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INCREASED NON-ENZYMATIC GLYCATION REPORTED IN APIACEAE FAMILY EXTRACTS

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Abstract

Prevention and treatment of diabetic complications implicates inhibition of free radicals and advanced glycation end products (AGEs) generated during diabetes. The present work is an investigation of *In vitro* anti-glycation activity of hydro-alcohol (HE), hot and cold aqueous extracts (HAE and CAE) of Moroccan plant species from Apiaceae family. Anti-glycation activity of seven plant species extracts (*Ammodaucus leucotrichus*, *Daucus carota*, *Petroselinum sativum*, *Pimpinella anisum*, *Carum carvi*, *Coriandrum sativum*, *Foeniculum vulgare*), performed on BSA-Methylglyoxal system, was measured by fluorescence and native electrophoresis. Anti-oxidant activity and quantitative detection of chemical contents were assessed as well. Statistical analysis of principal components (PCA) allowed determining the implication of different parameters studied on glycation effect. Apiaceae family showed a strong dose-dependent glycation effect, activating both formation of Amadori products and fluorescent AGEs. This effect was stronger in *Pimpinella anisum* and *Foeniculum vulgare* extracts. Anti-glycation activity was recorded only at 1.5 mg/mL of extracts, with a significant correlation from antioxidant power that was between 65.29% and 81.05%. This family was generally poor in polyphenols and flavonoids with maximal values recorded in CAE (98.47±4.42 mg GA/g Dm) and HE (49.29±3.20 mg Qu/g Dm) respectively. The maximum value of tannins was 14.96±1.21 mg TA/g Dm. However, there was a significant richness in reducing sugar content. Anti-glycation effect of Apiaceae family lost its effectiveness with the increase of extract concentration. Glycation activity can be attributed to richness of this family by reduced sugar.

Key words: Antioxidant, Polyphenols, Flavonoids, Glycation, Apiaceae.

Introduction

Treatment of diabetic complications such as, coronary heart disease, stroke, cataract, neuropathy and atherosclerosis implicates inhibition of free radicals and advanced glycation end products (AGEs) (Jung *et al*., 2009; Grzegorczyk-Karolak *et al*., 2016). These AGEs are synthesized by non-enzymatic reactions between electrophilic carbonyl groups of reducing carbohydrates (such as fructose and glucose) and free amino groups of amino acids (proteins, lipids or nucleic acids) forming a non-stable Schiff base AGEs. Further rearrangement leads to formation of Amadori product, that can undergo further oxidation, dehydration, polymerization and oxidative breakdown reactions to give rise to AGEs (Thorpe & Baynes, 2003; Ahmed, 2005).

Medical field knows an important expansion in the treatment of different diseases. Nevertheless, these treatments are always expensive and present side effects. Medicinal plants constitutes an important alternative to replace classical drugs because of the richness of secondary metabolites such as phenolic and flavonoid compounds. These bioactive compounds are known to have high antioxidant capacity (Anderson *et al*., 2001), and a wide range of therapeutic properties such us anticancer and anti-diabetic effect (Wink, 2015). Several plant species were reported to possess anti-glycation properties (Jafari *et al.*, 2015; Ramdan *et al*., 2017, 2019). In fact, different studies reported that natural molecules considered as strong glycation inhibitors are present in herbs, vegetables and fruits (Pinto *et al*., 2009).

Apiaceae family is one of the largest plant families in the world and encloses about 450 genera and 3700 species worldwide (Amiri & Joharchi, 2016). This family comprises many important species that are very used as medicinal, vegetables and culinary plants such as *Carum carvi* (caraway), *Cuminum cyminum* (cumin) and *Foeniculum vulgare* (fennel) (Amiri & Joharchi, 2016). In this context, this study contributes to the scientific understanding on the quantitative chemical composition of ethanolic and aqueous extracts of Apiaceae family and the role of antioxidants compounds in non-enzymatic glycation management.

Materials and Methods

Chemicals: All reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Extract preparation: Plants were collected in March 2017, from different regions of Morocco (Table 1). They were dried at 40°C for 24 hour, ground into a fine powder that was extracted (40 g) with H_2O (400 mL) for 45 min, thrice, at 80°C (HAE) or at 25°C (CAE). Hydroalcoholic extract (HE) was obtained by extraction of 30 g of every sample with 300 mL of ethanol solution (70%) for 24 hour, thrice. The macerates were filtered, centrifuged for 20 min (4000 t/min) and the supernatants were evaporated (Ramdan *et al.*, 2018).

Table 1. Species description.

Botanical name	Genus	Origin	Harvest period	Used part	Spontaneous / cultivated	Voucher specimen No.
Ammodaucus leucotrichus (*A. leucotrichus*)	*Ammodaucus*	Marrakech	May	Fruit	Cultivated	73/20
Daucus carota (*D. carota*)	*Daucus*	Khmiss anjra	February	Seeds	Cultivated	73/27
Petroselinum sativum (*P. sativum*)	*Petroselinum*	Khmiss anjra	February	Seeds	Cultivated	73/32
Pimpinella anisum L. (*P. anisum*)	*Pimpinella*	Meknès	September	Seeds	Cultivated	73/36
Carum carvi L. (*C. carvi*)	*Carum*	Marrakech	July	Seeds	Cultivated	73/38
Coriandrum sativum L. (*C. sativum*)	*Coriandrum*	Gharb	July	Seeds	Cultivated	73/41
Foeniculum vulgare Mill (*F. vulgare*)	*Foeniculum*	Sidi kassem	July	Seeds	Spontaneous	73/49

To determine the extracts yield, the subsequent formula was used:

$$R = (Px / Py) * 100$$

R: Extract yield (%), Px: Extract weight (g), Py: Plant weight (g).

Quantification of total phenolic contents (TPC): Total Phenolic Contents (TPC) was determined by Folin-Ciocalteu method with some modifications (Checkouri *et al*., 2020). Briefly, 100 µl of extracts (1 mg/mL) were added to 500 µl of 1:10 Folin–Ciocalteu reagent. Sodium carbonate 7.5% (400 µl) was added after 4 min. Optical density was measured after 30 min at 765 nm (VARIAN Cary 50 UV-Vis). A gallic acid solution (GA) (5 mg/mL) was used to prepare the standard range (0 to 150 µg/mL). Results were reported in Gallic Acids Equivalents (GAE) per g of extract.

Quantification of total flavonoid contents (TFC): Total Flavonoid Contents (TFC) was determined by aluminum trichloride colorimetric method (AlCl3), with modifications (Cherbal *et al*., 2012). Briefly, 250 µl of extracts (2 mg/mL) were added to 1.4 mL of H_2O, 50 µl of aluminum trichloride 10% (m/v), 50 µl of potassium acetate (1 M) and 750 µl of absolute ethanol. After 30 min, absorbance was measured at 415 nm (VARIAN Cary 50 UV-Vis). A quercetin solution (10 mg/mL of ethanol 80%) was used to prepare the standard range (0 to 150 µg/mL). Results were reported in Quercetin Equivalents (QE) per g of extract.

Quantification of total tannins contents (TTC): Total Tannins Contents (CTT) was determined by Folin-Denis method, with some modifications (Braca *et al*., 2002). Briefly, 100 µl of extracts (1 mg/mL) were added to 500 µl of Folin–Denis reagent (1:10). After 5 min, 400 µl of Na_2CO_3 7.5% (m/v) were added. After 30 min, absorbance at 760 nm was measured. A solution of tannic acid (5 mg/mL) was used to prepare the standard range (0 to 150 µg/mL). Results were reported in Tannic Acids Equivalents (TAE) per g of extract.

Quantification of reducing sugar content: Reducing sugar content was determined by Nelson Somogyi method, with some modifications (Chiu & Le, 2007). Briefly, 100 µl of extracts (1 mg/mL) were added to 1 mL of alkaline copper tartrate reagent and 1.9 mL of H_2O. Arsenomolybdic reagent was added after 10 min at 100°C, then distilled water to reach a volume of 10 mL. Absorbance at 620 nm was measured. A glucose solution (100 mg/mL) was used to prepare the standard range (0 to 100 mg/mL). Results were reported in Glucose Equivalents (GE) per g of extract.

Antioxidant activity

Radical DPPH-scavenging activity: Free radical-scavenging activities were determined according to Wong & Kitts (2006), with modifications. For this, DPPH (4 mg) were dissolved in methanol (100 mL) and incubated in the dark for 3H. Then, 750 µl of DPPH solution were added to 250 µl of each extract (0.06, 0.125, 0.25, 0.5, 1 and 10 mg/mL) and incubated in the dark for 30 min. Absorbance was measured at 517 nm. A solution of ascorbic acid (5 mg/mL) was used to prepare the standard range (0 to 500 µg/mL).

Fe^{++} chelating assay: Chelating capacity of extracts was measured according to Akroum (2011) with some modifications. Briefly, 250 µl of extracts (0.25, 0.5, 1, and 10 mg/mL) was added to 450 µl of methanol and 50 µl of $FeCl_2$ (0.6 mM). After 5 min, 50 µl of Ferrozine (5 mM) was added. After 10 min, absorbance at 562 nm was measured (VARIAN Cary 50 UV-Vis). In negative control, methanol replaced the extract. A solution of quercetin (10 mg/mL of ethanol at 80%) was used to prepare the standard range (0 to 150 µg/mL).

Antiglycation activity

***In vitro* glycation of serum bovine albumin:** Bovine serum albumin (5 mg/mL) was incubated with methylglyoxal (10 mM) in 0.1 M phosphate buffer (pH 7.4). Extracts (1.5, 3.5 and 10 mg/mL) were added to the reaction mixture, then incubated for 24 hour at 60°C in the dark (Ramdan *et al.,* 2017). Metformine (30 mM) was used as positive control.

Electrophoretic migration in native conditions: The samples were separated on a 7% polyacrylamide gel then stained for 1 hour with coomassie blue. Destaining was for 1 hour with a solution of acetic acid.

Spectrofluorimetric measure: Fluorescence measure (excitation at 370 nm and emission 423 nm) was performed through a spectrofluorimeter type (VARIAN Cary 50 UV-Vis).

Statistical analysis

All the experiments were conducted in triplicate and the data are presented as mean values ± S.D. Results were subjected to a one-way analysis of variance (ANOVA) followed by Fischer's and Tukey test. Bivariate correlation was assessed by the test of Pearson using SPSS statistics (version 21, Chicago, IL, USA). The p values ≤ 0.05 were considered significant.

Results and Discussion

Quantification of polyphenols, flavonoids, tannins and reducing sugars: Apiaceae family was generally poor in polyphenols and flavonoids with maximal values recorded in CAE (from 47.32±0.25 mg GAE/g Dm in *A. leucotrichus* to 165.86±0.31 mg GAE/g Dm in *C. carvi*) and HE (from 20.44±0.77 mg Qu/g Dm in *C. sativum* to 96.17±0.43 mg Qu/g Dm in *P. anisum*) respectively. The maximal value of tannins was 14.96±1.21 mg TA/g Dm. However, there was significant amounts of reducing sugars (Tables 2 and 3). In addition, positive and highly significant correlation (r> 0.917) between polyphenol and flavonoid levels meant that more than 91% of polyphenols were flavonoids.

Principal component analysis (PCA) showed the presence of two groups positively correlated (55,45% and 23,18% of variance in component 1 and 2 respectively). These groups characterized *P. anisum* and *F. vulgare* extracts (Fig. 1), which were the richest in the quantified phytochemical compounds. However, the other species were characterized by high contents of reducing sugars. Results of this study were higher than those of literature (Shan, 2007; Il-Suk, 2011; Liga & Daina, 2013; Bekara, 2016).

Antioxidant activity: The maximum inhibitory effects in DPPH test ranged from 69.90% in HE to 81.05% in HAE. While maximum chelation rates ranged from 65.29% in HE to 71.44% in HAE (Fig. 2). The inhibitory effect was significantly higher than the chelating effect of iron-ferrous (p<0.05) (Table 4, Fig. 2). In addition, the correlation study between inhibitory and chelating effect showed high indices for HE and CAE with r = 0.791 and r = 0.936 respectively (p<0.000).

The strongest inhibitory effect was recorded in aqueous extracts of *C. carvi* (IC_{50} = 0.27±0.009 mg/mL) and HE of *P. anisum* (IC_{50} = 0.08±0.002 mg/mL). The highest chelating effect was nevertheless attributed to the aqueous extracts of *P. sativum* and HE of *F. vulgare* and *P. anisum* (p>0.05).

On the other hand, PCA showed that *C. carvi*, *F. vulgare* and *P. sativum* were characterized by the group 2. However, *A. leucotrichus* and *D. carota*, which did not show significant differences, were rather characterized by the group 1 (Fig. 3).

The study of Wong & Kitts, (2006) on a genus of *Petroselinum* reported that the chelating activity in the leaves and stems reached 26.0±2.4% and 40.5±5.1% respectively. According to Akroum, (2011), the DPPH test chowed 33.60% of inhibition in *P. sativum*.

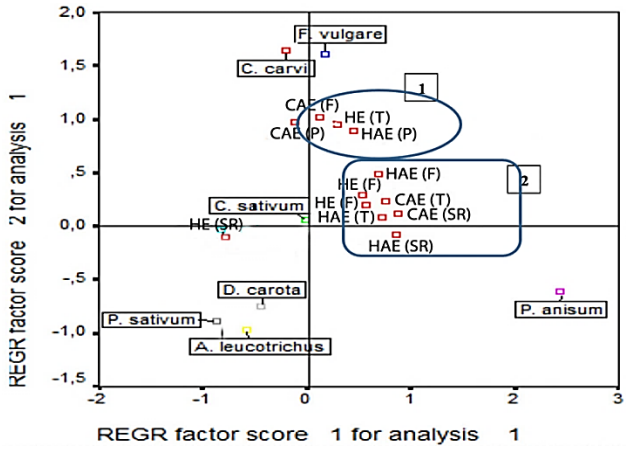


Fig. 1. Characterization of plant species of the Apiaceae family in chemical compounds. Variable projection according to the two components, 59, 04% and 26, 10% of variance in component 1 and 2 respectively. P: Polyphenols; F: Flavonoids; T: Tannins; RS: Reducing sugar; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; HE: Hydro-alcoholic extract.

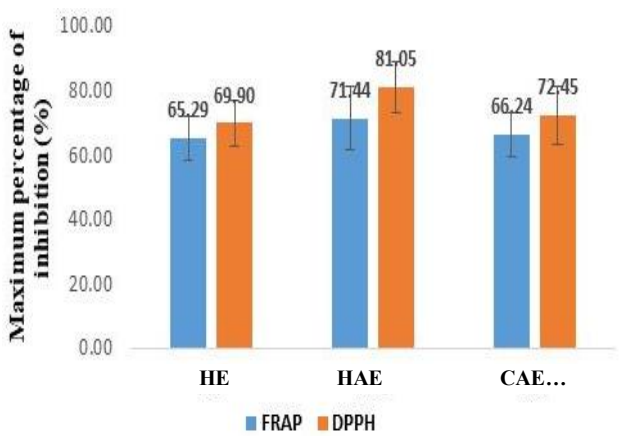


Fig. 2. Graphical representation of the maximum inhibition percentages means for DPPH and FRAP tests in the Apiaceae family. DPPH: Free radical-scavenging capacity test; FRAP: Fe++ chelating assay.

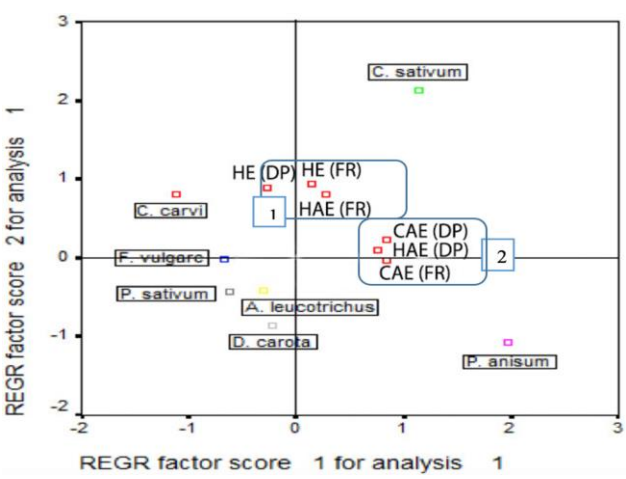


Fig. 3. Antioxidant capacity of extracts from Apiaceae family. Variable projection according to the two components. DP: Free radical-scavenging capacity test; FR: Fe^{++} chelating assay; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; HE: Hydro-alcoholic extract.

Table 2. Comparative study of total polyphenol and flavonoid concentrations of HA, CAE and HE among the seven species of Apiaceae family.

Species	Polyphenols (mg GAE/g Dm)			Flavonoids (mg QuE/g Dm)			Literature correspondence		
	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er	Polyphenols (mg GA/g Dm)	Flavonoids (mg Qu/g Dm)	Reference
C. carvi	131.18±0.47 (a)	157.7 ± 0.28 (b)	56.38 ± 0.19 (g)	65.98 ± 0.50 (b)	72.45 ± 0.41 (b)	22.61 ± 0.49 (f)	0.6 (HE) 9.92 ± 0.11 (HAE)	- 45.01 ± 2.27 HAE	(Shan *et al*. 2007) (Il-Suk, 2011)
C. sativum	108.55 ± 0.47 (c)	89.81 ± 0.34 (d)	58.39 ± 0.60 (f)	50.68 ± 0.46 (d)	42.13 ± 0.54 (d)	20.44 ± 0.77 (g)	9.22 ± 0.09 (HAE)	3.38 ± 0.09 HAE	(Il-Suk, 2011)
F. vulgare	116.31 ± 0.28 (b)	165.86 ± 0.31 (a)	192.50 ± 0.69 (b)	53.98 ± 0.45 (c)	76.92 ± 0.32 (a)	90.37 ± 0.14 (b)	9.36 ± 0.21 (HAE)	44.76 ± 2.32 HAE	(Il-Suk, 2011)
P. anisum	109.46 ± 0.31 (c)	67.29 ± 0.25 (g)	193.14 ± 0.30 (a)	67.43 ± 0.29 (a)	32.17 ± 0.50 (e)	96.17 ± 0.43 (a)	2,38 ± 0,01 (HAE)	8,06 ± 0,08 HAE	(Bekara *et al.*, 2016)
A. leucotrichus	70.87 ± 0.24 (f)	47.32 ± 0.25 (h)	74.05 ± 0.43 (d)	33.11 ± 0.24 (e)	26.2 ± 0.24 (f)	28.36 ± 0.32 (e)	-	-	-
D. carota	75.73 ± 0.27 (e)	77.83 ± 0.30 (f)	56.29 ± 0.41 (g)	34.35 ± 0.21 (e)	22.6 ± 0.25 (g)	55.58 ± 0.19 (d)	-	-	-
P. sativum	62.14 ± 0.47 (g)	83.69 ± 0.45 (e)	66.76 ± 0.22 (e)	25.74 ± 0.28 (f)	23.60 ± 0.16 (g)	28.65 ± 0.23 (e)	1812.81	-	(Liga & Daina, 2013)
Total	95.32 ± 2.59	98.47 ± 4.42	97.83 ± 6.25	47.3717 ± 1.61	42.77 ± 2.32	49.29 ± 3.20		----	
Fisher	4397.67 (p<0.000) **	16065.58 (p<0.000) **	18569.81 (p<0.000) **	1694.55 (p<0.000) **	3639.36 (p<0.000) **	5110.94 (p<0.000)**			

Groups with the same letters do not differ significantly by tukey test; Std. Er: Standard Error; **: Very highly significant difference

Table 3. Comparative study of tannins and reducing sugar concentrations of HAE, CAE and HE among the seven species of Apiaceae family.

Species	Tannins (mg TAE/g Dm)			Reducing sugar (mg GE/g Dm)		
	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er
C. carvi	16.51 ± 0.51 (c)	14.77 ± 0.24 (b)	13.34 ± 0.26 (b)	13.65 ± 0.24 (d) (c)	12.61 ± 0.31 (b)	11.59 ± 0.24 (c)
C. sativum	19.44 ± 0.37 (b)	14.04 ± 0.26 (c) (b)	8.69 ± 0.30 (d)	12.32 ± 0.31 (d) (c)	10.92 ± 0.17 (c) (b)	11.36 ± 0.25 (c)
F. vulgare	11.78 ± 0.23 (d)	13.59 ± 0.17 (c)	15.75 ± 0.24 (a)	14.64 ± 0.32 (b)	13.48 ± 0.17 (b)	13.41 ± 0.21 (b)
P. anisum	28.06 ± 0.36 (a)	23.52 ± 0.18 (a)	10.11 ± 0.37 (c)	62.64 ± 4.76 (a)	25.65 ± 1.89 (a)	10.18 ± 0.2 (d)
A. leucotrichus	9.30 ± 0.35 (e)	6.8 ± 0.20 (e)	6.27 ± 0.20 (e)	6.43 ± 0.16 (d)	8.43 ± 0.29 (d) (c)	12.31 ± 0.24 (c)
D. carota	7.44 ± 0.32 (f)	8.44 ± 0.27 (d)	6.12 ± 0.14 (e)	8.47 ± 0.26 (d) (c)	8.33 ± 0.25 (d) (c)	9.27 ± 0.19 (d)
P. sativum	9.46 ± 0.14 (e)	7.66 ± 0.32 (e) (d)	5.52 ± 0.21 (e)	7.38 ± 0.23 (d) (c)	5.52 ± 0.23 (d)	23.54 ± 0.2 (a)
Total	14.96 ± 1.21	12.14 ± 0.59	9.00 ± 0.41	16.63 ± 2.06	11.94 ± 0.68	14.28 ± 0.59
Fisher	425.08 (p<0.000) **	482.58 (p<0.000) **	194.93 (p<0.000) **	122.72 (p<0.000) **	74.25 (p<0.000) **	546.78 (p<0.000) **

Std. Er: Standard Error; **: Very highly significant difference

Table 4. DPPH reducing power and iron-ferrous chelator activity (FRAP) of HAE, CAE and HE among the Apiaceae family species.

SPECIES	IC_{50} DPPH (mg/mL)			IC_{50} Fe^{++} chelating assay (mg/mL)		
	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er	Mean HAE ± Std.Er	Mean CAE ± Std.Er	Mean HE ± Std.Er
C. carvi	0.27 ± 0.009 (f)	0.30 ± 0.001 (f)	0.74 ± 0.001 (a)	0.45 ± 0.004 (c)	0.40 ± 0.0019 (d)	1.06 ± 0.011 (b)
C. sativum	0.47 ± 0.007 (a)	0.66 ± 0.005 (b)	0.74 ± 0.003 (a)	0.63 ± 0.007 (a)	0.65 ± 0.005 (b)	2.10 ± 0.031 (a)
F. vulgare	0.30 ± 0.001 (e)	0.31 ± 0.005 (f)	0.24 ± 0.008 (c)	0.57 ± 0.006 (b)	0.42 ± 0.004 (d) (c)	0.29 ± 0.001 (e)
P. anisum	0.47 ± 0.004 (a)	0.73 ± 0.005 (a)	0.08 ± 0.002 (d)	0.44 ± 0.005 (c)	0.91 ± 0.019 (a)	0.27 ± 0.007 (e)
A. leucotrichus	0.37 ± 0.002 (d) (c)	0.40 ± 0.003 (c)	0.39 ± 0.003 (b)	0.42 ± 0.002 (d)	0.44 ± 0.004 (c)	0.40 ± 0.004 (d)
D. carota	0.38 ± 0.25 (d)	0.37 ± 0.001 (d)	0.11 ± 0.02 (d)	0.42 ± 0.003 (d)	0.44 ± 0.006 (c)	0.47 ± 0.007 (c)
P. sativum	0.36 ± 0.003 (d)	0.33 ± 0.006 (e)	0.38 ± 0.002 (b)	0.40 ± 0.001 (d)	0.37 ± 0.005 (e)	0.39 ± 0.004 (d)
Total	0.37 ± 0.78	0.43 ± 0.01	0.38 ± 0.02	0.47 ± 0.008	0.51 ± 0.019	0.67 ± 0.066
Fisher	207.69 (p<0.000) **	1316.09 (p<0.000) **	564.25 (p<0.000) **	291.53 (p<0.000) **	517.22 (p<0.000) **	2439.00 (p<0.000) **

Std. Er: Standard Error; **: Very highly significant difference

Table 5. Comparative study of glycation inhibition percentages after treatment with HAE, CAE and HE, among 7 species of the Apiaceae family.

		Concentration (mg/mL)	*C. carvi*	*C. sativum*	*F. vulgare*	*P. anisum*	*A. leucotrichus*	*D. carota*	*P. sativum*
Fluorescence intensity	Mean HAE	1.5	38.09	50.99	23.81	34.00	57.83	59.19	60.90
		3.5	26.83	47.56	23.34	30.44	56.92	51.79	49.11
		10	18.53	43.76	20.25	14.02	40.17	38.92	40.36
	Mean CAE	1.5	34.81	40.44	-16.13	35.21	55.98	55.58	58.67
		3.5	29.16	37.21	5.96	29.10	44.61	56.25	50.84
		10	22.84	36.87	70.11	25.82	40.77	55.30	45.81
	Mean HE	1.5	49.77	37.82	28.87	27.46	61.86	60.00	59.18
		3.5	48.84	31.08	28.89	25.24	51.86	42.16	53.11
		10	44.21	-2.04	58.37	9.27	41.71	21.99	38.27
	Contrôle BSA-MG-Met					84.01			
Migration distance	Mean HAE	1.5	6.06	-13.94	9.30	10.42	59.86	25.21	31.13
		3.5	-4.65	-14.08	35.92	-0.28	31.69	20.85	15.92
		10	-9.58	-14.65	35.63	-9.44	10.00	6.20	9.86
	Mean CAE	1.5	31.13	-13.10	15.35	25.49	24.93	14.65	9.44
		3.5	25.21	-14.23	5.35	23.66	8.87	-4.37	5.77
		10	9.86	-14.93	-10.42	12.68	-3.80	-10.00	-4.23
	Mean HE	1.5	24.93	25.07	10.85	21.27	39.72	39.15	44.93
		3.5	15.07	17.18	10.42	9.58	30.85	35.21	0.28
		10	-0.14	5.77	6.06	5.49	9.72	9.72	-3.24
	Contrôle BSA-MG-Met					67.94			

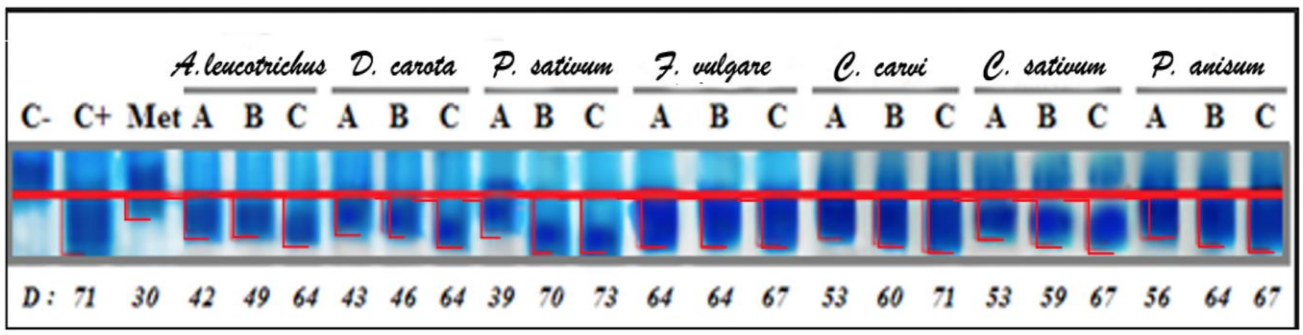
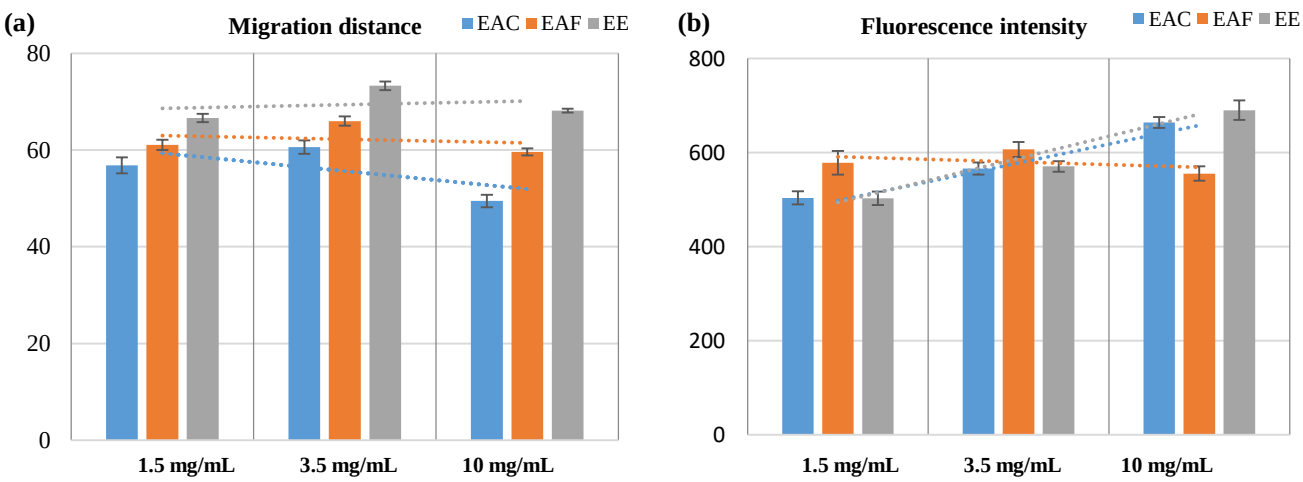


Fig. 4. Effect of HE on the migration of glycated BSA in Apiaceae Family.
C -: Not glycated BSA; C +: MG + BSA; Met: MG + BSA + Metformin; D: Migration distance compared to the not glycated BSA band in mm; A: 1.5 mg/ml; B: 3.5 mg/ml; C: 10 mg/ml.



a: HAE (y = -3.675x + 62.98 ; R^2 = 0.4216); CAE (y = -0.735x + 63.68 ; R^2 = 0.0487); HE (y = 0.755x + 67.823; R^2 = 0.0468)
b: HAE (y = 80.13x + 417.76 ; R^2 = 0.9835); CAE (y = -11.435x + 603.05 ; R^2 = 0.1979); HE (y = 93.65x + 400.65; R^2 = 0.975)

Fig. 5. Representation of anti-glycation effect of the HAE, CAE and HE mean concentrations in Apiaceae family. HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; HE: Hydro-alcoholic extract.

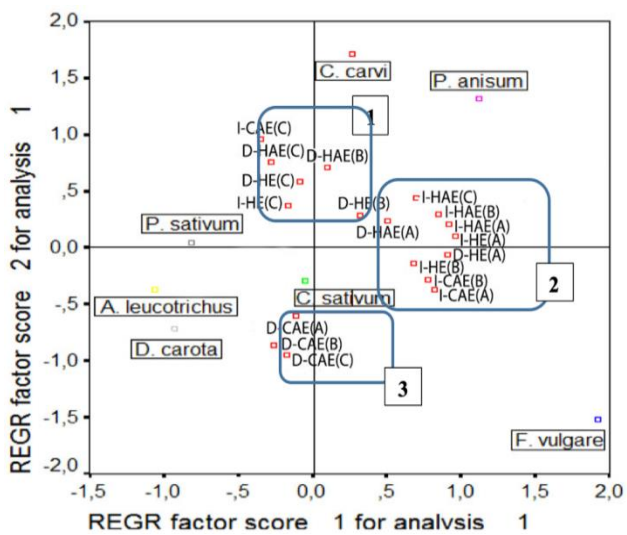


Fig. 6. Anti-glycation power of extracts from Apiaceae family. Variable projection according to the two components (38, 61% and 23, 18% of variance in component 1 and 2 respectively). I: fluorescence intensity; D: migration distance in PAGE-Native gel; A: 1.5 mg/ml; B: 3.5 mg/ml; C: 10 mg/ml; HAE: Hot Aqueous Extract; CAE: Cold Aqueous Extract; HE: Hydro-alcoholic extract.

Antiglycation activity: The study of anti-glycation activity revealed some cases of AGEs formation inhibition by using the lowest extract concentration (1.5 mg/mL). This can be explained by a synergistic relationship between antioxidant and anti-glycation activity of PAMs extracts (Ramkissoon *et al.*, 2013).

In general, in high doses, the three types of extracts were losing their effectiveness, which results in a dose-dependent increase in the fluorescent AGEs formation ($0.983 \leq R^2 \leq 0.975$) (Table 5). In PAGE-Native gel migration profiles, this was elucidated by an increase in the apparent molecular weight of the band (Fig. 4).

The lowest level of glycation was observed in HAE, with a non-significant difference from HE in native PAGE test. At 10 mg/mL of extract, this effect was significantly reduced using CAE (Fig. 5).

At low concentrations, HAE and HE acted simultaneously by formation of fluorescent AGEs and Amadori products. While at 10 mg/mL, they mainly leaded to fluorescent AGEs formation. Regarding CAE, the absence of significant correlation indicates that this extract increases *In vitro* glycation, especially by AGEs formation (Fig. 5).

Factorial projection of Apiaceae species showed that, at low doses of extract (1.5 and 3.5 mg/mL), only *P. sativum*, *A. leucotrichus* and *D. carota* acted as anti-glycants by inhibiting fluorescent AGEs formation. While the other species studied had a dose-dependent glycation power. *Pimpinella anisum* and *F. vulgare* increased both the formation of Amadori products and fluorescent AGEs. However, the methanolic extracts of some *F. vulgare* ecotypes have shown high anti-glycation activity (Gutiérrez *et al*., 2010). In contrast, *C. sativum* effect was variable depending on the extract type and the concentration used, which was in agreement with previous studies that revealed anti-glycation activity in the range of 50% at 0.42 mg/mL and 38.8% at 10 mg/mL in its methanolic and ethanolic extracts respectively (Gutiérrez *et al.*, 2010; Lacinová *et al*., 2010; Yoshika *et al*., 2015) (Fig. 6).

Lacinová *et al.*, (2010) indicates that migration of different extracts towards the PAGE anode has been increased with conditions that accelerate glycation such as increasing the concentration of sugar and / or protein, incubation period and incubation temperature. This may partly explain the effect of Apiaceae species with high concentration of reducing sugars compared to the other families studied previously (Ramdan *et al.*, 2017). On the other hand, they may contain a significant amount of vegetable protein which can in turn undergo glycation. In addition, there are molecules with reducing functions involved in the reactions of glycation and participated largely in their increase, such as aldehydes from caramelization of sugars. Indeed, peroxidized lipids form aldehydes can react with amino acids to give Schiff bases (Rowan *et al*., 2018). It should be noted that this Apiaceae family has low levels of polyphenols, flavonoids and tannins.

Another study has shown that modification of protein charge during glycation is particularly important when the proteins are glycated with a carboxyl group containing sugars such as glucuronic acid, or still with phosphorylated sugars (Yaylayan & Huyghues-Despointes, 1994; Rowan, *et al*., 2018). These derived sugars add negative charges to proteins, hence the increase in net density of the negative charge, with a resulting increase in their electrophoretic mobility. In addition, amine groups of Amadori products are more basic and can be easily protonated than the primary amine groups of non-glycated proteins. Therefore, the increase in net negative charge may also result from p-elimination and enolization of the sugar moiety of Amadori products under the alkaline conditions of PAGE to form an enolate anion (Rowan, *et al*., 2018).

Conclusion

The present study has highlighted the glycation effect of some species belonging to Apiaceae family. These species had an anti-glycation effect by using low extract concentrations. In fact, they lost their effectiveness with increasing extract concentration and became activators of the glycation reaction especially in its early stages. The extracts obtained had low levels of antioxidants such as polyphenols, flavonoids and tannins. On the other hand, they contain a considerable amount of reducing sugars, which may explain their average antioxidant capacity and their glycation process activation at high extract concentrations. As a result, plant extracts from Apiaceae family should be used with caution in diabetic patients. Considering the antiglycation effect observed at 1.5 mg/mL of extract, it would be interesting to conduct a thorough study on the pharmacokinetics and pharmacodynamics of these active ingredients to determine the preventive and therapeutic doses. Results of this study are therefore a crucial step in the search for biologically active substances, the choice of the extract type and the concentration to use.

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