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ORIGINAL ARTICLE

Phytochemical and Bioactivities of Male Flower Buds of Fruit Trees from the Southern Tunisia: Polyphenols UPLC-MS Profiles and Antioxidant Enzymatic Potential in Human Plasma of Parkinson's Disease Patients

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Abstract
Strong evidence supports that oxidative stress represents one of the most important pathogenic molecular events in the development of Parkinson's disease (PD). Phenolic compounds have been widely known by their antioxidant properties. In this context, phenolic extracts from three different southwest Tunisian male flower buds of pistachio "*Pistacia vera* L.", fig "*Ficus carica* L." and date palm "*Phoenix dactylifera* L.", were extracted using ethanol in 13.20%, 4.88% and 8.31% yields, respectively. The total phenolic and flavonoids content analysis were evaluated. Besides, the phenolic profiling was performed using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS). Additionally, in vitro superoxide dismutase (SOD), glutathione reductase (GR) enzymatic activity and 4-hydroxynonenal (4-HNE) formation in human blood plasma of PD patients of the three phenolic extracts were assayed for the first time (0.5–50 µg/mL—the incubation time 25 min). The results showed that the three phenolic extracts tested presented a significant antioxidant enzymatic property expressed by the increase of plasma SOD activity of Parkinsonian patients at different stages of the disease. On the other hand, the phenolic extracts from pistachio and fig showed important increases of plasma GR activity of PD patients at stage 2. Pearson correlation coefficients were established between the total phenolic and flavonoids content, the common phenolic compounds detected in the tested extracts and their effects on SOD, GR activities as well as on the 4-HNE formation.

Keywords *Pistacia vera* · *Ficus carica* · *Phoenix dactylifera* · Phenolic extracts · UPLC-MS · Antioxidant enzymatic properties · SOD · GR,4-HNE · Parkinson's disease

Abbreviations

TPC	Total phenolic content
TFC	Total flavonoid content
SOD	Superoxide dismutase
4-HNE	4-Hydroxynonenal
GR	Glutathione reductase
UPLC-MS	Ultra-performance liquid chromatography coupled to mass spectrometry
AD	Alzheimer's disease
PD	Parkinson's disease
PD1	First stage of Parkinson's disease according to Hoehn and Yahr scale
PD2	Second stage of Parkinson's disease according to Hoehn and Yahr scale
PD3	Third stage of Parkinson's disease according to Hoehn and Yahr scale
PD4	Fourth stage of Parkinson's disease according to Hoehn and Yahr scale
C	Control (healthy individual without signs of neurological disorders)

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1 Introduction

Parkinson's Disease (PD), is the second most common neurodegenerative disease after Alzheimer's disease (AD) [1]. Oxidative stress is a hallmark of neurodegenerative disorders

and plays the most important role in the development of PD [2–6].

Oxidative stress favors α-synuclein aggregation and reduces dopamine production in substantia nigra dopaminergic neurons. In addition, α-synuclein has been proposed to regulate negatively the function of dopamine transporter in dopaminergic nerve terminals by controlling its concentration in the plasma membrane [7]. The pathological hallmarks of PD, as mentioned above, are cytoplasmic inclusions consisted mainly of α-synuclein aggregates known as Lewy bodies. α-Synuclein phosphorylation and fibrillization processes lead to the Lewy bodies formation and induce neuronal death (Fig. 1).

Although there is not currently a curative treatment for PD, many novel therapies are focused on neuroprotection, comprising ROS scavengers, non-vitamin natural antioxidants as phenolic compounds, non-steroidal anti-inflammatory drugs, transition metal (e.g., iron and copper) chelators, antiapoptotic drugs such as calcium channel and caspase inhibitors and bioenergetics drugs [8].

Antioxidant defense mechanisms remove the free radicals to prevent oxidative damage of biomolecules [9]. The antioxidant system comprises of different types of functional components such as enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase), also including the formation of 4-HNE by enzymatic catalysis which are all biomarkers of oxidative stress [9]. Also, this system comprises the non-enzymatic antioxidants such as reduced glutathione, vitamin E, vitamin C, uric acid, and various exogenous antioxidants including polyphenols [9].

The side effects of synthetic antioxidants have been discussed in different studies [10, 11]. Consequently, there is an increasing interest toward the use of natural substances as alternatives to the synthetic ones [10, 11]. Polyphenolic compounds, are natural antioxidants which have been recently proposed to be useful prophylactics for the treatment of excitotoxic and oxidative neuronal death [12–15]. Moreover, they possess a wide spectrum of biological properties such as anti-inflammatory, antioxidant, neuroprotective and pro-autophagic activities [12, 16–18]. As polyphenols are multi-targeting drugs, they could be potent therapeutic agents in combating PD [19]. Therefore, natural polyphenols are gaining considerable interest in the treatment of this disease [20].

This study aimed to characterize the phenolic extracts from three different male flower buds from "*P. vera* L.", "*F. carica* L." and "*P. dactylifera* L." growing in the

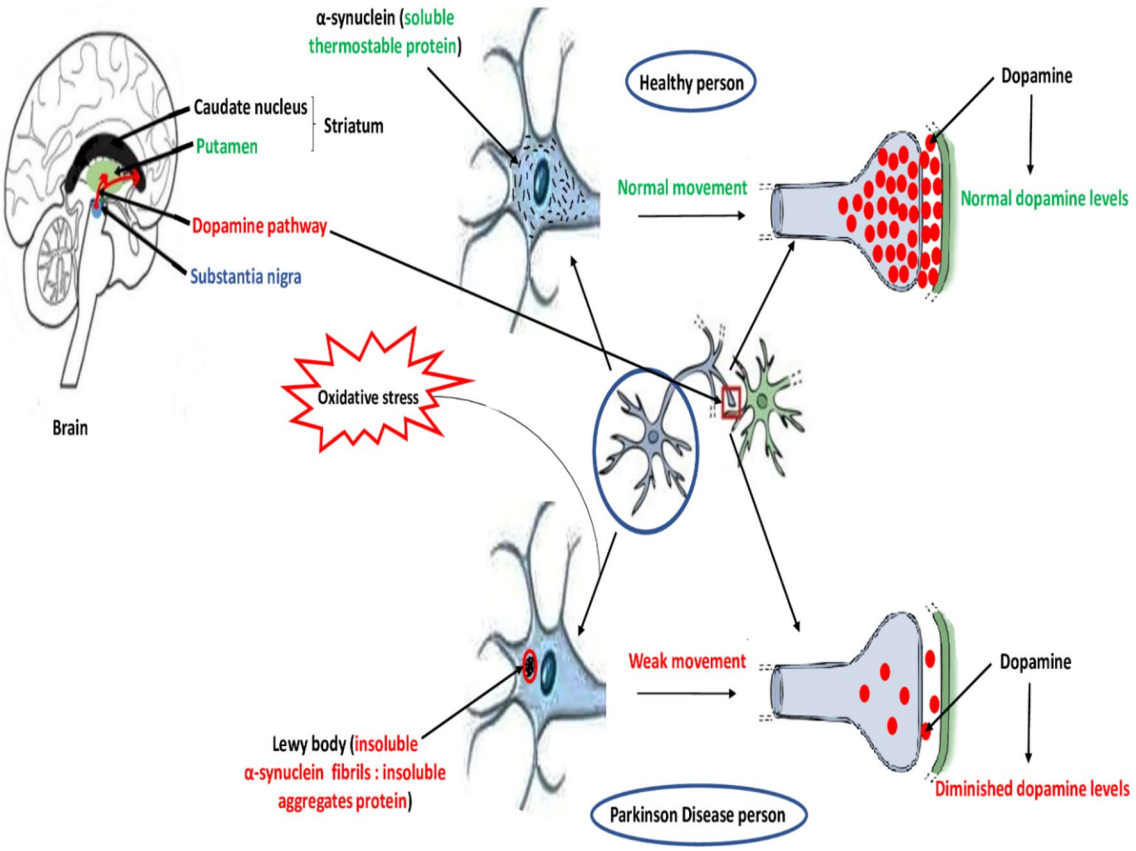


Fig. 1 Oxidative stress and PD

southwest of Tunisia. Afterwards, to examine their effects on three essential biomarkers of oxidative stress superoxide, dismutase (SOD), 4-hydroxynonenal (4-HNE) production and Glutathione reductase (GR) in human blood plasma from PD patients at the four stages of the disease. Then, to establish Pearson correlation between the total phenolic and flavonoids content, the common phenolic compounds of the examined extracts and their effects on the three aforementioned biomarkers.

2 Material and Methods

2.1 Chemical Reagent

Chloroform, ethanol, folin–Ciocalteu reagent (sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate), gallic acid (3,4,5-trihydroxybenzoic acid), sodium nitrite, aluminum chloride, sodium hydroxide, quercetin and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvents for ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) analyses were UPLC grade. All other reagents represented analytical grade and were provided by commercial suppliers.

2.2 Plant Material and Preparation of the Phenolic Extracts

Male flower buds of "*P. vera* L.", "*F. carica* L." and "*P. dactylifera* L." were collected in April 2019 from the region of EL Guettar-Gafsa (S 32° 09′ 22″/W 52° 06′ 01″) in the southwest of Tunisia. The botanical identification was accomplished by Dr. Asma EL Ayeb-Zakhama, in the Laboratory of Genetic, Biodiversity and Valorization of Bioresources (LR11ES41), Higher Institute of Biotechnology of Monastir, Tunisia. Voucher specimens (No. P.v1; F.c2; P.d3) were deposited in the Laboratory of Interfaces and Advanced Materials (LR11ES55), Faculty of Sciences of Monastir, Tunisia for future reference.

Male flower buds were dried at 45 °C in a forced convection laboratory oven until the stabilization of its weight then reduced to powder using a mill. Powdered male flower buds were defatted by chloroform in Soxhlet apparatus. After that, the defatted residues were soaked for 24 h, at room temperature, in ethanol. The solid residues were filtered and re-extracted under the same conditions. The resultant filtrates were mixed and concentrated with *in vacuo*. The phenolic extracts were weighed and kept at + 4 °C until further studies.

2.3 Total Phenolic and Flavonoids Content Analysis

The analysis of total phenolic content (TPC) in the three phenolic fractions was performed based on the Folin–Ciocalteu method according to Nur-Izzati et al. [21]. TPC was calculated using a calibration curve from gallic acid standard solution (fig. S1.a) and expressed as mean of gallic acid equivalents (mg GAE/g dried extract). Total flavonoid content (TFC) was determined using aluminum chloride ($AlCl_3$) reagent according to Elshamy et al. [22]. TFC was calculated from quercetin calibration curve (fig. S1.b) and expressed as mean of quercetin equivalents (mg QE/g dried extract). All the experiments were performed in triplicate.

2.4 UPLC-MS Analysis of Phenolic Compounds

The Phenolic composition of the extracts was estimated as described by Kenny et al. [23]. Firstly, Ultra-Performance Liquid Chromatography (Waters Acquity) was performed using Acquity system (Waters Corporation, Milford, MA) coupled to a Waters XEVO G2 Q-TOF mass spectrometer (UPLC-MS) was carried out on the prepared ethanolic extracts. The extracts were dissolved in methanol (20 mg/mL), centrifuged at 15,000 rpm, and filtered with syringe by 0.22 µm PVDF filters. The chromatographic separations were carried out at 40 °C using a Waters Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 µm) column accompanied by a Waters C18 VanGuard (5 mm × 2.1 mm, 1.8 µm). The starting condition for each run was 98:2 mobile phase A (water + 0.5% formic acid): mobile phase B (ACN + 0.5% formic acid) held for 1 min, with a ramp to 90:10 (A:B) by 2.5 min, then to 80:20 by 3 min and held for 3 min. Further gradient increases to 65:35 by 7.5 min with a final change to 10:90 by 8.5 min and held for 1 min and finally reconditioned to initial starting conditions. The mass spectrometry was used in negative ion mode (ESI^-) and Nitrogen gas was used as both auxiliary and sheath gas. Identification of phenolic compounds was carried out by library search and the proposed elemental composition by the MassLynx V4.1 program and in the cases of not identified compounds by comparing their mass spectra (*m/z*) and their relative retention times with available literature. Quantification of individual polyphenolic compounds was directly performed also using MassLynx V4.1 that calculate the pics area and give the area percentage of each pic.

2.5 Subjects Under Study

PD patients at stages 1 to 4 according to Hoehn and Yahr scale were recruited from the Unit of Movement Disorders of the University Hospital "San Cecilio" of Granada, Spain. A healthy subject without sings of disease was assigned as control. The main demographic data are shown in Table 1.

Table 1 Demographic data of subjects under study

Subjects	Gender	Age (years)[a]
PD1	Man	61
PD2	Man	78
PD3	Man	66
PD4	Man	55
Control	Woman	69

PD1 patient at first stage of PD, *PD2* patient at second stage of PD, *PD3* patient at third stage of PD, *PD4* patient at fourth stage of PD

[a]Age at sample collection

2.6 Plasma Isolation and Incubation with the Extracts

Blood samples were drawn from the cubital vein into EDTA tubes and refrigerated at + 4 °C until be processed. Plasma was isolated by centrifugation for 15 min at 1400×*g* and + 4 °C, and then stored at − 80 °C until analysis.

Previous to measure oxidative stress markers, plasma from subjects under study was incubated with the three phenolic extracts (pistachio, fig and date palm) at concentrations of 0.5–50 µg/mL (µg of dried extract per mL of plasma) for 25 min, at 37 °C [24]. Stock solutions of the dried phenolic extracts were made in 50% DMSO. The final concentration of DMSO in samples was less than 0.05% and its effects were determined in all experiments [24, 25].

2.7 Enzyme Inhibitory Assays in Blood Plasma from Parkinson's Disease Patients

2.7.1 Superoxide Dismutase (SOD)

SOD belongs to a group of metalloenzymes that constitute a very important antioxidant defense against oxidative stress in the body. Several studies have revealed the therapeutic potential and physiological importance of SOD [26, 27]. Plasma SOD activity was measured by a colorimetric assay according to the manufacturer's instructions (EIASODC, Thermo Fisher Scientific) [28]. The results were expressed in U/mL, and the experiments were carried out in triplicate.

2.7.2 4-Hydroxynonenal (4-HNE)

4-HNE is regarded as a specific biomarker of lipid peroxidation. It has been found to be elevated in brain tissues and body fluids in several neurodegenerative disorders, included PD [29–31]. Plasma 4-HNE levels were determined by an ELISA assay from a commercial source (E-EL-0128, Elabscience) following the manufacturer's instructions [32]. The results were expressed in U/mL and the experiments are conducted in triplicate.

2.7.3 Glutathione Reductase

Glutathione reductase (GR) is one of the enzymes thatserve to maintain glutathione in the reduced form. GR deficiency is considered as a good biomarker of oxidative stress. Plasma Glutathione reductase activity was measured by a colorimetric assay according to the manufacturer's instructions (STA-812, OxiSelect™) [33]. The results were expressed in mU/mL and the experiments are performed in triplicate.

2.8 Statistical Analysis

Statistical analyses were carried out by two-way ANOVA for repeated measurements. The level of significance was set at 5%. GraphPad Prim 5.0 software was used for calculations.

Pearson correlation coefficient was employed using Matlab R2019a (MathWorks, Massachusetts, USA) to establish the correlation coefficients among TPC, TFC, the common phenolic compounds detected in the samples and their 4-hydroxynonenal, glutathione reductase and superoxide dismutase activities.

3 Results and Discussion

3.1 Total Phenolic and Flavonoids Content

The phenolic extracts yield based on dried flower buds weight, the TPC and TFC are given in the Table 2. As shown in this Table, the extraction yields from *P. vera F. carica* and *P. dactylifera* are 13.20%, 4.88% and 8.31%, respectively. The qualitative composition of the extracts evaluated via the determination of TPC and TFC in the three ethanolic extracts divulgated the presence of significant amounts of TPC (512.96, 76.98 and 227.36 mg GAE/g) and TFC (68.04, 20.13 and 29.24 mg QE/g) for *P. vera*, *F. carica* and *P. dactylifera*, respectively. For *P. vera*, the results showed that TPC and TFC values were in good agreement with those found by Boumaiza et al. (2016): 513.77 (mg GAE/g) and 67.94 (mg catechin equivalents (CE)/g), respectively [34]. For *F. carica*, the results showed that TPC value was higher than the that revealed in literature (6.18 and 6.86 (mg GAE/g)) for the hydroethanolic extract from leaves respectively from the cultivars 'Tounsi' and 'Temri', and for the hydroethanolic fruits extract from the same cultivars are respectively 2.00 and 1.24 (mg GAE/g) [35]. For the black Tunisian fig cultivar 'Kohli', the peel juice showed a TPC and TFC values comparable to our value, they are varied between 50.57 GAE/g and 74.16 mg GAE/g for TPC and

Table 2 Phenolic composition (%), total phenolic and flavonoids content of the three male flower buds extracts

Peak	Rt (min)	$[M-H]^-$ (*m/z*)	Formula	Identification	Identified compound (%)		
					Pis[a]	Fig[b]	DP[c]
1	0.49	341.1077	$C_{12}H_{21}O_{11}$	Caffeoyl-*O*-hexoside	ND	ND	7.35
2	0.59	191.0538	$C_7H_{11}O_6$	Quinic acid	ND	9.44	ND
3	0.60	331.0654	$C_{13}H_{15}O_{10}$	1-*O*-galloyl β-D-glucopyranose (β-glucogallin)	8.66	ND	ND
4	7.35	197.0424	$C_9H_9O_5$	Syringic acid	4.92	ND	ND
5	8.25	625.1405	$C_{27}H_{29}O_{17}$	Dihexosyl quercetin	ND	ND	4.23
6	8.74	479.0823	$C_{21}H_{19}O_{13}$	Myricetin 3-*O*-galactoside	0.54	ND	ND
7	8.91	639.1581	$C_{28}H_{31}O_{17}$	Isorhamnetin dihexoside	ND	ND	5.72
8	9.19	609.1465	$C_{27}H_{29}O_{16}$	Quercetin-3-*O*-rutinoside	3.02	2.34	ND
9	9.50	615.0995	$C_{28}H_{23}O_{16}$	Quercetin-(galloyl)glucoside	1.06	ND	ND
10	9.83	463.0870	$C_{21}H_{19}O_{12}$	Quercetin 3-*O*-galactoside	1.91	ND	ND
11	10.07	463.0866	$C_{21}H_{19}O_{12}$	Quercetin-3-*O*-glucoside	2.89	ND	ND
12	10.20	623.1610	$C_{28}H_{31}O_{16}$	Isorhamnetin-3-*O*-rutinoside	ND	ND	7.30
13	10.73	939.1113	$C_{41}H_{31}O_{26}$	Pentagalloyl hexoside	3.46	ND	ND
14	11.39	197.0433	$C_9H_9O_5$	Ethyl gallate	2.50	ND	ND
15	11.42	477.1020	$C_{22}H_{21}O_{12}$	Rhamnetin 3-glucoside	ND	ND	0.46
16	11.88	607.1638	$C_{28}H_{31}O_{15}$	Peonidin 3-rutinose	ND	ND	0.26
17	12.26	349.0546	$C_{16}H_{13}O_9$	Ethyl digallate	2.58	ND	ND
18	12.87	737.5160	$C_{33}H_{38}O_{19}$	Apigenin-6,8-di-C-hexose-*O*-hydroxymethyl glutaric acid	ND	ND	2.03
19	12.91	737.5168	$C_{36}H_{34}O_{17}$	Procyanidin A-type hexoside	ND	1.37	ND
20	13.11	629.2448	$C_{28}H_{21}O_{17}$	Trigalloylshikimic acid	3.08	ND	ND
21	13.54	301.0334	$C_{15}H_9O_7$	Quercetin	ND	ND	0.27
22	13.81	615.2495	$C_{28}H_{23}O_{16}$	Myricitrin *O*-gallate	0.54	ND	ND
23	13.98	644.2615	–	Not identified	0.53	ND	ND
24	14.35	598.2578	–	Not identified	0.39	ND	ND
25	15.86	909.4504	–	Not identified	ND	ND	0.60
26	16.03	893.4662	$C_{47}H_{41}O_{18}$	Dimethyl-*O*-procyanidin B trimer	ND	ND	0.48
27	16.59	747.3971	–	3-*O*-feruloyl-7-*O*-acyl-feruloyl-4-*O*-caffeoyl-quinic acid	ND	ND	0.58
28	16.76	463.2311	$C_{21}H_{19}O_{12}$	Quercetin-7-*O*-hexoside	1.23	3.76	ND
29	16.80	761.4103	–	Galloyl(epi)gallocatechin dimer	ND	ND	0.39
30	17.60	419.2789	$C_{25}H_{39}O_5$	p-coumaroyl-*O*-16-hydroxypalmatic acid	ND	ND	6.22
31	17.82	476.2766	–	Not identified	ND	ND	2.97
32	17.99	461.2659	$C_{21}H_{17}O_{12}$	Kaempferol-*O*-glucuronide	1.60	ND	3.68
33	18.36	461.2646	$C_{22}H_{21}O_{11}$	Chrysoeriol-O-glucuronide	22.47	26.35	21.57
34	18.77	309.1724	$C_{14}H_{13}O_8$	Feruloyl-malic acid I	6.85	18.32	4.91
35	19.04	369.2417	$C_{15}H_{13}O_9S$	Sulfated epicatechin	0.87	4.16	1.29
36	19.12	319.2262	$C_{14}H_7O_9$	Luteic acid	1.00	ND	ND
37	19.38	309.1720	$C_{14}H_{13}O_8$	Feruloyl-malic acid II	1.25	1.22	2.40
38	19.72	353.1981	$C_{16}H_{18}O_9$	Caffeoylquinic acid isomer	1.53	2.59	0.47
39	20.22	775.5342	–	Acacetin derivative	ND	ND	1.65
40	20.39	793.5422	–	Hydroxycinnamic derivative	1.44	1.87	ND
41	20.73	265.1464	–	Not identified	ND	1.01	ND
42	21.24	819.6302	–	Coumaroylquinic acid derivative	23.27	26.66	21.99
43	22.03	459.2507	–	Pterostilbene *O*-acetylglucoside	1.76	ND	2.49
44	22.46	217.0004	$C_7H_5O_8$	Cyclopropane-1,1,2,2-tetracarboxylic acid	0.63	0.89	0.67
				Yield (%)	13.20	4.88	8.31
				TPC (mg GAE/g)	512.96	76.98	227.36
				TFC (mg QE/g)	68.04	20.13	29.24

ND not detected, *TPC* total phenolic content, *mg GAE/g* milligrams of gallic acid equivalents per gram of the sample, *TFC* total flavonoid content, *mg QE/g* milligrams of quercetin equivalents per gram of the sample

[a] *P. vera*

[b] *F. carica*

[c] *P. dactylifera*

12.75 mg CE/g for TFC [36]. For *P. dactylifera*, our recorded TPC and TFC values were found to be comparable to data reported by Daoud et al. (2019) for the cultivar of "Tozeur, southern Tunisia" for the ethanolic male floral buds extract with values of 211.11 (mg GAE/g) and 22.25 (mg QE/g), while for the same extract from the cultivar of "Kerkennah, an island to the southeast of Tunisia" were 13.42 (mg GAE/g) and 4.29 (mg QE/g) [37]. These differences could be attributed to the genetic factors, geographical variations, and climatic changes [37]. Overall, our findings indicated that male flower buds of *P. vera*, *F. carica* and *P. dactylifera* are rich in phenolic compounds, including flavaonoids, which could offer good antioxidant property, and can be used as promising sources of beneficial bioactive compounds for human health and nutrition.

3.2 Identification and Quantification of Phenolic Compounds

In the present study, ethanolic phenol rich extracts from male flower buds of *P. vera*, *F. carica* and *P. dactylifera* were analyzed for their phenolic profile (Fig. 2). The UPLC-MS analysis results are illustrated in the Table 2.

For *P. vera*, the analysis revealed the existence of 24 phenolic compounds (Fig. 3a) grouped into 11 flavonoids, 5 phenolic acids, 2 phenolic acid derivatives and 6 tannins. Flavonoids were the main polyphenolic compounds detected in this species with 37.89% of the total detected polyphenols, followed by phenolic acid derivatives (24.71%), tannins (21.28%) and phenolic acids (15.18%). Coumaroylquinic acid derivative was found to be the most abundant phenolic acid representing 24.71% of the total polyphenols. It has been reported that coumaroylquinic acid derivatives were identified in nuts [38, 39] and in pin pollen [40]. Chrysoeriol-*O*-glucuronide was the main flavonoid detected in *P. vera* with 22.47% and 1-*O*-galloyl β-D-glucopyranose was the main tannin with 8.66% of the total identified polyphenols. 1-*O*-galloyl β-D-glucopyranose also named β-glucogallin is an hydrolysable tannin, was identified in pistachio hulls [41]. Besides, Feruloyl-malic acid I was identified as the most abundant phenolic acid in *P. vera* (6.85%). In a previous study on male flower buds of pistachio from another Tunisian cultivar "Mateur" [34], the phenolic composition showed the presence of 15 compounds of which the phenolic acids constitute the major fraction (91.60%) and represented mainly by gallic acid (63.29%) and ellagic acid (20.63%). Rutin (flavonoid) was also highlighted (4.74%) among the phenolic compounds of this cultivar. The clear difference of these results compared to ours can be explained by the nature of the varieties and by non-identical climatic conditions [42].

To the best of our knowledge, this is the first study on the identification of phenolic compounds from male flower buds of *F. carica*. The UPLC-MS results showed the existence of 12 phenolic compounds (Fig. 3b) classified as 5 flavonoids (37.98%), 5 phenolic acids (32.46%) and 2 phenolic acid derivatives (28.53%). Coumaroylquinic acid derivative was found to be the most abundant phenolic acid with 26.66% from the total phenolic compounds identified in *F. carica*. Petruccelli et al. (2018) have reported the presence of coumaroylquinic acid in ten Italien fig leaves varieties [43], while Palmeira et al. (2019) have detected 5-*p*-coumaroylquinic acid in the peel of a Portuguese common fig variety [44]. Chrysoeriol-*O*-glucuronide was identified as the major flavonoid (26.35%) in *F. carica* whereas feruloyl-malic acid I was the most abundant phenolic acid (18.32%). A previous study has reported the presence of feruloyl-malic acids I and II in Tunisian *F. carira* leaves [35]. Quinic acid was also detected in *F. carica* with a relatively high amount (9.44%). Oliveira et al. have reported the presence of quinic acid only in the leaves of Pingo de mel and Branca Tradicional varieties [45].

The investigation of the phenolic profile of *P. dactylifera* revealed the presence of 22 phenolic compounds (Fig. 3c) represented by 13 flavonoids (54.65%), 4 phenolic acid derivatives (30.82%), phenolic acids (8.45%) and 1 stilbene (2.49%). Chrysoeriol-*O*-glucuronide was the preeminent flavonoid in *P. dactylifera* (21.57%), followed by caffeoyl-*O*-hexoside (7.35%) and isorhamnetin-3-O-rutinoside (7.30%). A previous study has reported the presence of chrysoeriol-*O*-glucuronide and caffeoyl-*O*-hexoside in Saoudien male flowers of *P. dactylifera* [46]. While Hammouda et al. (2015) have detected isorhamnetin-3-*O*-rutinoside in a Tunisian variety (*Ghars Mettig*) at Kimri and Tamar stages. Coumaroylquinic acid derivative was the main phenolic acid derivatives in *P. dactylifera* (21.99%) followed by p-coumaroyl-*O*-16-hydroxypalmatic acid (6.22%). These results are in accordance with those found by Ben Said et al. (2017) [46]. Feruloyl-malic acids I and II were the major phenolic acids identified in *P. dactylifera* representing 4.91 and 2.40%, respectively. Pterostilbene *O*-acetylglucoside was the unique stilbene recorded in male flower buds of date palm.

3.3 Enzyme Inhibitory Assays in Blood Plasma from Parkinson's Disease Patients

3.3.1 Superoxide Dismutase (SOD)

We evaluated the SOD activity in plasma from control and PD patients after incubation with the three extracts at different concentrations. The results are summarized in the Fig. 3. For *P. vera* (Fig. 4a), in absence of incubation, there were not significant differences in plasma SOD activity between control and PD patients. However, at concentrations of 1, 5, 10 and 50 µg/mL, plasma SOD activity was significantly

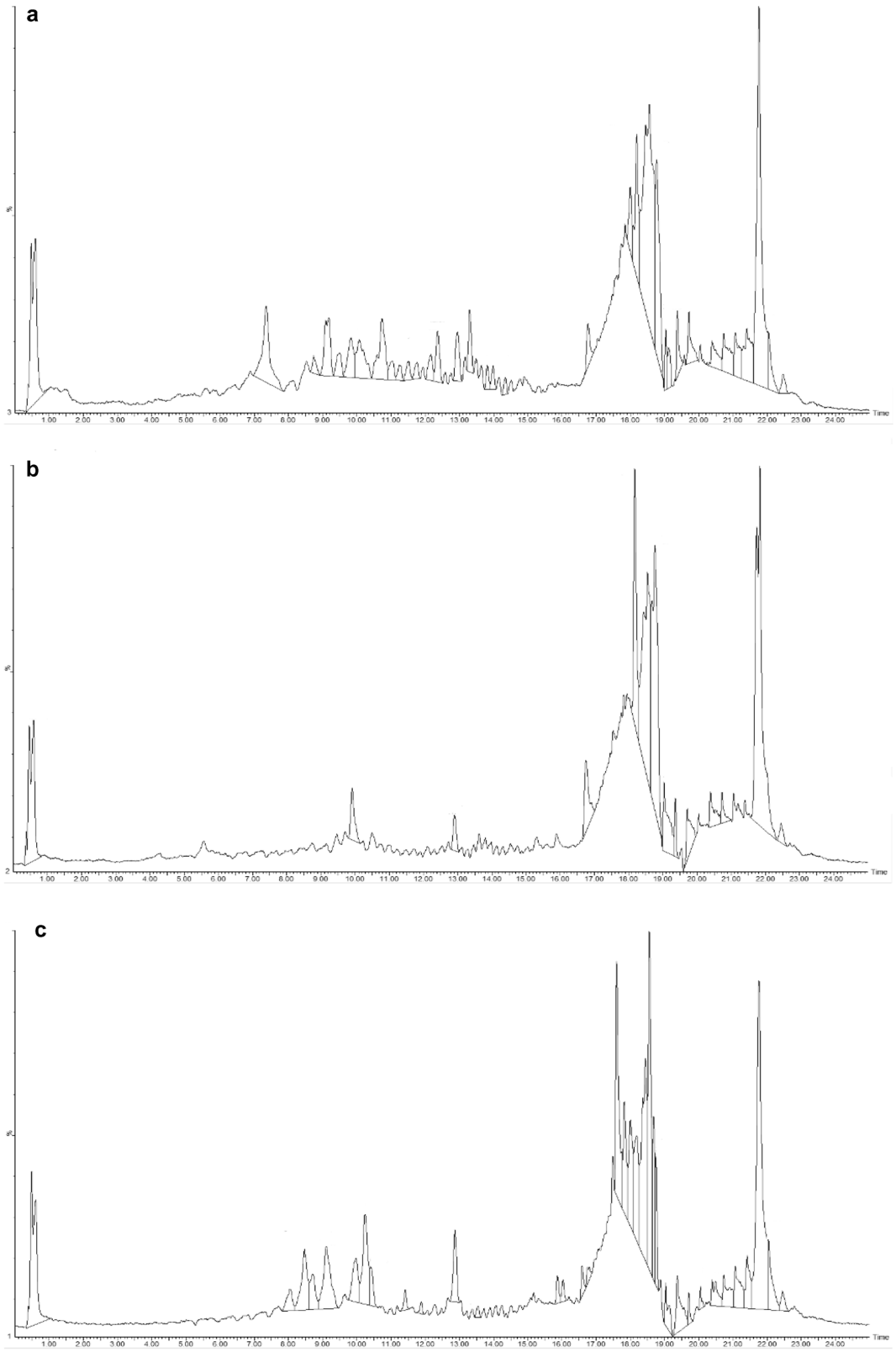


Fig. 2 UPLC-MS chromatograms of the three tested phenolic extracts: *P. vera* (**a**), *F. carica* (**b**) and *P. dactylifera* (**c**)

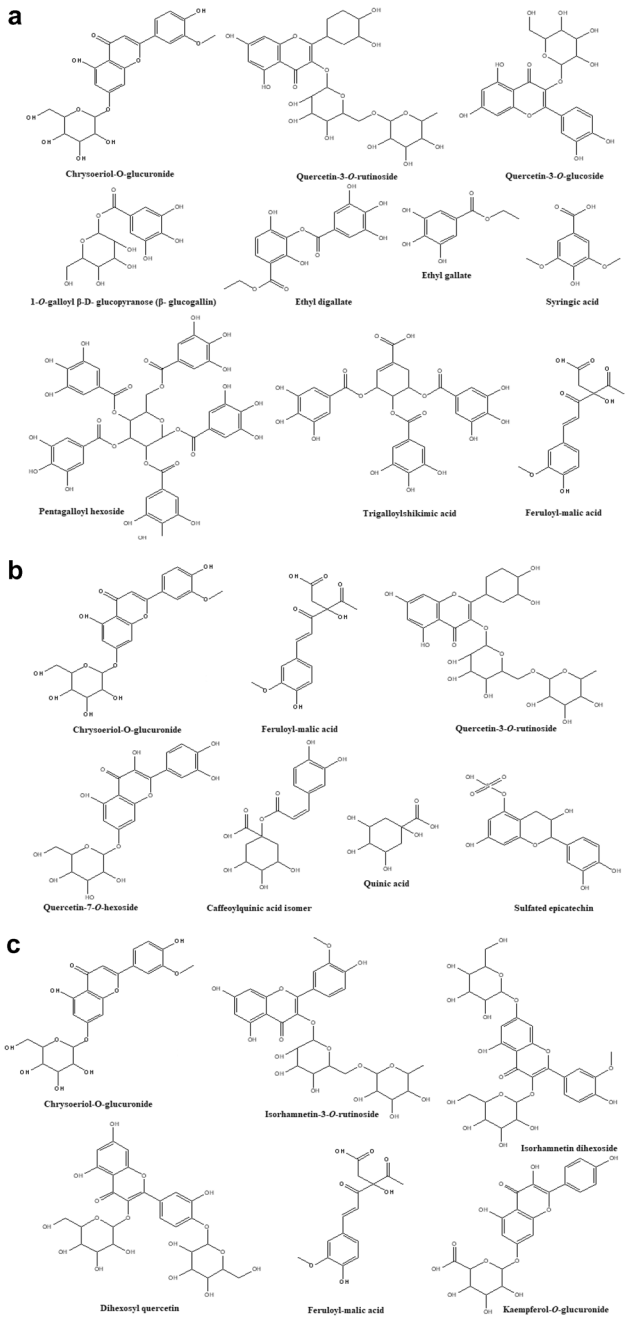


Fig. 3 Structure of major identified phenolic compounds: in *P. vera* (**a**), in *F. carica* (**b**) and in *P. dactylifera* (**c**)

higher in PD patients at stages 1 and 2 compared to control ($p < 0.05$). Furthermore, we found that plasma SOD activity significantly increased in control after incubation with *P. vera* at concentration of 0.5 µg/mL and in PD patients at stages 1, 2 and 4 after incubation with *P. vera* at the concentration of 50 µg/mL ($p < 0.001$, $p < 0.01$, $p < 0.05$, respectively).

In relation to *F. carica* extract (Fig. 4b) in absence of incubation, there were not significant differences in plasma SOD activity between control and PD patients. However, at concentrations of 5, 10 and 50 µg/mL, plasma SOD activity was significantly higher in PD patients at stage 2 compared to control ($p < 0.05$). Furthermore, examining the dose effect in every subject, we found a significant increase of plasma SOD activity in control after incubation with *F. carica* at concentration of 0.5 µg/mL compared to plasma without incubation ($p < 0.05$) and in PD patients at stage 2 after incubation with *F. carica* at concentrations of 5 and 10 µg/mL ($p < 0.05$).

The results with *P. dactylifera* extract (Fig. 4c) showed that plasma SOD activity was significantly higher in control after incubation with the *P. dactylifera* extract at both concentrations of 0.5 and 50 µg/mL compared to PD patients at stages 3 and 4 (all, $p < 0.05$). Moreover, these increases in plasma SOD activity were significant when compared to plasma from control in absence of incubation (all, $p < 0.05$).

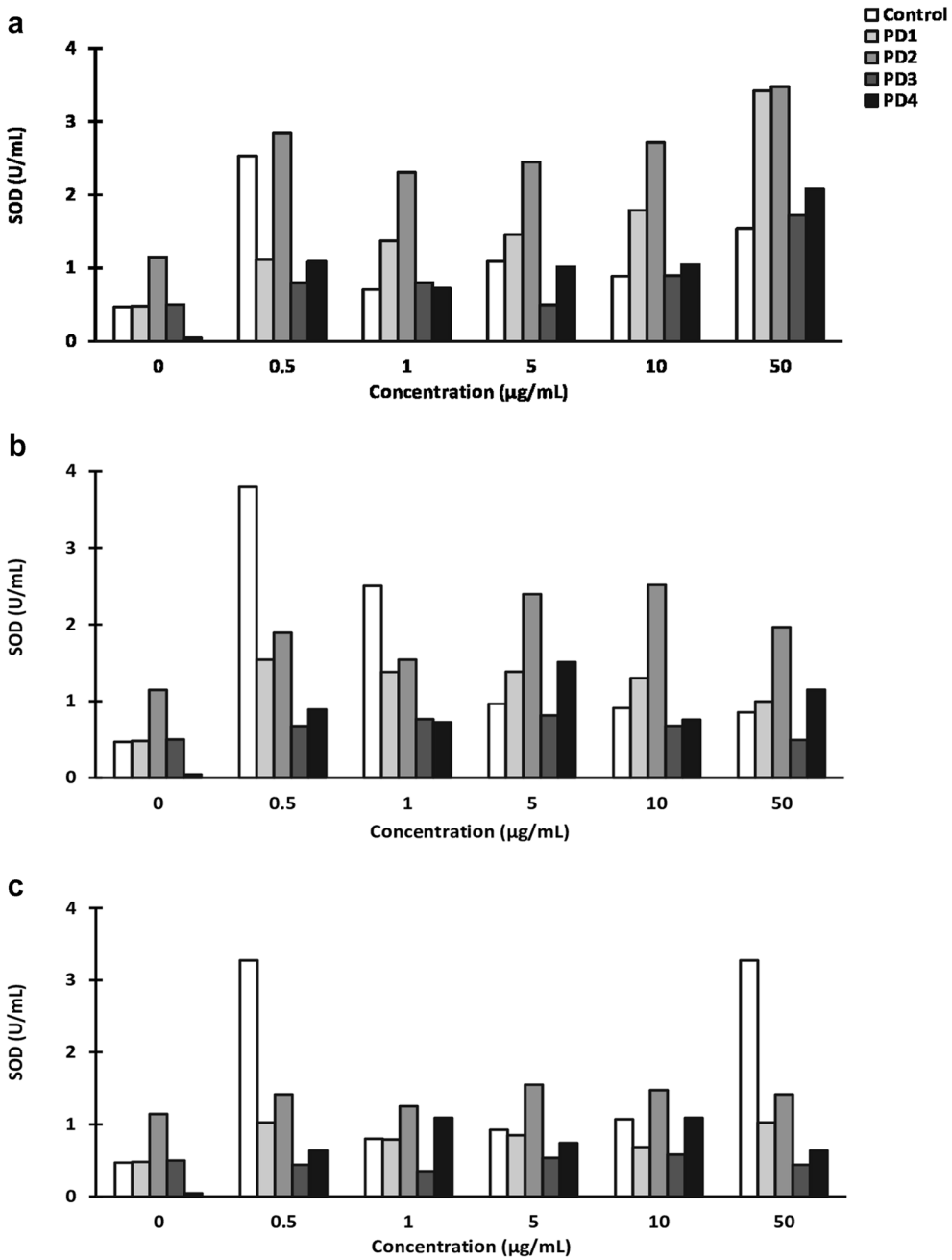
Overall, the three tested polyphenolic extracts increased the plasma SOD activity of the subjects under study, particularly in healthy control. *P. vera* extract was found to have the highest SOD-activating effect in PD patients at different stages of the disease, followed by *P. dactylifera*.

3.3.2 4-Hydroxynonenal (4-HNE)

The Fig. 4 shows the plasma 4-HNE levels in samples of control and PD patients incubated with the extracts at different concentrations. We only found a significant decrease for this marker in PD patient at stage 2 compared to control after incubation with *P. vera* (Fig. 5a) at concentration of 0.5 µg/mL. None dose effect was observed in all samples under study. When plasma samples were incubated with *F. carica* (Fig. 5b) and *P. dactylifera* (Fig. 5c) at different concentrations, we did not find any significant difference in the 4-HNE levels between control and PD patients. Moreover, this lipid peroxidation marker was not influenced by dose effect of both extracts in all analyzed samples. Our findings allowed concluding that none of the three phenolic extracts had a significant effect on reducing plasma 4-HNE levels.

3.3.3 Glutathione Reductase (GR)

The Fig. 5 shows the plasma GR activities of samples from control and PD patients incubated with the extracts. For *P. vera* (Fig. 6a), in absence of incubation, there were not significant differences in the plasma GR activity between control and PD patients. However, at concentrations of 10 and 50 µg/mL, plasma GR activity was higher in PD patients at stage 2 compared to control ($p < 0.05$). In relation to *F. carica* extract (Fig. 6b), we only found a significant increase of plasma GR activity in PD patient at stage 2 compared to control after incubation with the extract at the concentration of 5 µg/mL. When plasma samples were incubated with *P.*

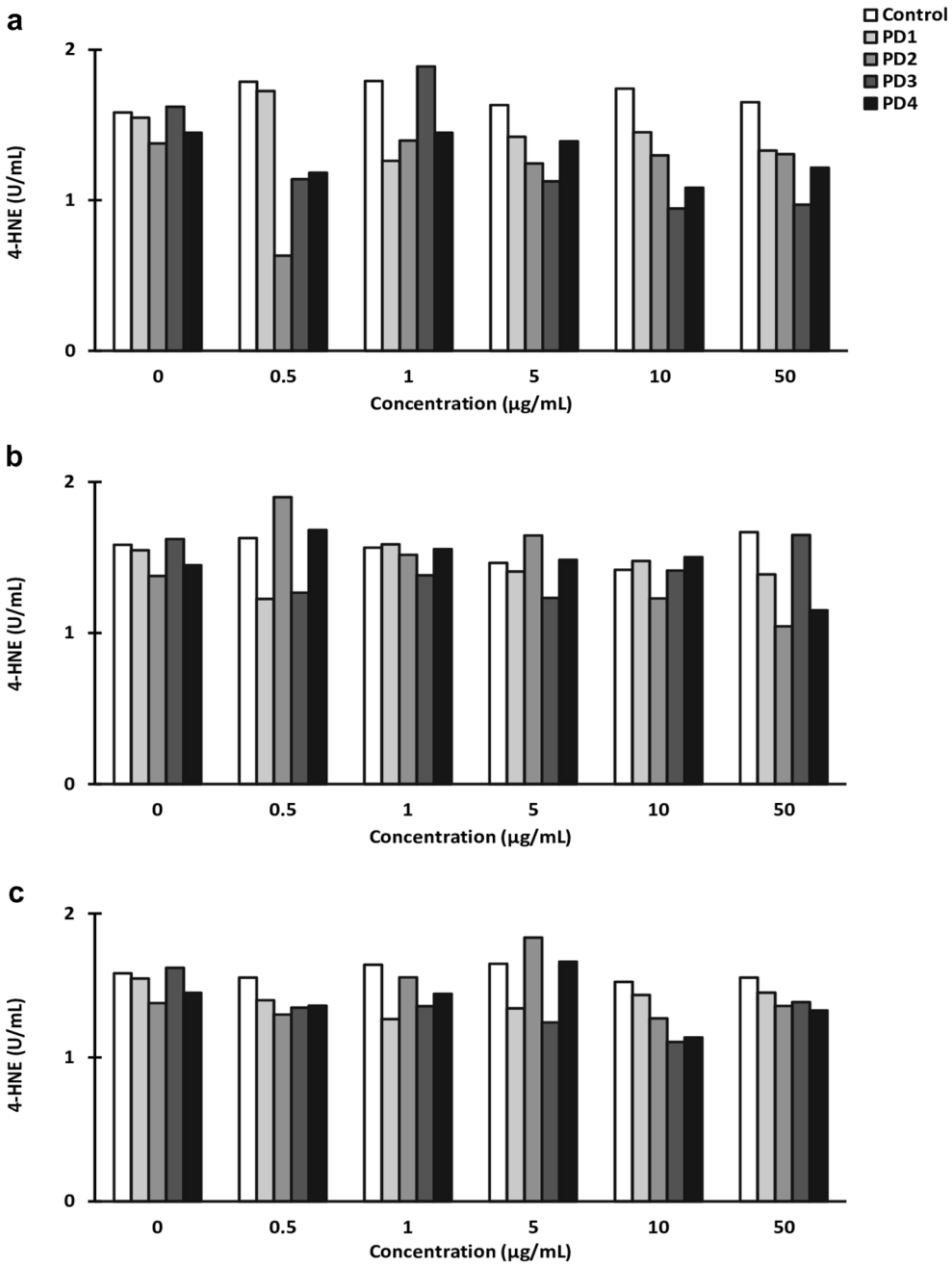


0 – 50 µg/mL: concentrations of phenolic extract analyzed. **PD1:** First stage of Parkinson's disease according to Hoehn and Yahr scale, **PD2:** Second stage of Parkinson's disease according to Hoehn and Yahr scale, **PD3:** Third stage of Parkinson's disease according to Hoehn and Yahr scale, **PD4:** Fourth stage of Parkinson's disease according to Hoehn and Yahr scale, **C:** Control (healthy individual without signs of neurological disorders).

Fig. 4 Effect of the three tested phenolic extracts at the different concentrations on plasma SOD activity (U/ml) of the subjects under study: *P. vera* (**a**), *F. carica* (**b**) and *P. dactylifera* (**c**)

dactylifera extract (Fig. 6c) at different concentrations, we did not find any significant difference in GR activity between control and PD patients. Likewise, plasma GR activity was not influenced by dose effect in all analyzed samples. *P. vera* and *F. carica* extracts seem to reinforce GR activity in PD patients at stage 2 of disease via their strong antioxidant powers due to their richness in phenolic compounds have a good antioxidant.

According to the literature, the male flower buds are known by their good antioxidant activity, related to their



0 – 50 µg/mL: concentrations of phenolic extract analyzed. **PD1:** First stage of Parkinson's disease according to Hoehn and Yahr scale, **PD2:** Second stage of Parkinson's disease according to Hoehn and Yahr scale, **PD3:** Third stage of Parkinson's disease according to Hoehn and Yahr scale, **PD4:** Fourth stage of Parkinson's disease according to Hoehn and Yahr scale, **C:** Control (healthy individual without signs of neurological disorders).

Fig. 5 Effect of the three tested phenolic extracts at the different concentrations on plasma 4-HNE levels (U/mL) of the subjects under study: *P. vera* (**a**), *F. carica* (**b**) and *P. dactylifera* (**c**)

high TPC and TFC. Nevertheless, there is still controversy over which compounds are actually responsible for their antioxidant properties, in part due to differences between species, varieties and geographic regions [47]. On the other hand, a previous studies have shown that consumption of high level of flavonoids decreases the risk of dementia by 50% [48]. These data agree perfectly with our results which show the high content of flavonoids in *P. vera* and *F. carica* (64.73 and 92.98%, respectively), and therefore explains the significant activity of *P. vera* and

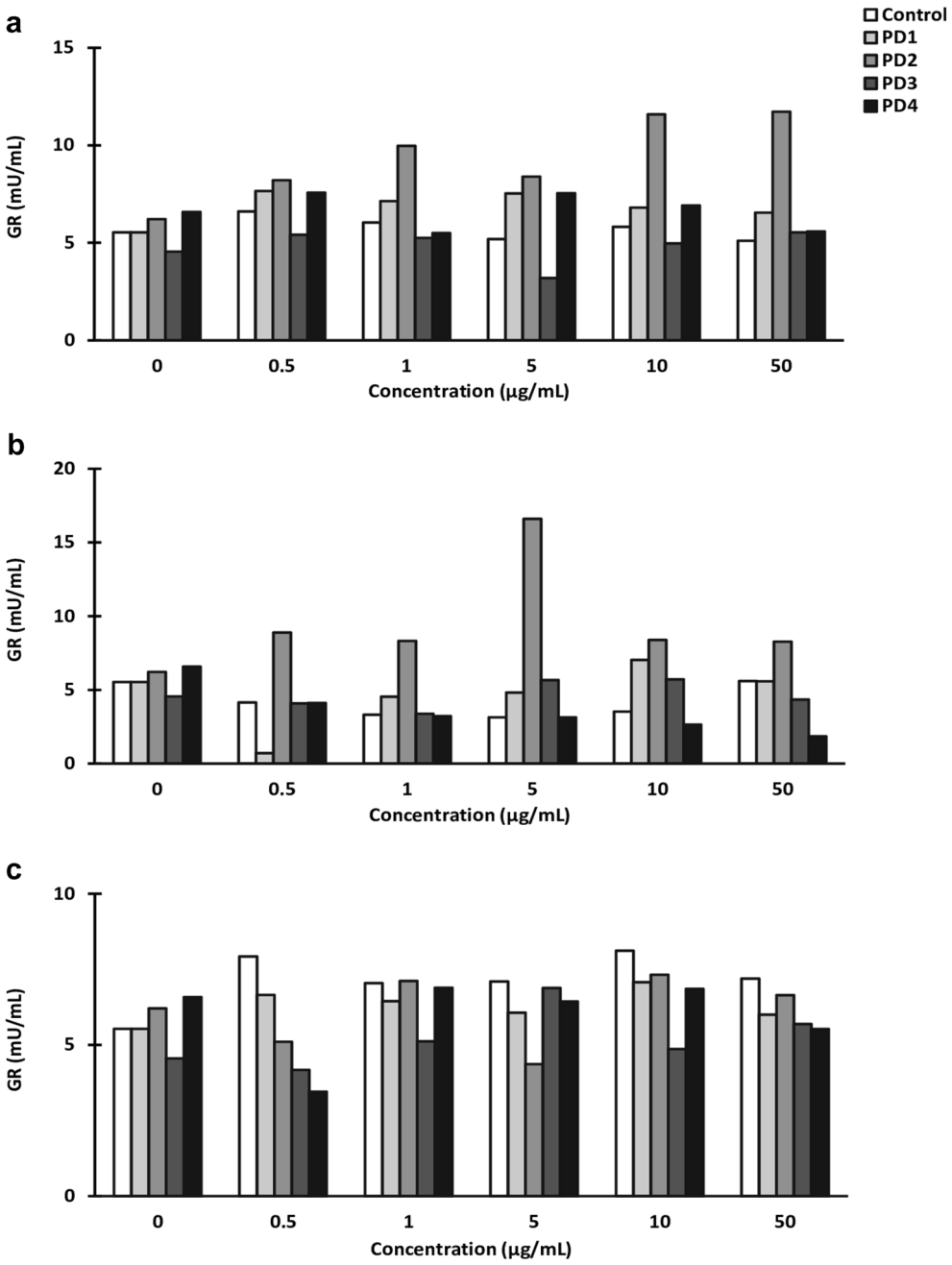


Fig. 6 Effect of the three tested phenolic extracts at the different concentrations on plasma GR activity (mU/mL) of the subjects under study: *P. vera* (**a**), *F. carica* (**b**) and *P. dactylifera* (**c**)

F. carica compared to that given by *P. dactylifera* with a TFC of only 14.31%.

A literature survey showed that *P. vera* via its different extracts from its different organs a high antioxidant activity [11, 49, 50]. Moreover, the ethanolic extract from male flower buds showed to have the strongest antioxidant activity when was evaluated by other oxidative stress markers (DPPH, β-carotene and ABTS) [41]. These data were found to be in good agreement with our results detailed above. For *P. dactylifera*, a previous study in Alzheimer's disease

Table 3 Pearson correlation shown by studied parameters in the three male flower bud tested extracts

	TPC	TFC	4-HNE	GR	SOD	ChGlu	FMA I	CoQA D	Q3R	Q7H	KGlu	SE	FMA II	CQA
TPC	1.00													
TFC	0.9606*	1.00												
4-HNE	−0.9998*	−0.9658*	1.00											
GR	0.9715*	0.9991*	−0.9759*	1.00										
SOD	0.5750*	0.7796*	−0.5907*	0.7525*	1.00									
ChGlu	−0.7225*	−0.5020	0.7089*	−0.5380	0.1503	1.00								
FMA I	−0.7521*	−0.5394	0.7392*	−0.5744	0.1068	0.9990*	1.00							
CoQA D	−0.6572*	−0.4219	0.6424*	−0.4598	0.2388	0.9959*	0.9910*	1.00						
Q3R	0.2744	0.5308*	−0.2930	0.4946*	0.9446*	0.4666*	0.4273	0.5444*	1.00					
Q7H	−0.6123*	−0.3685	0.5968*	−0.4074	0.2948	0.9890*	0.9890*	0.9890*	0.9890*	1.00				
KGlu	0.3772*	0.1051	−0.3591*	0.1469	−0.5409*	−0.9128*	−0.8940*	−0.9459*	−0.7871*	−0.9632*	1.00			
SE	−0.8926*	−0.7322*	0.8837*	−0.7603*	−0.1443	0.9566*	0.9685*	0.9264*	0.1886	0.9030*	−0.7542*	1.00		
FMA II	−0.8952*	−0.9838*	0.9037*	−0.9753*	−0.8793*	0.3387	0.3796	0.2524	−0.6742*	0.1958	0.0751	0.5981*	1.00	
CQA	−0.4457	−0.1794	0.4282	−0.2207	0.4762*	0.9409*	0.9252*	0.9676*	0.7385*	0.9807*	−0.9972*	0.8014	−0.01	1.00

ChGlu chrysoeriol-*O*-glucuronide, *CoQA D* coumaroylquinic acid derivative, *CQA* caffeoylquinic acid isomer, *FMA I* feruloyl-malicacid I, *FMA II* feruloyl-malicacid II, *GR* glutathionereductase, *KGlu* kaempferol-*O*-glucuronide, *Q7H* quercetin-7-*O*-hexoside, *Q3R* quercetin 3-*O*-rutinoside, *SE* sulfated-epicatechin, *SOD* superoxide dismutase, *TFC* total flavonoid content, *TPC* total phenolic content, *4-HNE* 4-hydroxynonenal

*Significant values ($p < 0.5$)

showed that fruits of this species protected against neuronal injury and oxidative stress [51]. However, our results with male flower buds extract (*P. dactylifera*) did not show a very high benefit for PD.

3.4 Correlation Among Phenolic Compounds and 4-Hydroxynonenal, Glutathione Reductase and Superoxide Dismutase Activities

To the best of our knowledge, this is the first report exploring the correlation among phenolic compounds of extracts from male flower buds of pistachio, fig and date palm; and 4-Hydroxynonenal, Glutathione reductase and Superoxide dismutase activities in blood plasma of Parkinson's disease patients. Since any correlation requires the presence of at least 3 values, only the common phenolic compounds present in the three extracts were considered. The average of each enzyme activity at different stages was calculated in order to determine the global activity of each enzyme. Based on Pearson's correlation (Table 3), the current study has recorded considerable positive correlations between TPC and GR (r = 0.9715), TFC and GR (r = 0.9991), however, negative correlations between independent phenolic compounds, except quercetin 3-*O*-rutinoside and GR (r = 0.4946), showing that phenolic compounds act synergistically to inhibit the potential of glutathione reductase. The results manifested a positive correlation between TFC and SOD (r = 0.7796) and quercetin 3-*O*-rutinoside and SOD (r = 0.9446) explaining that quercetin 3-*O*-rutinoside is the main flavonol responsible for the inhibition of Superoxide dismutase activity. TPC and 4-HNE; and TFC and 4-HNE are negatively correlated (r = -– 0.998 and − 0.9658, respectively), while positive correlations were recorded between feruloyl-malic acid II and 4-HNE (r = 0.9037), sulfated-epicatechin and 4-HNE (r = 0.8837) and chrysoeriol-*O*-glucuronide and 4-HNE (r = 0.7089). These findings are in accordance with the results found previously in the Sect. 3.3.2 that none of the three polyphenolic extracts had a significant effect in reducing levels of plasma 4-HNE taking in account all the phenolic compounds together but not their individual effect.

4 Conclusion

In this study, polyphenolic extracts from male flower buds of *Pistacia vera*, *Ficus carica* and *Phoenix dactylifera* revealed high levels of phenolic acids and flavonoids content. From the tested phenolic extracts, the *P. vera* and *F. carica* showed to have a significant antioxidant activity increasing plasma SOD and GR activities of PD patients. Our results suggest that pistachio and fig male flower buds might be regarded as an excellent source of antioxidants and have therapeutic potential preventing oxidative stress related neurodegeneration. Nevertheless, our study presents several limitations such as the identification of single or groups of phenolic compounds responsible for the antioxidant properties of *P. vera* and *F. carica*, which might be of great scientific relevance.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

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