Restorative antiaging influence and chemical profile of *Prunus domestica* L. (European plum) seed extract in a D-galactose-induced rat model

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Background
Prunus domestica L., also known as European plums or prunes, is a member of the Rosaceae family. Recently, fruit byproducts like seeds have been used as a novel and affordable source of bioactive compounds that may be an affordable source of substances. These substances hold potential benefit to the food and pharmaceutical industries.
Objective
The study aims to evaluate the chemical composition of *P. domestica* L. seeds and their safety and efficiency as an antiaging agent.
Materials and methods
The phenolic content in ethanolic seed extract was determined using the Folin–Ciocalteu reagent. Chlorophyll a, chlorophyll b, and β-carotene concentrations were measured by high performance liquid chromatography analysis. Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) negative ion mode was carried out to investigate the phytoconstituents, in addition to the isolation of the main active compounds on preparative TLC as well as evaluation of cognitive capabilities, measurement of antioxidants and neurotransmitters and histopathological brain examination.
Results and conclusion
The total phenolic and flavonoid contents of ethanolic extract were 136.55 mg GAE/g and 89 mg CE/g, respectively. Also, high performance liquid chromatography analysis led to the identification of β-carotene as the main natural pigment (59.376 mg/100 g), followed by chlorophyll b (41.745 mg/100 g) and finally chlorophyll a (20.824 mg/100 g). In addition, UPLC/ESI-MS analysis using the negative ion mode led to the identification of 24 compounds, including two flavonols, one flavanone, two flavones, 10 flavonoid-*O*-glycosides, two methylated flavonoids, one isoflavonoid*C*-glycosides, two phenolic acids, two aldehydes, one stilbene, and one coumarin. Preparative TLC chromatographic technique for ethanolic *P. domestica* seed extract resulted in the isolation of γ-tocopherol, kaempferol-3-*O*-rutinoside, luteolin, and luteolin-7-*O*-glucoside. The ethanolic extract proved to have a remarkable scavenging effect against 1,1-Diphenyl-2-picryl-hydrazyl in a dose-dependent manner compared with vitamin C. Improvement in behavioral assessment was noticed posttreatment of rats with the extract suggesting amelioration in cognitive ability and spatial memory of rats compared with standard drug. In addition, noticeable improvements were observed in neurotransmitter levels, acetylcholinesterase, noradrenaline, dopamine, and serotonin, in the brain tissue of aged rats. Besides, remarkable improvements in oxidative stress biomarkers (nitric oxide, reduced glutathione and lipid peroxide, total antioxidant capacity) and in histopathological pictures of different brain regions posttreatment of aged rats with the extract compared with donepezil standard drug were recorded.

Keywords:
antiaging, antioxidant, flavonoids, *Prunus domestica* seeds

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Introduction

Brain cellular impairment could result from either uncontrollable age-related deterioration with or exposure to environmental or genetic stimuli, eventually leading to brain tissue damage and

deteriorated neurological conditions in a time-dependent pattern [1]. Moreover, brain aging is one of the most popular neurological syndromes that prompts massive physiological damage [2]. It could be diagnosed by diminished cognitive function. The impairment in neurological parameters includes mitochondrial malfunctioning, lipid peroxidation, oxidative stress, beta-amyloid (Aβ) elevation, and crucial brain tissue modification [3]. Multiple research studies have used D-galactose (D-gal.)-induced aging animal models to investigate the antiaging mechanism [4–6]. Numerous studies have demonstrated that long-term D-gal injection would significantly increase ROS production, decrease the activity of antioxidant enzymes in different organs, and increase free radicals. It also enhances apoptosis and neurodegeneration [6].

The most significant contributors to preventing chronic illnesses and providing a healthful life have been recognized by consuming a variety of nutritious foods [7]. *Prunus domestica* L., also known as European plums or prunes, is a member of the Rosaceae family [8]. According to Jaiswal *et al.* [9], fruits are considered to be a particularly high source of polyphenols. Recently, fruit byproducts like seeds have been utilized as a novel and affordable source of bioactive compounds. Because seeds are high in proteins and lipids, they may be an affordable source of substances that could be beneficial to the food, cosmetic, and pharmaceutical industries [10]. The traditional and medicinal activities of *Prunus domestica* L. have been widely proved including antioxidant, antimicrobial, antihemolytic, cytotoxic, hepatoprotective, GIT laxative, and anti-inflammatory [11].

The current research study aims to evaluate the chemical composition of *P. domestica* L. (European plum) seed ethanol extract (80%), which is considered a plant waste product, and its safety and efficiency as an antiaging agent.

Materials and methods

Phytochemical study

Chemicals and reagents

All the chemicals and reagents were of analytical grade. Folin–Ciocalteu reagent and catechin were purchased from Sigma-Aldrich (USA) and ChemFaces (Wuhan, China), respectively. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was acquired from Sigma-Aldrich. Ascorbic acid was bought from E. Merck (India). The authentic chlorophylls and carotene from Sigma-Aldrich. Donepezil (reference drug) was obtained from Sanofi, Egypt.

Plant material

P. domestica L. (European plum) fruits were collected in May 2021 from the local markets in Cairo, Egypt. The specimen of the fruit was identified by Dr Gamal Farag, (National Agriculture Centre, Egypt). The seeds were separated, dried in shades, and then ground and kept in sealed bags.

Extraction

The dried, powdered *P. domestica* seeds (500 g) were extracted with 80% ethyl alcohol several times till exhausted. The obtained total extracts were concentrated under reduced pressure at 45°C using the rotary evaporator and kept in a refrigerator for further studies.

Determination of total phenolic and flavonoid contents

The total phenolic content was determined using the Folin–Ciocalteu reagent, and the values were expressed in terms of gallic acid equivalent (μg gallic acid/g extract). In addition, the total flavonoid content was assigned as stated by Zilic *et al.* [12], and expressed as catechin equivalent (μg catechin/g extract).

High performance liquid chromatography analysis of natural pigment content

High performance liquid chromatography (HPLC) analysis procedures of chlorophyll a, chlorophyll b, and β-carotene content of *P. domestica* seeds were performed using HPLC Agilent Packed and ultraviolet detector according to Siong and Lam [13]. The peak area was calculated in comparison with pigment standards in triplet injections.

UPLC-QTOF-MS/MS analysis

UPLC-QTOF-MS/MS in negative ion mode was carried out on a Vendor/Specstriple quadruple instrument. The HPLC-MS system was composed of an autosampler injector (Switzerland) and a mass spectrometer from waters corporation (Milford, Massachusetts, USA), and. The column used was the ACQUITY UPLC-BEH Waters (X select HSS T3) C18 with dimensions of 1.7 μm–2.1×50 mm. The mobile phase elution was made with a flow rate of 0.3 ml/min using the gradient mobile phase comprising two eluents: eluent A is 5 mM ammonium formate buffer pH 3 containing 1% methanol and eluent B is 100% acetonitrile. The peaks and spectra were processed using the Analyst TF 1.7.1 software and tentatively identified by comparing its retention time (Rt) and mass spectrum with the reported data.

Isolation of the main active compounds

The total extract (80% ethanol) was applied on preparative TLC using two developing systems:

chloroform–methanol (90 : 10 v/v) and (toluene : ethyl acetate 80 : 20 v/v). The plates were examined under the UV light at 254 and 365 nm, then separately subjected to $AlCl_3$ solution and 10% H_2SO_4 spraying reagents for the detection of flavonoids and terpenoids, respectively. The selected bands were marked and scratched and then collected. The isolated compounds were identified by different spectral analyses (UV, H^1-NMR, ^{13}C-NMR, IR, and MS spectrometry). Determination of the melting point and different spectral analyses were done using: Koffler's heating stage microscope to determine the melting point, UV–visible spectrophotometer double-beam UVD–3500, Labomed Inc., FT-IR spectrophotometer, Perkin-Elmer 283 (Germany), nuclear magnetic resonance spectrometers JEOL EX-270, Mass spectrometer, Finnigan Model 3200 at 70 eV.

Biological study

In-vitro study

In-vitro antioxidant assay

The free radical scavenging activity of two concentrations (0.01 and 0.05 μg/ml) of ethanolic *P. domestica* extract was assessed against the DPPH inhibition activity (20 mg dissolved in 5 ml ethyl alcohol). The inhibition percentage of DPPH was spectrophotometric assessed using measuring color intensity at 518 nm according to Rahman *et al.* [14].

$$\text{Percentage of DPPH scavenging effect} = [(\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{extract}})/\text{absorbance}_{\text{control}}] \times 100$$

In-vivo study

Acute toxicity studies

LD_{50} value of different oral doses of the ethanolic *P. domestica* extract was performed up to a dose of 5000 mg/kg body weight. Observation of the rat's morality and behavior was estimated for 2 weeks. An acute toxicity study was performed according to the method described by Hu *et al.* [15].

Animals

Using conventional experimental circumstances with temperatures ranging from 25°C and humidity level of 55°C, t32 male rats with an average weight of 120 g were sorted into four categories of eight rats in each group. The first group is the control, second group consists of animals intraperitoneally administered with 100 mg/kg D-gal. for two consecutive months and served as a positive control group [16]. The third group, post 2 months of D-gal. administration, animals will be orally treated daily with 500 mg/kg, body weight (according to acute toxicity study) for 1 month. The fourth group is aging-induced rats, which will be treated with donepezil as a reference drug at a dosage of 10 mg/kg for 1 month [17].

Ethical procedures

All animals were handled under academic ethical standards of the Medical Research Ethics Committee of the National Research Centre in Egypt ensuring that they did not suffer any pain during treatment and adhering to the safety procedures during their killing (ethical approval no: 19288).

Behavior evaluation

Evaluation of cognitive capabilities

At NRC., a T-maze is performed. After 60 days of continuous D-gal. handling, the cognitive function and spatial memory deficits in rats were assessed [18].

Serum preparation and brain tissue homogenate collection

At the end of the period of D-gal. induction and post 1 month of treatments as well as after 24 h of behavioral testing, blood was obtained by puncturing the sublingual vein. Serum was subsequently extracted by a centrifuge at 4000 revolutions per minute for 15 min. Following slight anesthesia with diethyl ether, rats were killed and the entire brain was quickly dissected and cleaned with isotonic saline. This is followed by weighing a part of the brain, homogenizing it (1 : 10 w/v) in icy cold phosphate buffering saline, centrifugation for 15 min at 5000 revolutions per minute, and storing the supernatant at −80°C. For histological analyses, a 10% formalin solution was used to fix the brain tissue.

Biochemical findings

Assessment of antioxidants

The nonenzymatic, glutathione-reduced (GSH), malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (TAC) in brain tissues were assessed in all animal groups using standard diagnostic kits (Biodiagnostic Chemical Co., Cairo, Egypt).

Measurement of neurotransmitters

Acetyl cholinesterase (AchE) levels were determined in brain tissue homogenate by colorimetric assay according to the method developed by Ellman *et al.* [19]. Noradrenaline (NA), dopamine (DA), and serotonin (5-HT) were measured in accordance with Giday *et al.* [20] in brain tissue homogenate by the HPLC system, agilent technologies 1100 series (Santa Clara, California, USA), equipped with a quaternary pump (G131A model). Separation was achieved on

octadecylsilyl-reversed phase column (C18, 25 0.46 cm^2; internal diameter: 5 mm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1.5 ml/min. Ultraviolet detection was performed at 270 nm, and the injection volume was 20 ml. The concentration of both catecholamines and serotonin was determined by the external standard method using peak areas. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve [21].

Nitric oxide estimation

NO was measured in brain homogenates using a spectrophotometric procedure [22]. The determination of the nitric oxide ($NO^{\bullet}$) radical scavenging method is based on the inhibition of the NO radical generated from sodium nitroprusside in phosphate buffer saline and determined by the Griess reaction.

Malondialdehyde estimation

MDA was assayed in brain tissues [23]. All animal groups were subjected to a determination of MDA and TBA reacts with MDA in an acidic medium at a temperature of 95 0C for 30 min to form the TBA reactive product. The absorbance of the resultant pink product can be measured at 534 nm.

Total antioxidant capacity estimation

The term TAC is an assay that measures the scavenging of free radicals by a test solution or suspension according to Koracevic *et al.* [24].

Histopathological examination

All animal brain samples were fixed in formalin and then embedded in paraffin. Hematoxylin and eosin stains were used to stain the paraffin blocks, which were then sliced to a thickness of 4–5 mm before being inspected under a light microscope (Olympus BX50, Japan). The brain's cerebral cortex and hippocampus' histopathological damage was rated on a scale of 0–4 based on the following criteria: (0) designated no changes, (1) represented a percentage of the affected area of less than 10%, (2) signified a percentage of affected parts in the range of 20–30%, (3) stated for percentages of damaged regions in the range of 40–60%, and (4) showed an affected area of more than 60% [25].

Histopathological lesion scoring

Histopathological alterations in the brain were recorded and scored as follows: no changes (0), mild (1), moderate (2), and severe (3) changes. The grading was determined by percentage as follows: less than 30% changes (mild change), less than 30–50% (moderate change), and more than 50% (severe change) [26].

Statistical analysis

Statistical comparisons between the tested groups were carried out by SPSS description 9.05 (USA). The significant differences were investigated using one-way analysis of variance followed by the Co-state computer program; different letters indicate significance at P value less than or equal to 0.05.

Results and discussions

Phytochemical study

Quantitative estimation of total phenolics and total flavonoid content

Total phenolics in the ethyl alcohol extract of *P. domestica* seed was spectrophotometrically determined and calculated at 136.55 mg gallic acid/g extract, while the total flavonoid content yielded 89 mg catechin/g extract.

High performance liquid chromatography analysis of the natural pigment content

HPLC analysis of the ethanolic *P. domestica* seeds extract led to the identification of β-carotene as the main natural pigment (59.376 mg/100 g), followed by chlorophyll b (41.745 mg /100 g) and finally chlorophyll a (20.824 mg /100 g). Pigment profile of the ethanolic *P. domestica* seed extract was assessed for the first time in the current study.

UPLC-QTOF-MS/MS analysis

The earlier study stated the presence of several flavonoids in *P. domestica* seeds, such as hesperidin, naringenin-7-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, and naringenin [27]. However, no brief investigation was conducted to study the full chemical composition of alcoholic extract of *P. domestica* seeds. UPLC/ESI-MS analysis using the negative ion mode was performed for the identification of flavonoids and phenolic constituents in the ethanolic *P. domestica* seed extract as illustrated in Table 1. From the molecular weight and mass fragments in addition to the review literature, 24 compounds were identified, including two flavonols, one flavanone, two flavones, 10 flavonoid-*O*-glycosides, two methylated flavonoids, one isoflavonoid C-glycosides, two phenolic acids, two aldehydes, one stilbene, and one coumarin.

The two identified flavonols were 3'-methoxy-4',5,7-trihydroxy flavonol and 3 5 7-trihydroxy-4'-

Table 1 LC-ESI-MS analysis of ethanolic *Prunus domestica* seed extract

Class	Name	Rt (min.)	[M-H]$^-$ (*m/z*)	Molecular formula	Main fragments (*m/z*)
Flavonols	3'-Methoxy-4',5,7-trihydroxyflavonol	5.09	315	$C_{16}H_{12}O_7$	153, 251, 229, 274
	3 5 7-Trihydroxy-4'-methoxyflavone	9.44	299	$C_{16}H_{12}O_6$	269, 207,143
Flavanone	Naringenin	4.91	271	$C_{15}H_{12}O_5$	91, 119, 153
Flavones	Apigenin	10.5	269	$C_{15}H_{10}O_5$	117, 151, 227
	Luteolin	9.38	285	$C_{15}H_{10}O_6$	147, 285
Flavonoid-O-glycosides	Hesperidin	7.6	609	$C_{28}H_{34}O_{15}$	301, 609
	Isorhamnetin-3-*O*-glucoside	6.89	477	$C_{22}H_{22}O_{12}$	285, 302, 317
	Kaempferol-3-*O*-arabinoside	6.28	417	$C_{20}H_{18}O_{10}$	202, 121
	Kaempferol-3-*O*-rhamnoside	1.08	431	$C_{21}H_{20}O_{10}$	255, 284, 431
	Kaempferol-7-neohesperidoside	6.5	593	$C_{27}H_{30}O_{15}$	285, 287
	Kaempferol-3,7-*O*-bis-rhamnoside	6.80	577	$C_{27}H_{30}O_{14}$	431, 577
	Luteolin-3', 7-di-*O*-glucoside	5.50	609	$C_{27}H_{30}O_{16}$	285, 447
	Myricitrin	6.5	463	$C_{21}H_{20}O_{12}$	129, 153
	Quercetin-3-D-xyloside	6.88	433	$C_{20}H_{18}O_{11}$	364, 433
	Quercitrin	6.85	447	$C_{21}H_{20}O_{11}$	401, 326
-O-methylated flavonoids	Acacetin	13.48	283	$C_{16}H_{12}O_5$	151, 211, 239
	Hesperetin	9.55	301	$C_{16}H_{14}O_6$	153, 177
Isoflavonoid C-glycosides	Daidzein-8-*C*-glucoside	4.91	415	$C_{21}H_{20}O_9$	267, 297
Phenolic acids	Quinic acid	1.09	191	$C_7H_{12}O_6$	191, 93, 85
	Rosmarinic acid	4.6	359	$C_{18}H_{16}O_8$	161, 179
Aldehydes	4-Hydroxy-3-methoxycinnamaldehyde	7.94	177	$C_{10}H_{10}O_3$	162, 107
	Sinapyl aldehyde	8.2	207	$C_{11}H_{12}O_4$	121, 145, 207
Stilbene	E-3,4,5'-Trihydroxy-3'-glucopyranosylstilbene	1.08	405	$C_{20}H_{22}O_9$	191, 404
Coumarin	6,7-Dihydroxycoumarin	7.5	177	$C_9H_6O_4$	177, 157

methoxyflavone with [M-H]$^-$ 315 and 299 for the molecular formula $C_{16}H_{12}O_7$ and $C_{16}H_{12}O_6$, respectively.

One flavanone (naringenin) and two flavones (apigenin and luteolin) were identified, in addition to 10 flavonoid-*O*-glycosides (hesperidin, isorhamnetin-3-*O*-glucoside, kaempferol-3-*O*-arabinoside, kaempferol-3-*O*-rhamnoside, kaempferol-7-neohesperidoside, kaempferol-3,7-*O*-bis-rhamnoside, luteolin-3', 7-di-*O*-glucoside, myricitrin, quercetin-3-D-xyloside, quercitrin). In addition, two identified *O*-methylated flavonoids were acacetin and hesperetin with [M-H]$^-$ 283 and 301, respectively. Futhermore, an isoflavonoid *C*-glycoside, daidzein-8-*C*-glucoside, was identified with [M-H]$^-$ 415 for $C_{21}H_{20}O_9$

Two identified phenolic acids were asquinic acid and rosmarinic acid with molecular formulas $C_7H_{12}O_6$ and $C_{18}H_{16}O_8$. Besides the two aldehydes, 4-hydroxy-3-methoxycinnamaldehyde and sinapyl aldehyde with molecular formulas $C_{10}H_{10}O_3$ and $C_{11}H_{12}O_4$, respectively, were identified.

The identified stilbene was E-3,4,5'-Trihydroxy-3'-glucopyranosyl stilbene with a molecular formula of $C_{20}H_{22}O_9$ and coumarin was 6,7-dihydroxycoumarin with a molecular formula of $C_9H_6O_4$.

Isolation of the main active compounds

The preparative TLC chromatographic technique for ethanolic *P. domestica* seed extract resulted in the isolation of four major compounds (1, 2, 3, 4). Compound 1 (30 mg) was eluted using toluene–ethyl acetate (80 : 20 v/v), which turned a dark brown color on adding 10% H_2SO_4, while compounds 2, 3, and 4 (19, 24, 27 mg) were isolated using a chloroform–methanol (90 : 10 v/v) solvent system, giving a yellow color upon exposure to the $AlCl_3$ spray reagent. Various spectral analyses (UV, IR, MS, H^1-NMR, and ^{13}C-NMR spectrometry) have been applied for identification and structure configuration.

Compound 1: γ-tocopherol was oily, with an R_f value of 0.61 in the toluene–ethyl acetate (80 : 20 v/v) developing system. MS (m/z) gave a molecular ion at *m/z* 430 representing the molecular formula $C_{29}H_{50}O_2$. Other main fragments were 415 (M^+-CH_3), 203 and 205, 165 (100%). FT-IR (cm^{-1}) showed peaks at 3150–2848, indicating asymmetric and symmetric stretching of the CH_2 and CH_3. The peak at 1469 characterizes methylene group bending; the peak at 1735 was for the conjugated ketone (C=O group), while the aromatic ring gave distinctive peaks in the range of 1426 and 722, and finally the peak at 1081 was attributed to the ether link of the pyran ring.

The above spectral data were in agreement with that previously published by Elbatanony *et al.* [28], as well as a comparison against the authentic reference material. It is to be mentioned that γ-tocopherol is abundant in Rosaceae seeds, and it is remarkably presented in plum seeds with almost 89% [29].

Compound 2: Kaempferol-3-*O*-rutinoside (nicotiflorin) was obtained as a crystalline yellow powder with an R_f of 0.38 in chloroform–methanol (90 : 10 v/v), with a melting point 177°C. $UV_{\lambda max}$, nm (MeOH): 342, 298, 268. The MS (*m/z*) showed a molecular ion *m/z* at 594 for the molecular formula $C_{27}H_{30}O_{15}$. Other distinctive fragments were 449, 285, 255, 227, and 151. ^{1}H-NMR(400 MHz, CD_3OD, *δ*/ppm) showed the following signals: 8.10 (2H, d, H-2′,6′ aromatic ring), 6.90 (2H, d, H3′,5′ aromatic ring), 6.38 (1H, d, H-8), 6.17 (1H, d, H-6), 5.12 (1H, d, glc H-1), 4.53 (1H, s, Rha H-1), ^{13}C-NMR (125 MHz, MeOD, *δ*/ppm) gave the following signals: 160.0 (C-2), 134.9 (C-3), 178.8 (C-4), 162.9 (C-5), 100.0 (C-6), 167.1 (C-7), 96.1 (C-8), 159.2 (C-9), 105.9 (C-10), 120.8 (C-1′), 131.7 (C-2′), 115.7 (C-3′), 162.1 (C-4′), 116.0 (C-5′), 133.1 (C-6′), 104.8 (C-1″), 75.3 (C-2″). The data are compatible with that present in Osw and Hussain [30]. This flavonoid glycoside is widely spread in other *Prunus* species, especially the peels [31].

Compound 3: Luteolin was isolated as a yellow powder with an R*f* of 0.72 in chloroform–methanol (90 : 10 v/v); it exhibited a melting point of 330°C, EI-MS exhibited molecular ion at *m/z* 286 for the molecular formula $C_{15}H_{10}O_6$ besides peaks at 269, 241, 161, 153, 135, and 67 which matched with the results of Tsimogiannis [32]. ^{1}H-NMR (400 MHz, CD_3OD, *δ*/ppm) 6.22 (1H, d, H-6), 6.39 (1H, d, H-8), 6.48 (1H, s, H-3), 6.77 (1H, d, H-5′), 7.29 (2H, m, H-2′, H-6′); ^{13}C-NMR (125 MHz, CD_3OD, *δ*/ppm) 165.4 (C-2), 102.6 (C-3), 184.1 (C-4), 162.5 (C-5), 99.8 (C-6), 165.3 (C-7), 93.2 (C-8), 158.7 (C-9), 104.6 (C-10), 124.3 (C-1′), 113.8 (C-2′), 146.8 (C-3′), 150.3 (C-4′), 117.2 (C-5′), 119.6 (C-6′) which are in agreement with Lin *et al.* [33].

Compound 4: Luteolin-7-*O*-glucoside (Cynaroside) was isolated as a yellow powder with an R*f* of 0.84 in chloroform–methanol (90 : 10 v/v), melting point 266°C; EI-MS showed a molecular ion at *m/z* 448 for $C_{21}H_{20}O_{11}$, other fragments appeared at 241, 199, 217, 243, 213, 257, 242, 267 as illustrated by Pikulski and Brodbelt [34]. ^{1}H-NMR (400 MHz, CD_3OD, *δ*/ppm) 5.12 (1H, d, H-1″), 6.50 (1H, d, H-6), 6.69 (1H, s, H-3), 6.73 (1H, d, H-8), 6.91 (1H, d, H-5′), 7.38 (1H, d, H-2′), 7.41 (1H, dd, H-6′); ^{13}C-NMR (125 MHz, CD_3OD, *δ* / ppm) 165.3 (C-2), 102.7 (C-3), 181.9 (C-4), 160.7 (C-5), 99.9 (C-6), 162.9 (C-7), 95.2 (C-8), 156.8 (C-9), 106.1 (C-10), 122.0(C-1′), 114.2 (C-2′), 146.1 (C-3′), 149.8 (C-4′), 115.6 (C-5′), 120.3 (C-6′), 100.3(C-1″), 72.8 (C-2″), 75.9 (C-3″), 68.4 (C-4″), 76.8 (C-5″), 61.4 (C-6″), which are in agreement with Lin *et al.* [33].

Biological study

In-vitro antioxidant assay

The free radical scavenging activity of two concentrations (0.01 and 0.05 μg/ml) of ethanolic *P. domestica* extract was assessed using DPPH radical, and the percentage of inhibition is illustrated in Tables 2 and 3, where the antioxidant activity of the ethanolic *P. domestica* extract was remarkable in comparison to vitamin C as standard, and the activity showed dose-dependent relationship (directly proportional to increasing the concentration of the extract).

In-vivo antiaging study

Behavioral assessment

T-maze: significant increase in the time taken by D-gal-induced rats to reach the food compared with control rats with a percentage increase reached of up to 66.67%. However, treatment of aging rats with *P. domestica* showed an improvement of 52.30%, compared with the standard drug, which recorded 81.10%.

The results in Table 4 showed a significant increase in AchE level in the brain tissue with a percentage increase of 109.86%, compared with control rats. Treatment of aging rats with *P. domestica* extract showed a significant reduction in AchE levels with an improvement of 67.11%, while treatment of aging rats with donepezil standard drug recorded improvement percentages of 100.71%. An insignificant difference was noticed between control and treated normal rats with the extract.

Table 2 ***In vitro*** **1,1-Diphenyl-2-picryl-hydrazyl scavenging activity of the ethanolic** ***Prunus domestica*** **seed extract**

	% Inhibition	
Samples	0.01 μg/ml	0.05 μg/ml
Vitamin C	81.00±0.82%[a]	89.00±0.82%[a]
Prunus domestica	35.25±0.91%[g]	57.58±0.82%[d]

Samples are three replicates in each group. Data are means percentage ±SD of three replicates in each group. Statistical analysis is carried out using the SPSS computer program coupled with Co-state, where different letters are significant at *P* value less than or equal to 0.05.

Table 3 Efficacy of the ethanolic *Prunus domestica* extract on the T-maze test in D-galactose-induced aging

Groups	Baseline (Sc)	Induction 2 months (Sc)	Treatment 4 weeks (Sc)
Control	11.60±0.60[a]	12.00±0.44[a]	11.10±0.50[a]
D-gal.		20.00±0.00[b]	–
%change		66.67	
D-gal.-*treated Prunus domestica*	–	–	14.20±1.11[c]
%improvement			52.30
D-gal. Donepezil standard drug	–	–	11.00±0.24[d]
%improvement			81.10

Data are expressed as mean SD (n=8) in seconds. Statistical analysis is carried out using the SPSS computer program coupled with Co-state, where different letters are significant at P value less than or equal to 0.05.

The results in Table 5 indicated an insignificant difference in neurotransmitter levels, NA, DA, and 5-HT, between normal rats treated with the extract compared with the control. Induced aging in rats showed a significant increase in NA in the brain tissue with a significant reduction in DA and 5-HT levels compared with control rats. Treatment of aging rats with *P. domestica* extract showed noticeable improvements in neurotransmitter levels: NA, DA, and 5-HT with improvement percentages 147.11, 48.16, and 30.00%, respectively, compared with the standard drug (182.92, 51.04, and 21.37%, respectively).

Table 4 AchE levels in aging and treated rats treated with *Prunus domestica* seed extract

Groups	Mean±SD (U/l)
Control	629.00±21.76[a]
Normal treated extract	667.00±12.45[a]
%change	6.04
D-gal. (aged rats)	1320. 98±23.00[b]
%change	109.86
D-gal. treated with *Prunus domestica*	897.90±23.00[c]
%change	31.98
%improvement	67.11
Donepezil+treated D-gal.	687.50±12.98[a]
%change	47.96
%improvement	100.71

Data are expressed as mean SD (n=8) in seconds. Statistical analysis is carried out using the SPSS computer program coupled with Co-state, where different letters are significant at P value less than or equal to 0.05.

D-gal.-induced rats showed a significant reduction in TAC (57.00%), while a significant increase in NO (126.19) and MDA (150.31%) levels compared with the control. However, the treatment of aged rats with the extract showed a significant increase in the TAC level with a percentage of amelioration recorded at 28.30%, compared with donepezil (37.37%). However, there was a significant reduction of both NO and MDA levels reaching 71.43 and 90.18%, respectively, compared with the standard drug (100.00 and 96.93%, respectively) as illustrated in Table 6.

The scoring system was designed as: score 0=absence of the lesion in all rats of the group (n=8), score 1=less than 30%, score 2=less than 30–50%, score 3=more than 50%. Group 1: control, group 2: aging rats, group 3: aging rats treated with extract, and group 4: aging rats treated with the drug.

Table 5 Neurotransmitter levels in aging and treated rats with the ethanolic *Prunus domestica* seed extract (μg/g tissue)

Sample name	NA	DA	5-HT
Control	4.86±0.02[a]	3.82±0.05[c]	2.90±0.03[e]
Normal+*Prunus domestica*			
Change %	4.55±0.22[a]	3.82±0.13[c]	2.87±0.04[e]
%improvement	6.37	0	1.03
D.gal			
Change %	16.89±1.12[b]	1.22±0.87[f]	1.03±0.02[f]
%improvement	247.53	68.10	64.48
D.gal-treated with *Prunus domestica*	9.74±0.25[c]	3.06±0.33[c]	1.90±0.06[g]
%change	42.33	150.82	84.50
%improvement	147.11	48.16	30.00
D.gal-treated with Donepezil	8.0±0.85[d]	3.17±0.078[c]	1.65±0.65[h]
% Change	52.63	159.84	60.19
%Improvement	182.92	51.04	21.37

Data are expressed as mean± SD (n=8). Statistical analysis is carried out using the SPSS computer program (one-way analysis of variance) coupled with the co-state computer program, where different letters are significant at P value less than or equal to .05.

Table 6 Oxidative stress levels in aging and treated rats with the seed extract

Parameters groups	Control	*Prunus domestica* treated normal rats	Aged rats	Treated *Prunus domestica* aging rats	Treated drug-aging rats
TAC (mM/g) tissue	0.90[a]±0.0	0.92±0.11[a]	0.42±0.09[c]	0.70±0.20[b]	0.79±0.07[b]
%Change	3	7.10	57.60	66.67	88.10
%Improvement				28.30	37.37
NO (μmol/g tissue)	42.00±0.	40.00±0.11[d]	95.00±0.12[a]	65.00±0.10[b]	53.00±0.16[c]
%Change	07[d]	4.76	126.19	31.58	44.21
%Improvement				71.43	100.00
MDA (nmol/g tissue)	326.00±0	316.00±0.06[c]	816.00±0.12[a]	522.00±0.16[b]	500±0.18[b]
%Change	0.17[c]	3.10	150.31	36.10	38.73
%Improvement				90.18	96.93

Data are expressed as mean±SD (n=8). Statistical analysis is carried out using the SPSS computer program (one-way analysis of variance) coupled with co-state computer program, where different letters are significant at P value less than or equal to 0.05.

Table 7 Scoring of histopathological alterations in the brain of all therapeutic groups

Lesions	G1	G2	G3	G4
Cerebral capillary congestion and hypertrophy	0	3	1	2
Neuronal degeneration with the formation of NFT	0	3	0	2
Meningeal congestion and hemorrhage	0	3	0	1
Encephalomalacia	0	3	1	1
Astrocytosis	0	3	1	0
Degeneration of the hippocampus neurons	0	3	1	1
Degeneration of the cerebellum Purkinje cells	0	3	1	1

Group 1: control, G2: aging rats group 3: aging rats treated with *Prunus domestica* extract, and G4: aging rats treated with the drug.

Table 7 illustrates the potent effect of the extract compared with the standard drug in reducing the lesion score in different brain regions as represented by the absence of (0 score) neuronal degeneration with the formation of NFT, and meningeal congestion and hemorrhage.

Histopathological examination showed the presence of capillary congestion, neuronal degeneration, and the formation of neurofibrillary tangles, endothelial hypertrophy in the cerebellum, cerebral cortex, and the hippocampus of the aging rat brains (group 2) compared with the control one (group 1). Treatment of D-gal.-induced aging rats with the extract and donepezil showed slightly hypertrophied capillaries, few degenerated Purkinje cells, and neurons in different brain regions (Fig. 1).

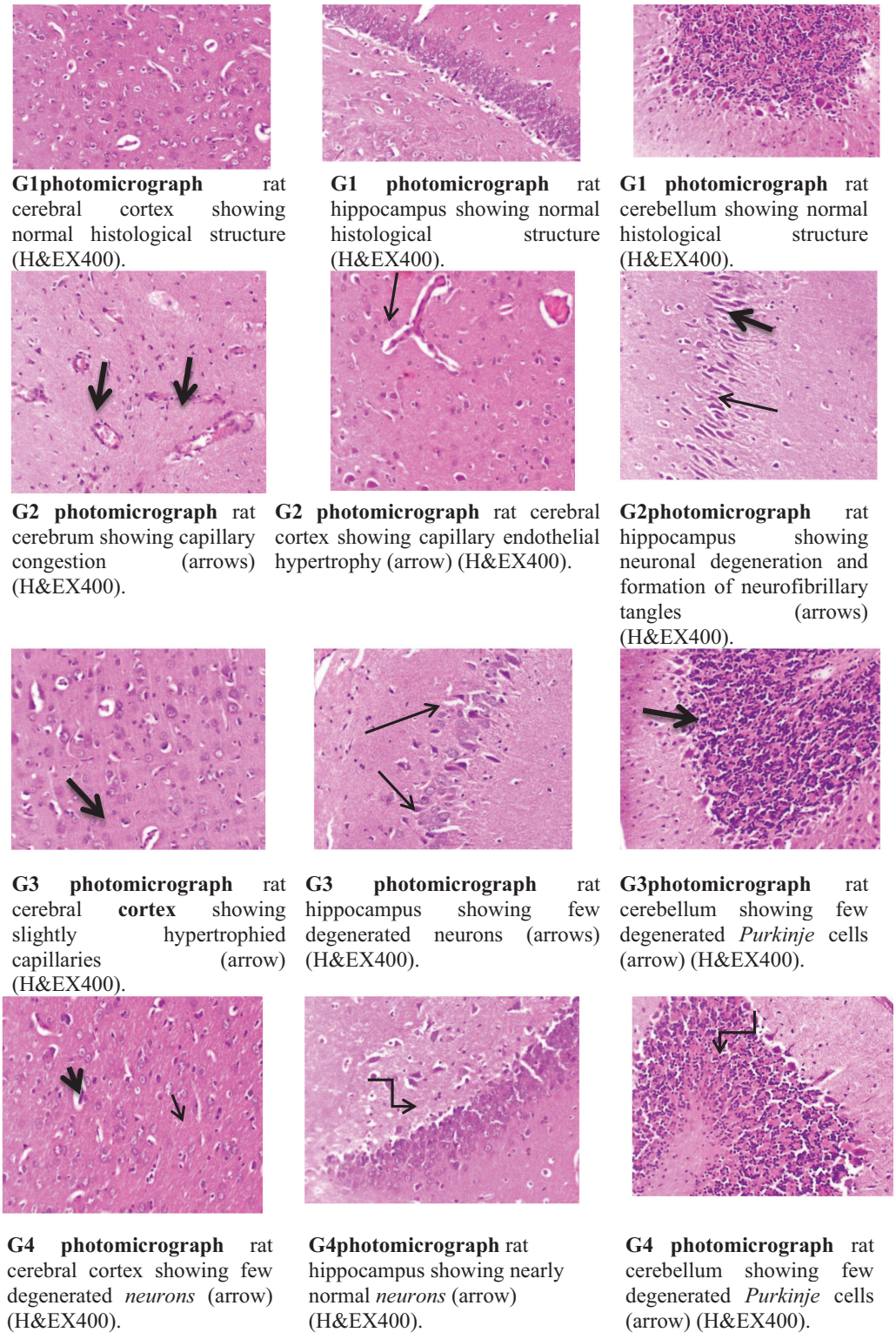
Age-related disease susceptibility and vulnerability are biological processes consisting of decreased physiological responsiveness to external stimuli, changes in chemical substance [35], and cell damage due to oxidative stress, which is characterized by a difference between the rate of production of reactive oxygen species and destruction generated by the antioxidant defense mechanism [36].

P. domestica has an elevated level of free radical scavengers, such as numerous flavonoids and phenolics, which have several positive effects, such as antioxidant, anti-inflammatory, and immunomodulatory properties that shield cells and small molecules like DNA from free radical damage and strengthen the antioxidant defense system [37].

The degree of brain aging can be determined by a variety of behavioral tests. As indicated in Table 4, the T-maze test used in our investigation demonstrated that D-gal significantly deteriorated brain neural processes. However, treatment of rats with donepezil or the seed extract led to an improvement in the behavioral state as shown by an improvement in cognition. These findings are consistent with earlier findings showing that $AlCl_3$-neurointoxicated rats took longer to capture food in the T-maze than control rats, indicating decreased neurocognitive performance [38,39].

Past investigations have demonstrated that D-gal accelerated cognitive aging and caused a variety of neurodegenerative effects. The blood–brain barrier allows D-gal to reach the brain. In distinct parts of the brain that are important in the aging process, an excess of D-gal causes mitochondrial malfunction, oxidative stress, reduced respiratory chain enzymes, and the time course of aging markers to accelerate [40]. Remarkably, D-gal. initiates the intrinsic and extrinsic apoptotic pathways and produces the inflammation markers [41]. The research findings

Figure 1



G1photomicrograph rat cerebral cortex showing normal histological structure (H&EX400).

G1 photomicrograph rat hippocampus showing normal histological structure (H&EX400).

G1 photomicrograph rat cerebellum showing normal histological structure (H&EX400).

G2 photomicrograph rat cerebrum showing capillary congestion (arrows) (H&EX400).

G2 photomicrograph rat cerebral cortex showing capillary endothelial hypertrophy (arrow) (H&EX400).

G2photomicrograph rat hippocampus showing neuronal degeneration and formation of neurofibrillary tangles (arrows) (H&EX400).

G3 photomicrograph rat cerebral **cortex** showing slightly hypertrophied capillaries (arrow) (H&EX400).

G3 photomicrograph rat hippocampus showing few degenerated neurons (arrows) (H&EX400).

G3photomicrograph rat cerebellum showing few degenerated *Purkinje* cells (arrow) (H&EX400).

G4 photomicrograph rat cerebral cortex showing few degenerated *neurons* (arrow) (H&EX400).

G4photomicrograph rat hippocampus showing nearly normal *neurons* (arrow) (H&EX400).

G4 photomicrograph rat cerebellum showing few degenerated *Purkinje* cells (arrow) (H&EX400).

Histopathological examination of control, aging, and *Prunus domestica* treated rats. Group 1: control, group 2: aging rats, group 3: aging rats treated with *P. domestica* extract, and group 4: aging rats treated with the drug.

submit that D-gal. achieve remarkable elevation in AChE action (Table 5) and NA level, while DA and 5-HT documented a noticeable decrease in D-gal. aged rats in comparison with the control group (Table 6). The disturbance in the antioxidant defense process and ROS extreme production are reflected as the major reasons for mitochondrial dysfunction producing intracellular destruction. Therefore, the use of antioxidants is considered as a beneficial treatment for ROS-induced brain damage [42].

After D-gal. administration, MDA and NO concentrations were elevated but TAC levels were reduced (Table 4), which agreed with the oxidative stress and related diseases produced by D-gal.. Management by the plant seed extract proved significant antioxidant effects as supported by the elevation of the antioxidant defense system, TAC, and the reduction of the oxidative stress mediated by MDA and NO. Ameliorations in antioxidant levels and the lowering effects in oxidative stress post *P. domestica* extract administration may be explained on the basis of the richness of a diversity of compounds such as quinic acids, sinapaldehyde, and many flavonoids, which are responsible for the antioxidant activity of the fruits [43,44]. Our data also declared improvements in AChE activity and neurotransmitter levels posttreatment with *P. domestica* extract. A recent study evaluated the acetylcholinesterase (AChE) and butyrylcholinesterase inhibitory activity of three *Prunus* species leaves oil while *P. domestica* leaf oil was the most active one [45]. But to our knowledge, there is no scientific research study concerned with the restorative antiaging influence of *P. domestica* seed extract.

Plenty of studies illustrated the mode of action of antiaging activity of plant extracts enriched with flavonoids. Taking tocopherols, for example, one of the isolated compounds in our study, have been acknowledged to prohibit the expression of the enzymes responsible for oxidation, for example, oxidases, cyclooxygenase, and lipoxygenase, which are extensively engaged in oxidative processes [46].

However, hesperidin acts by reducing glutathione peroxidase activity, thus leading to potential decline of total reactive antioxidants [47]. In addition, myricitrin and quercetin glycoside derivatives possess antioxidative, anti-inflammatory, and neuroprotective activities [48] through improving mitochondria malfunction and restoring the mitochondrial impairment besides their prophylactic action on DNA neurotoxicity [49]. Moreover, many flavonoids exert their antioxidant and neurodegenerative effects by way of MDA reduction and elevation of superoxide dismutase and glutathione levels [3]. Nevertheless, they play a crucial role in ceasing the accumulation of Aβ plaque accumulation [50].

It is noteworthy that stilbenes exhibit neuroprotection by effectively reducing the expression of proinflammatory cytokines tumor necrosis factor-α and interleukin-1β [51]. However, the phenolic acid mechanism of action is by means of augmenting the antioxidant action by reducing oxidative stress and increasing the inflammatory status by decreasing proinflammatory parameters [52].

Conclusion

This study affirmed the curative antiaging effect of *P. domestica* in D-gal-induced rats, suggesting that plum seed extracts could be tolerated as promising antiaging agents, and this could be attributed to the richness of the extract in a diversity of phenolics and flavonoids. Also, the extract's safety profile encourages further biological and chemical studies.

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Conflicts of interest

There are no conflicts of interest.

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