an increased interest in recent years. On the basis of their nutritional values (rich in protein, fat, dietary fiber, ash, and minerals, especially calcium and sodium and contain a higher amount of lysine than conventional cereals), amaranth and quinoa have been used as important ingredients primarily in bread, pasta, and baby's food.

In this work, the seed derived-flours of two ecotypes of C. quinoa and three varieties belonging to two species of Amaranthus were extracted with hexane and ethanol successively; and the ethanolic extract was further partitioned using solvents of increasing polarity, namely diethyl ether, ethyl acetate, n-butanol, and water. The Folin–Ciocalteu method was used to determine the total phenolics content of each one of the ethanolic extracts. The antioxidant activity of various extracts and fractions was assessed using following methods: (i) DPPH assay to determine the free radicals scavenging activity, (ii) FRAP assay to assess the metal (iron exclusively) ions binding ability, and (iii) β -carotene bleaching assay to determine the inhibiting effect on the oxidation of fat-containing food.

The aim of this work was then to assess the total phenolics content and the antioxidant properties of studied plant materials. Furthermore, the UV–VIS spectrum of several extracts and fractions, and the correlation between total phenolic content and antioxidant activities were examined in order to give an orientation to the search of antioxidant compounds. Such study would contribute to further knowledge relating to the screening of antioxidant compounds into these plant materials.

2. Materials and methods

2.1. Plant materials

Two ecotypes of *C. quinoa*: The Japan sea-level type (JQ) and the Bolivia Altiplano type (BQ) and three varieties of Amaranthus: Amaranthus hypochondriacus (AH) K-343; Amaranthus cruentus (AC) R 104; Amaranthus cruentus (AC) M 7 were used as plant materials in this work.

2.2. Extraction procedure

The extraction was performed using solvents of increasing polarity (hexane, diethyl ether, ethyl acetate, n-butanol) in order to fractionate antioxidant compounds contained in the crude materials according to their polarity. Flour of studied seeds, as available in their commercial packing conditions, were mixed with ten-fold (w/v) of solvents at each extraction step. First, the samples were extracted by n-hexane to remove lipophilic compounds, then the resulting residues were extracted with ethanol. The ethanolic extracts were further portioned in separating funnel using diethyl ether, ethyl acetate, n-butanol, and water as described by [Mellidis and Papageorgiou \(1993\)](#page-6-0) with a slight modification, yielding one extract and four fractions as explained above.

2.3. Total phenolics content

The content of phenolic compounds in the extracts was determined according to the method of Jayaprakasha and his co-workers [\(Singh, Murthy, & Jayaprakasha, 2002\)](#page-6-0). The extracts were dissolved in ethanol. Aliquots of 0.5 mL were mixed with 2.5 mL of the 10-fold diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature before measuring spectrophotometrically the absorbance at 760 nm. The final results were expressed as mg of tannic acid equivalents per gram of dry weight (dw).

2.4. Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing ability of studied plant materials was assessed following the method described by [Benzie and](#page-6-0) [Strain \(1996\)](#page-6-0). Briefly, the FRAP reagent contained 2.5 mL of 10 mM of 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM FeCl₃ and 25 mL of 0.3 M acetate buffer, pH 3.6, which was freshly prepared and warmed prior to the analysis. Aliquots $(40 \mu L)$ of sample (studied extracts or fractions) were mixed with 0.2 mL distilled water and 1.8 mL of FRAP reagent was added. The final results were expressed as the concentration of antioxidants having ferric reducing ability equivalent to that of $1 \text{ mM } \text{FeSO}_4$, particularly expressed as mol Fe(II) equivalent/g sample in dry weight.

2.5. β -Carotene bleaching assay

The β -carotene method was carried out according to the method developed by [Wettasinghe and Shahidi \(1999\).](#page-6-0) In short, $2 \text{ mL of } \beta$ -carotene solution $(0.2 \text{ mg/mL}$ in chloroform) were pipetted into a round-bottom flask containing $20 \mu L$ linoleic acid and $200 \mu L$ Tween 20. The mixture was then evaporated at 40 \degree C for 10 min to remove solvent, immediately followed by the addition of distilled water (100 mL). After agitating vigorously the mixture, 5 mL aliquots of the resulting emulsion were transferred into test tubes containing $200 \mu L$ of extract. The mixture was vortexed and placed in a water bath at 50° C for 2 h while the absorbance of tested sample was repeatedly measured every 15 min at 470 nm using a UV–VIS spectrophotometer. The blank solution contained the same concentration of sample without β -carotene. All determinations were performed in triplicates and the total antioxidant activity was calculated based on the following equation:

$$
AA = 1 - (A_0 - A_t)/(A_0^0 - A_0^t),
$$

where AA is antioxidant activity, A_0 and A_0^0 are the absorbance values measured at initial time of the incubation for samples and control, respectively, while A_t and A_0^t are the absorbance in the samples and control at $t = 120$ min.

2.6. Free radical scavenging ability on 1,1-diphenyl-2 picrylhydrazyl

To assess the scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH), each extract (1–20 mg/mL) in ethanol was mixed with 1 mL of ethanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a blank ([Shimada,](#page-6-0) [Fujikawa, Yahara, & Nakamura, 1992\)](#page-6-0). Then the scavenging ability was calculated using the following equation:

Scavenging ability $(\%) = [(\Delta A_{517} \text{ of control} - \Delta A_{517} \text{ of sample})$ ΔA_{517} of control| \times 100.

2.7. Statistical analysis

All experiments were conducted in triplicate and statistical analysis was done using the Statistical Package for Social Science (SPSS) programme. Results are expressed as a mean of three determinations \pm SD

3. Results and discussion

3.1. Total phenolics content

The total phenolics content was determined according to the colorimetric Folin–Ciocalteu method with tannic acid as a standard compound $(R^2 = 0.98, v = 0.0063 + 0.083)$ (data not shown). A wide range of total phenolics content was found in studied plant materials as shown in Table 1. Their content ranged from 94.3 ± 3.0 to 148 ± 1.9 mg/g tannic acid equivalent (TAE) of dried samples, with an average of 121 mg/g TAE. Among the five plant materials, the ethanolic extract of the Japan sea-level type of C. quinoa demonstrated the highest phenolics content $(148 \pm 1.9 \text{ mg/g} \text{TAE})$, followed by A. hypochondriacus K-343 (133.2 \pm 3.0 mg/g TAE), *A. cruentus* R 104 $(130.4 \pm 1.1 \text{ mg/g} \text{TAE})$, *A. cruentus* M 7 (99.8 \pm 4.3 mg/ g TAE), and the Bolivia altiplano type of C. quinoa $(94.3 \pm 3.0 \text{ mg/g} \text{ TAE})$. One way ANOVA showed signifi-

Table 1 Characterization of the ethanolic extracts of plant materials cant differences ($p \le 0.05$) in total phenolics content among the five studied samples. The difference in the phenolics concentration between the two ecotypes of C. quinoa might be explained by the difference in the environmental conditions or genetic background. Plant phenolics are biosynthesized following different routes: the shikimic acid pathway being the most biosynthetic route involved. This pathway, as reported by [Rivero et al. \(2001\)](#page-6-0), is thought to be an acclimatization mechanism of plants to external stress (temperature, injury, infections, etc.), the cold having the highest phenylalanine ammonia-lyase activating effect and the highest peroxidase and polyphenol oxidase inhibiting effect. It is important to note that [Amin, Nor](#page-6-0)[azaidah, and Emmy Hainida \(2006\)](#page-6-0) reported the ''bayam merah" (Amaranthus gangeticus) to possess the highest total phenolics content $(107 \pm 1.08 \text{ g/kg dw})$ among Amaranthus blitum, Amaranthus viridis, and Amaranthus paniculatus. Despite of the difference in the standard compounds used (tannic acid in this work and gallic acid in the study of [Amin et al., 2006](#page-6-0)) and in the Amaranthus species studied, two values obtained in this work, A. hypochondriacus (133.2 \pm 3.0 mg/g TAE dw) and A. cruentus R 104 $(130.4 \pm 1.1 \text{ mg/g dw})$ showed nearest phenols content as compared to A. gangeticus.

3.2. Antioxidant activities of the ethanolic extract

The principle of the antioxidant activity is the availability of electrons to neutralize any so-called free radicals. In this work, the antioxidant activity of the ethanolic extract between selected plant materials was evaluated using DPPH scavenging, FRAP, and β -carotene bleaching assays. As shown inTable 1, a big difference was observed among respective values obtained. The DPPH values varied from 59.2 \pm 1.4 to 85.6 \pm 0.9%, with the lowest activity for the Japan sea-level type of C. quinoa, which in contrast possessed the highest phenolics content. Interestingly, the FRAP assay and the β -carotene method demonstrated the higher antioxidant activity for the Japanese C. quinoa. Related FRAP values ranged from 7.3 to 8.2 mmol L^{-1} , with the following decreasing order Japanese C. quinoa

Values are expressed as a mean of three determinations \pm SD. TPC: total phenolics content was measured using the Folin–Ciocalteu method and is expressed as mg/g tannic acid equivalent (TAE). Antioxidant capacity was determined as % of DPPH free radicals scavenged, mmol L^{-1} of extracts or fractions having a ferric reducing ability equivalent to that of 1 mM FeSO4, and as the percent of b-carotene non-oxidized during 2 h of incubation in a water bath kept at 50 °C. % of 1,1-diphenylpicrylhydrazyl free radicals scavenged, FRAP: ferric reducing ability of plasma, BC: % inhibition of β -carotene oxidation. Values not sharing common letters are significantly different ($P \le 0.005$).

 $(8.2 \pm 3.0 \text{ mmol L}^{-1})$ > A. hypochondriacus K-343 (7.6 \pm 0.7 mmol L^{-1}) > Bolivian *C. quinoa* (7.5 \pm 1.7 mmol L^{-1}) > A. cruentus R 104 (7.3 \pm 1.1 mmol L^{-1}) and A. cruentus M 7 (7.3 \pm 0.8 mmol L⁻¹). Although values of FRAP were not significantly different, all studied samples showed high ferric reducing ability.

3.3. Total phenols content versus the radical scavenging activity

In this work, the correlation between the radical scavenging activity and phenols content of the five samples was studied using a linear regression analysis. As demonstrated in Fig. 1, the correlation coefficient between total phenolics and FRAP values ($R^2 = 0.4833$, $y = 0.0103X +$ 6.3218), DPPH scavenging activity $(R^2 = 0.2049, y =$ $-0.2677X + 107.09$, and β -carotene assay ($R^2 = 0.489$, $y = 0.1185X + 53.594$ was found to be very weak, less than 0.5. These low correlation values between total phenols and the antioxidative activity suggest that the major antioxidant compounds in studied seeds might be nonphenolics. Although total phenolics content showed a relative high amount, other non-phenolic compounds, such as ascorbic acid and phytic acid (in water soluble compounds), tocopherols, sterols, and carotenoids (lipophilic compounds), saponins and ecdysteroids (butanol soluble compounds), and others might be the most probable contributors to the antioxidant activity of the five samples studied on this experiment.

3.4. The concentration of extracts or fractions giving 50% inhibition (IC₅₀) related to FRAP, DPPH, and β -carotene assays

The IC_{50} of extracts and fractions of studied plant materials are summarized in [Table 2.](#page-4-0) Ranged from 0.9 ± 0.9 to 23.9 ± 2.3 mg/mL, the IC₅₀ values showed various inhibiting efficacy against β -carotene oxidation. In each plant material, the hexane extract and the ethyl acetate and butanol fractions demonstrated the highest inhibition potency. In contrast, a general tendency was also observed that the diethyl ether and water fractions had the lowest inhibitory ability on the β -carotene oxidation with IC₅₀ values arising from 5.7 ± 1.2 to 23.9 ± 2.3 mg/mL as shown in [Table 2](#page-4-0). These results indicated that the hexane extract, ethyl acetate, and butanol fractions of studied seeds contain antioxidant compounds with high potency in inhibiting the oxidation of lipids and could be used as preservatives to delay or limit lipids oxidation, which leads to the rancidity into fat-containing foods.

The DPPH scavenging activity of extracts and fractions, expressed in the term of IC_{50} was in the range of 0.1– 24.8 mg/mL ([Table 2\)](#page-4-0), with the strongest antioxidant potency for the ethyl acetate fractions of both A. cruentus R 104 and C. quinoa (Japan) and the weaker for the diethyl ether fraction of A. cruentus R 104. These results suggest

Fig. 1. Correlation between total phenolics content and FRAP (a), DPPH (b), and β -carotene assays (c). Values are mean of three determinations \pm SD. TAE: tannic acid equivalent.

that the ethyl acetate fractions might contain the strongest free radical scavenger compounds.

The IC_{50} values (mg/mL) related to FRAP assay showed the strongest ferric reducing ability on both the ethyl acetate and butanol fractions, with the highest activity found in ethyl acetate fraction of the sea-level type of Japan C. quinoa (1.1 \pm 0.9 mg/mL) and the butanol fraction of Alti-

Table 2 The half-inhibition (IC₅₀) values of the antioxidant activities measured using FRAP, DPPH, and β -carotene assays

Extracts and fractions	Yield $(\%)$	BC	DPPH	FRAP
Chenopodium quinoa (Bolivia)				
Hexane extract	5.90	1.2 ± 0.8	22.4 ± 0.3	4.8 ± 2.5
Diethylether fraction	0.80	10.4 ± 1.4	5.0 ± 0.1	8.2 ± 1.2
Ethyl acetate fraction	0.20	1.8 ± 0.5	0.3 ± 0.8	2.3 ± 1.2
Butanol fraction	0.40	2.5 ± 1.5	ND	4.6 ± 2.3
Water fraction	0.40	20.9 ± 2.2	15.8 ± 1.1	6.2 ± 2.4
Chenopodium quinoa (Japan)				
Hexane extract	6.80	1.4 ± 1.2	7.5 ± 0.7	4.0 ± 2.7
Diethylether fraction	0.03	5.7 ± 1.2	5.3 ± 0.3	6.1 ± 3.1
Ethyl acetate fraction	0.04	0.9 ± 0.9	0.1 ± 1.2	1.1 ± 0.9
Butanol fraction	0.70	1.4 ± 0.6	ND	4.9 ± 2.2
Water fraction	1.03	4.6 ± 2.1	ND	7.8 ± 1.4
Amaranthus hypochondriacus K-343				
Hexane extract	7.30	5.2 ± 0.9	13.6 ± 0.7	6.6 ± 1.6
Diethylether fraction	0.03	10.8 ± 2.3	16.2 ± 1.9	9.2 ± 2.2
Ethyl acetate fraction	0.03	2.0 ± 0.9	0.2 ± 1.1	4.7 ± 1.5
Butanol fraction	0.40	2.3 ± 0.7	0.2 ± 0.8	4.9 ± 2.1
Water fraction	0.80	23.9 ± 2.3	0.6 ± 1.2	7.9 ± 1.2
Amaranthus cruentus R 104				
Hexane extract	6.80	1.4 ± 0.8	15.9 ± 0.3	7.7 ± 1.9
Diethylether fraction	0.40	11.2 ± 2.1	24.8 ± 1.3	10.5 ± 1.5
Ethyl acetate fraction	0.06	1.3 ± 0.5	0.1 ± 0.9	4.2 ± 2.1
Butanol fraction	Insig.	1.1 ± 1.0	ND	5.3 ± 1.8
Water fraction	0.73	20.1 ± 2.3	ND	8.2 ± 2.2
Amaranthus cruentus M 7				
Hexane extract	5.50	5.4 ± 1.2	14.7 ± 0.8	7.2 ± 3.2
Diethylether fraction	0.40	12.8 ± 2.3	7.8 ± 1.1	11.7 ± 2.7
Ethyl acetate fraction	0.02	5.2 ± 1.2	0.3 ± 1.1	1.8 ± 1.7
Butanol fraction	0.07	4.9 ± 1.3	ND	5.1 ± 1.0
Water fraction	0.71	23.8 ± 2.8	ND	9.1 ± 1.9

The IC-50 value is expressed as mg/mL and represents the concentration of extracts or fractions that is required for 50% of free radicals inhibition (DPPH method), 50% inhibition of β-carotene oxidation, and 50% of ferric ions reduction. Lower IC50 value indicates the higher antioxidant activity. Each value in the table was obtained by calculating the average of three determinations \pm standard deviation. DPPH: 1,1-diphenylpicrylhydrazyl scavenging activity, FRAP: ferric reducing ability of plasma, BC: b-carotene/linoleate oxidation system. % yield gives the amount (g) of extracts or fractions produced if our starting material (seeds) was 100 g. Insig.: insignifiant; ND: not determined.

platino type of the Bolivia C. quinoa $(4.6 \pm 2.3 \text{ mg/mL})$. These data shows that both the above ethyl acetate and butanol fractions might contain either the higher concentration or the most potent compounds having the most potent efficacy in reducing metal ions, which act as catalysts of oxidative reactions.

We here report on a different trend in antioxidant capacity between the FRAP, DPPH, and β -carotene assays. Thus, it is important that results of antioxidant capacity of plant samples determined from different methods to be interpreted with caution. It may, therefore, be important to assess the quality of antioxidants for consideration, as plasma polyphenol antioxidant concentrations in vivo are less than $1 \mu M$ after consumption of fruits, vegetables, and beverages ([Vinson, Proch, & Bose, 2001\)](#page-6-0). Briefly, this study showed that β -carotene bleaching assay, DPPH scavenging activity, and FRAP assay can be used for convenient monitoring of the antioxidant capacities in seeds of studied pseudocereals in one hand. In the other, these experimental results led to think that various extracts and

fractions could contain different antioxidants, which demonstrated varying reactivity in the three in vitro models used in this work.

3.5. The UV–VIS spectrum of various extracts and fractions (in term of λ_{max})

The UV–VIS spectrum recorded from the ethanolic extract of both C. quinoa ecotypes [\(Fig. 2\)](#page-5-0) showed two peaks respectively at 264 nm and 354 nm suggesting the presence of flavonoids. In contrast, the spectrum of the ethanolic extract of the three varieties of Amaranthus showed a single λ_{max} at 270 nm, which suggests to be related to the presence of tocols and other phenolic acids.The UV–VIS spectrum resulting from the hexane fraction of both C. qui*noa* and A. cruentus R 104 and M 7 showed unique λ_{max} at 278 nm, while that taken recorded from A. hypochondriacus K-343 demonstrated its λ_{max} at 288 nm. Both λ_{max} values orientate the analysis toward tocols. Although, this extract did not show characteristic λ_{max} related to other lipophilic

Fig. 2. UV/VIS spectrum of extracts and fractions made from the five studied seeds. The UV/VIS spectrum was recorded from 200 nm to 450 nm and related λ_{max} determined. Each extract or fraction demonstrated typical λ_{max} which gives an approximate idea on the compound to search from EtOH: ethanol, HEX: hexane, DE: diethylether, E: ethyl acetate, BU: butanol, and W: water fractions of 1. C. quinoa from Bolivia, 2. C. quinoa from Japan, 3. A. hypochondriacus K-343, 4. A. cruentus R-104, 5. A. cruentus M-7.

antioxidants, such as carotenoids and sterols, those cited compounds will be evident target during the analytical works. The UV/VIS spectrum of the diethyl ether fraction, considered as remaining triglycerides, showed unique λ_{max} at 275 nm, which is supposed to be related to tocols. That from the ethyl acetate fraction exhibited one peak at 329 nm from both C. quinoa, this λ_{max} gives an idea to the search of flavonoids, while other plant materials have shown a peak at 258 nm, which is thought to be from phenolic acids. The butanol fraction demonstrated two peaks: the first at 355 nm which is thought to be from residual flavonoids, as compounds were not fully extracted with previous solvent (ethyl acetate). The other peak at 262 nm suggests the presence of butanol soluble triterpenoidal or steroidal saponins ([Itabashi, Segawa, & Ikeda,](#page-6-0) [2000; Regiantto, Kauffman, & Schripsema, 2001; Soth](#page-6-0)[eeswaran, Bokel, & Kraus, 1989](#page-6-0)). The water fraction showed a single λ_{max} at 263 nm, which is expected to be due to the presence of either vitamin C (in Amaranthus sp. exclusively) as this compound absorbs at a nearest wavelength (265 nm) in a neutral medium ([Fontannaz, Kil-](#page-6-0) [inc, & Heudi, 2006](#page-6-0)) or some low molecular weight phenolics and lignans.

4. Conclusion

Both the two ecotypes of C. quinoa and the three varieties of Amaranthus seeds might be rich in several phytonutrients that act as powerful dietary antioxidants. Total phenolics content and antioxidant capacity had different values among the samples and there was observed a weak correlation between phenolics concentration and antioxidant potency. Various extracts and fractions might provide different antioxidants, which in general demonstrated strong activities within the ethyl acetate and butanol fractions. The UV/VIS spectrum as recorded gave a slight idea on possible antioxidant compounds contained in various extracts and fractions studied. Therefore, to better manage clinical consequences arising from oxidative damage, detailed information on the structure of the most active compounds in both fractions are now under investigation. That work will be the core of the next scientific communications.

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