Phytochemical profiling of three Egyptian *Phoenix dactylifera* L. cultivars' leaves with comparative positive impacts in controlling the streptozotocin diabetic rats

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Background
Date palm, *Phoenix dactylifera* L., is a tropical plant of the Arecaceae family. No sufficient studies have been directed to evaluate leaves' phytochemistry and bioactivity.
Objective
The major aim of this study is to assess the phytochemical configuration and antidiabetic effect of palm leaf extracts cultivars.
Materials and methods
It included total phenolic and flavonoid contents determination, ultra-performance liquid chromatography coupled with mass analysis, and isolation of the main compounds while, the biological investigation targeted *in vitro* bioactivities and detailed *in vivo* antidiabetic activity. Animals were inducted with streptozotocin dose (40 mg/kg b.wt) and treated with different extracts dose (200 mg/kg b.wt).
Results and conclusion
Phenolic and flavonoid contents in three cultivars of Egyptian date palm leaves, Samani, Hayani, and Zaghloul were 545.652, 90.609, and 2231.304 mg GAE/g for phenolics, and 535.63, 118.00, and 2185.00 mg CE/g for flavonoids, respectively. Additionally, UPLC-QTOF-MS/MS elicited the recognition of 28, 27, and 40 phytoconstituents in three extracts, respectively. However, Zaghloul extract exhibited higher *in vitro* antioxidant activity compared with vitamin C. *In vivo* treatment with the extracts in contrast to glibenclamide as a standard drug for 4 weeks caused a significant glucose level decrease by 64.81, 65.61, 69.61, and 66.87%, respectively. The tested extracts showed significant *in-vitro* and *in-vivo* anti-diabetic activity and ameliorated the other accompanying diabetes parameters e.g. liver and kidney. Histopathological investigation showed that treatment with Zaghloul leaf extract has better results than reference drug and nearly similar effects for other leaf extracts.

Keywords:
antidiabetic, antihyperlipidemic, antioxidant, flavonoids, Phoenix dactylifera

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Introduction

Date palm, Latin name *Phoenix dactylifera* L. (*P. dactylifera* L), is a palm tree that belongs to the Arecaceae family. Their original habitat is in the hot and dry regions: Southern Asia including Saudi Arabia and North Africa as in Egypt [1]. *Phoenix* genus includes about 14 species where the name *dactylifera* states the formed fruit clusters meaning 'finger-bearing' [2]. Dates are nutritious diets since they include a wealth of phytonutrients, vitamins, and minerals [3]. Yearly, the accumulation of date palm fruits causes the annual generation of a massive amount of palm leaves. Although there are many different date palm cultivars, only a limited number of them have been studied in terms of chemical composition and nutritious benefits [4]. Some recent studies highlighted *P. dactylifera* L. and the therapeutic activities of the plant. A study evaluated the plant seeds aqueous extract on melanogenesis where intracellular reactive oxygen species level decreased and the expressions of melanocortin 1 receptor in addition to other inflammatory markers [5]. In Arabic folk medicine, *P. dactylifera* L. seeds were consumed as a diabetes remedy [6]. On the other hand, the seeds have been stated to contain a diversity of phenolics and flavonoids that are responsible for their antioxidant activities [7]. Furthermore, seeds showed potential protective effects against some serious diabetic complications in the liver and kidney[6].

Type 2 diabetes mellitus (T2DM) is commonly related to obesity, resulting in a clustering of interrelated biochemical and metabolic variables shown as hyperglycemia, central obesity, hypercholesterolemia, and elevated triglycerides (TG) [8]. The development of T2DM with obesity and streptozotocin (STZ) induction depends on mimicking the natural pathologic phases of diabetes through guiding the model that is brought by feeding rats a high-fat diet, based on the hypothesis that obesity is characterized as a low constant inflammation phase, which can lead to the propagation of insulin resistance, ending to diabetes.T2DM and hyperlipidemia are commonly managed by taking both anti-diabetic and hypolipidemic drugs at the same time. Glibenclamide is successful hypoglycemic treatment for T2DM, whereas fenofibrate is broadly used as a lipid control drug [9].

The major study objective is the assessment of the chemical composition (especially phenolic) of Samani, Hayani, and Zaghloul palm leaf extracts, which are date palm cultivars growing in Egypt, in the management of T2DM, as well as other metabolic consequences, which could spot the light on upgrading a safe and efficient natural antidiabetic source.

Materials and methods

Phytochemical investigation

Chemicals and reagents

Altogether chemicals as well as solvents were of analytical grade: Merck and Co., Inc., USA and Sigma-Aldrich, USA products.

Plant material and extraction

The fresh leaf of the three Egyptian *P. dactylifera* L. cultivars; Samani, Hayani, and Zaghloul date palm were collected in 2020 from a private farm 60 km. at Alexandria-Cairo, desert road, Egypt identification of the three leaves cultivars was performed by Prof. Dr. Abdelhalim Muhamed, (Flora department, National Agriculture Centre, Egypt). The original specimens are given voucher specimens: (JSAP-2019 a), (JSAP-2019 b), and (JSAP-2019 c), respectively. Each species was separately dried in shades, grinded, and kept in closed containers. The dried powdered leaves (500 g) of Samani, Hayani, and Zaghloul date palm were separately extracted with 80% methyl alcohol several times till complete extraction. The three total extracts were separately prepared under reduced pressure at 45°C using the rotary evaporator and kept in refrigerator for comparative chemical and biological studies.

Total phenolic and flavonoid content evaluation

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) [10]. Additionally, the total flavonoid content was assigned as stated by [11] and expressed as catechin equivalent (μg catechin/g extract). The analysis was based on colorimetric assay where flavonoids and phenolics were complexed with specific reagents and the percentage was calculated with the previously mentioned references to a standard calibration curve.

UPLC-QTOF-MS/MS analysis

The analysis was processed at 57357 hospital metabolomics unit, El Sayeda Zeinab, Cairo Governorate. UPLC-QTOF-MS/MS positive ion mode was resolved using a Vendor/ Specs triple quadruple instrument. Analyst TF 1.7.1 software was utilized for peaks and spectra interpretation where tentative identification was accomplished by relating the retention time (R_t) and mass spectrum to informed data [12].

Isolation of the main compounds

The conventional column chromatography technique was applied. Elution was consecutively underdone by chloroform ($CHCl_3$) and increased polarity with ethyl acetate (EA). R_f values were used to group the related fractions that emerged from the column together. Compound 1 was isolated from chloroform: ethyl acetate (80:20 v/v), whereas compounds (2 and 3) were obtained from chloroform: ethyl acetate (40:60 v/v). On the other hand, compounds 4, 5 were obtained from fractions (30:70 v/v) and were spotted on the TLC plate using ($CHCl_3$: CH_3OH, 90:10 v/v) alongside with available flavonoid authentic flavonoids. The compounds were quite purified using preparative TLC. Several spectral techniques were employed to identify the obtained compounds; FT-IR using Perkin-Elmer 283 spectrophotometer (Germany), ^{1}H-NMR and ^{13}C-NMR analyses (JEOL EX-400 MHz and, 125 MHz spectrometers) and MS analysis using 3200 Finnigan Model spectrometer, and melting point determination using Koffler's heating stage microscope (Wagner and Munz Kofler, ID-number: 017365).

Biological investigation

In vitro antioxidant activity

The evaluation of the three leaf extracts as antioxidant was determined using α, α-diphenyl-β-picrylhydrazyl (DPPH) [13]. Percent of the DPPH inhibition recorded using following equation: (A0–A1) A0

×100: A0 =control absorbance and A1 is the extract absorbance. Readings were recorded as mg vitamin C equivalents (vitamin C)/g sample).

In vitro anti-diabetic activity

All the used kits in addition to the test enzymes; α–amylase, α-glucosidase and β-galactosidase (EC3.2.1.1, EC3.2.1.20, and EC3.2.1.23, respectively), were attained from (Sigma Chemical Company (USA), Biosystems (Spain) and Biodiagnostic (Egypt). The followed method for carbohydrate hydrolyzing enzymes determination was described in [14].

In vivo anti-diabetic activity

Animals

The experiment was underdone on 54 adult male albino, Wistar rats weighing 130±10 g. The study was authentified by the Medical Research Ethics Committee following the Guide for the Care and Use of Laboratory Animals [15]. They were accommodated in laboratory for a week.

Induction of T2DM and experimental design

Induction of type 2 DM was achieved through the administration of a high-fat diet (HFD), by addition of lard fat to the normal diet (20%) besides, administration of cholesterol orally at a dose of 30 mg/0.3 ml olive oil/kg of animal body weight (b. wt.) five consecutive times/week. After 12 weeks, rats were injected with a single intra-peritoneal STZ dose (40 mg/kg b.wt., dissolved in 0.01 M citrate buffer, pH 4.5). Two hours later, they were supplied with 40% glucose solution to guard against possible hypoglycaemia, and 5% glucose solution *ad libitum* at night [16]. Post three days of injection, rats were checked for hyperglycemia (≥250 mg/dl is considered diabetic). Animals were distributed to nine groups (6 rats/group). Group I: assisted as control where rats were fed with normal nutrition. Groups: 2 (Normal-Samani extract), 3 (Normal-Hayani extract), and 4 (Normal- Zaghloul extract), separately, received orally normal diet and were given a daily oral dose of each extract (200 mg/kg b.wt), correspondingly, per diem for 4 weeks. Group 5 (HFD-STZ diseased group) received high-fat regimen and a single STZ injection intra-peritoneally. Groups 6–8: were classified according to the following order: (HFD-STZ/Samani extract), (HFD-STZ/Hayani extract), (HFD-STZ/Zaghloul extract) where, the HFD-diabetic induced rats treated with the orally administration of daily dose 200 mg/kg b.wt. of extracts, respectively, for 4 weeks. Group 9 (HFD-STZ/reference drugs) co-administered oral dose of glibenclamide (10 mg/kg b.wt) [17] and fenofibrate (50 mg/kg b.wt) day-to-day [18].

Sample preparation

Post 12 weeks of induction disease and post 4 weeks of treatment with respective diet, rats were fasted, each rat was anesthetized using diethyl ether, and blood samples were assembled by rupture of subtingual vein into clean and sterilize test tubes, centrifuged at 4000 rpm 15 min for collecting serum for biochemical examinations. Then rats were subjected to sacrifice via cervical dislocation, their livers instantly detached, washed in saline, dried on filter paper, weighed, and homogenized in 50 mM phosphate buffer, pH 7.4 using an Ultra-Turrax homogenizer where resultant homogenate (10% w/v) then centrifuged at 3,000 rpm for 15 min at 4°C. The resulting supernatant was kept at −80°C.

Biochemical analyses

It is worth mentioning that the accompanying biological parameters to diabetes including lipid profile, hepatic markers, kidney functions, and oxidative stress markers were also assigned.

Lipid profile parameters

Lipid profile valuations were piloted via estimating the serum total cholesterol (TC), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), triglycerides (TG), total lipids (TL), and phospholipids (PL) according to the method of [19,20] using kits supplied from Biodiagnostics Chemical Company, Cairo, Egypt, where: LDL in the sample precipitate with polyvinylsulphate and concentration is calculated from the difference between the (TC) and cholesterol in the supernatant after centrifugation. TG in the sample originates by the means of certain reactions and then, a colored complex that can be measured by spectrophotometer. PL are precipitated with trichloroacetic acid and oxidized to phosphate with sulphuric acid and perchloric acid. Inorganic phosphorus present as phosphate forms a complex with molybdic acid that is reduced by stannous chloride to a blue color which can be measured colorimetrically. TL Lipids react with sulphuric, phosphoric acids and vanillin to form pink-colored complex. Body weight calculation was performed by weekly body weighting using a digital scale beside blood glucose levels using rats' tail veins by a portable glucometer (Accu-Chek Active, Roche Diagnostics Ltd., Mannheim, Germany) periodically at given days post diabetes induction; (day: 0, 3, 7, 14, 21, and 28).

Hepatic markers

Serum aspartate, alanine aminotransferases (AST and ALT), and Alkaline phosphatase (ALP) were measured according to [21]. Total bilirubin were preceded as method stated by [22], using Biodiagnostics (Egypt) kits. However, γ-Glutamyltransferase (GGT) was kinetically assessed in light of [23] using Egyptian Company of Biotechnology, Egypt kits.

Kidney functions markers

Urea was colorimetrically assayed using Biodiagnostics kits (Egypt) [24]. Serum creatinine was determined according to [25].

Oxidative stress markers

Hepatic malondialdehyde (MDA) and glutathione (GSH) were assigned up to [26].

Pro-inflammatory markers

Serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were tested using enzyme-linked immunoassay (ELISA), Quantikine, R and D systems (MN, USA). Assessment of serum soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) were processed through the former ELISA tool and EIAab (USA).

Histopathological investigation

Liver and pancreas samples were preserved in 10% formalin, embedded into paraffin, beeswax tissue blocks, and cut into 4 μm thickness units, and stained with hematoxylin and eosin stain for examination using the light electric microscope [27].

Statistical analysis

Calculations were articulated as mean±SD. The outcomes were analyzed by SPSS (Windows 7, version 8, Chicago, IL) software. A computer program one-way analysis of variance (ANOVA), *post-hoc* followed by co-state computer program for different group's means evaluation. Contrasting letters were considered significant at P less than or equal to 0.05 .

Percentage of change= mean of control–mean of test/ mean of control ×100.

Percentage of improvement= mean of diabetic–mean of treatment/mean of control ×100.

Results and discussions

Phytochemical investigation

Yield percentage, quantitative total phenolics, and flavonoids evaluation

Extraction yield and the quantitative determination of the total phenolics and flavonoids are illustrated in Table 1. The comparative HPLC analysis of the three cultivars revealed that Zaghloul total leaf extract displayed the greatest phenolic content (2231.304 mg GAE/g) followed by Samani (545.652 mg GAE/g) and Hayani (90.609 mg GAE/g). On the other hand, flavonoid content in Zaghloul's total leaf extract was 2185.00 mg CE/g followed by Samani (535.63 mg CE/g) and Hayani (118 mg CE/g), separately.

UPLC-QTOF-MS/MS analysis

The analysis was performed for comparative characterization of different phytochemical classes in the total leaf extracts of the three cultivars under study.

Tables 2–4 revealed the presence of 28, 27, and 40 compounds in Samani, Hayani, and Zaghloul leaf extracts, respectively. Flavonols, flavones, flavonoid-*O*- hexoside, flavonoid-*C*- hexoside, and flavonoid di-hexoside were abundantly presented in the three cultivar total leaf extracts. On the other hand, anthocyanidin-*O*- hexoside, catechins, and coumarins are the major common classes in Samani and Hayani total leaf extracts. Nevertheless, flavanones are exclusively found in Samani, and Zaghloul leaf extracts. It is to be mentioned that isoflavonoid *C*-hexoside, isoflavones, and stilbenes existed in Samani leaf extract. While flavanonol, isoflavonoid *C*-glycosides, alkaloids, *O*-methylated flavonoids, phenolics, stilbenes, bicyclic monoterpenoids were presented in Zaghloul leaf extract.

Structural elucidation of the isolated compounds from Zaghloul leaf extract

Compound 1(Lutein): It was isolated as an orange amorphous powder from a fraction (CH_3Cl: EtOAc, 80:20), with a melting point of 184°C. R_f= 0.70. Mass spectrum (ESI): M^+ 568 for molecular formula $C_{40}H_{56}O_2$, other distinctive fragments were at m/z 550 for [M- H_2O], 429 for [550-C_9H_{12}] [28]. In addition, *m/z* 135 product ion corresponds to the endpoint ring dehydration due to ring A cleavage between 7 and 8 carbons. Furthermore, *m/z* 153 ion peak appeared upon carbons 7' and 8' cleavage of B-ring including the hydroxyl group, while peaks at *m/z* $[M-92]^+$ and $[M-106]^+$expressed side chain conjugated

Table 1 Extraction yield, total phenolics, and flavonoids in three *P. dactylifera* L. leaf extracts

Total leaf extract	Extraction yield %	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)
Samani	28	545.652	535.630
Hayani	20	90.6090	118.000
Zaghloul	34	2231.304	2185.00

Table 2 Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) results of the principal phytoconstituents in Samani leaf extract

Class	Name	Rt (min)	$[M+H]^+$	Molecular formula	Fragments (m/z)
Flavonols	Taxifolin	1.40	305	$C_{15}H_{12}O_7$	107, 184, 305
	Methoxy-trihydroxyflavonol	7.19	317	$C_{16}H_{12}O_7$	153, 251, 229, 274
	Quercetin	7.02	303	$C_{15}H_{10}O_7$	153, 229, 257
Flavanones	Tetrahydroxyflavanone	14.29	289	$C_{15}H_{12}O_6$	135, 151
	Naringenin	21.4	273	$C_{15}H_{12}O_5$	91, 119, 153
Flavones	Tetrahydroxy-7-methoxyflavone	17.47	317	$C_{16}H_{12}O_7$	121, 179
	Trihydroxy-methoxyflavone	6.42	301	$C_{16}H_{12}O_6$	151,252,285
	Myricetin	9.21	319	$C_{15}H_{10}O_8$	153, 217, 245
	Luteolin	7.32	287	$C_{15}H_{10}O_6$	153, 287
Isoflavones	Genistein	5.07	271	$C_{15}H_{10}O_5$	153, 215, 253
Flavonoid-*O*- hexoside	Isorhamnetin 3-*O*-hexoside	6.52	479	$C_{22}H_{22}O_{12}$	285, 302, 317
	Isorhamnetin 3-*O*-rutinoside	7.8	625	$C_{28}H_{32}O_{16}$	317, 479
	Diosmin (diosmetin 7-*O*-rutinoside)	6.58	609	$C_{28}H_{32}O_{15}$	313, 343, 367, 397, 409, 427, 445, 609
	Baicalein –*O*-glucuronide	5.6	447	$C_{21}H_{18}O_{11}$	341, 311, 269, 175
	Rutin (quercetin 3-*O*-rutinoside)	6.73	611	$C_{27}H_{30}O_{16}$	303, 354, 503, 465
Flavonoid-*C*-hexoside	Luteolin –*C*-hexoside	6.18	449	$C_{21}H_{20}O_{11}$	299, 311, 325, 329, 339, 353, 395, 413, 431
	Apigenin –*C*-hexoside	11.50	433	$C_{21}H_{20}O_{10}$	283, 312
	Vitexin-*O*-hexoside	6.3	579	$C_{27}H_{30}O_{14}$	313, 337, 367, 415, 433
Flavonoid-dihexoside	Luteolin –di-*O*-hexoside	5.7	611	$C_{27}H_{30}O_{16}$	278, 449
Anthocyanidin-*O*- hexoside	Cyanidin –*O*- hexoside	7.68	499	$C_{21}H_{21}O_{11}$	287, 499
	Cyanidin-*O*-rutinoside	6.62	211	$C_{12}H_{18}O_3$	109, 133, 151, 193
Catechin	Catechin	4.74	291	$C_{15}H_{14}O_6$	123, 139, 147, 165, 291
Coumarins	Scopoletin	9.88	193	$C_{10}H_8O_{44}$	193
Methylated flavonoids	Dihydroxy-methoxyflavone	13.76	287	$C_{16}H_{14}O_5$	147, 167, 287
	Hesperetin	5.55	303	$C_{16}H_{14}O_6$	153,177
Isoflavonoid *C*- hexoside	Daidzein –*C*-hexoside	11.9	417	$C_{21}H_{20}O_9$	133, 194, 223, 267
	Acacetin	14.2	285	$C_{16}H_{12}O_5$	151, 211, 239
Stilbenes	Resveratrol	6.35	229	$C_{14}H_{12}O_3$	95, 131, 229, 229

Table 3 Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) results of the principal phytoconstituents in Hayani leaf extract

Class	Name	Rt (min)	$[M+H]^+$	Molecular formula	Fragments (m/z)
Flavonols	Methoxy-trihydroxyflavonol	7.435	317.0635	$C_{16}H_{12}O_7$	153, 229, 246, 274, 285, 302, 317
	Quercetin	6.683	303	$C_{15}H_{10}O_7$	153, 229, 257
	Isorhamnetin (3'-methoxyquercetin)	17.185	317	$C_{16}H_{12}O_7$	285, 245, 229
Flavones	Trihydroxy-4'-methoxyflavone	20.196	301	$C_{16}H_{12}O_6$	181, 149, 301
	Luteolin	7.264	287	$C_{15}H_{10}O_6$	153, 287
	Myricetin	9.87	319	$C_{15}H_{10}O_8$	153, 217, 245
	Trihydroxy-methoxy-flavone triacetate	5.097	427	$C_{22}H_{18}O_9$	301, 343, 385
	Hydroxy-methoxyflavone	15.650	269	$C_{16}H_{12}O_4$	226, 237, 254
Flavonoid-*O*- hexoside	Isoquercitrin (quercetin *O*-hexoside)	7.040	465	$C_{21}H_{20}O_{12}$	127, 145, 303,465
	Isorhamnetin 3-*O*-hexoside	7.840	479	$C_{22}H_{22}O_{12}$	285, 302, 317
	Rutin (quercetin 3-*O*-rutinoside)	6.708	611	$C_{27}H_{30}O_{16}$	303, 354, 503, 465
	Kaempferol –*O*-hexoside	6.017	449	$C_{21}H_{20}O_{11}$	121, 153, 287
	Kaempferitrin (Kaempferol 3,7-*O*-dihexoside)	6.033	579	$C_{27}H_{30}O_{14}$	287, 433, 579
	Querciturone (quercetin 3-*O*-glucuronoide)	7.402	479	$C_{21}H_{18}O_{13}$	153, 229, 257, 303
	Vitexin- *O*-hexoside (2-*O*-Rhamnosylvitexin)	6.623	579	$C_{27}H_{30}O_{14}$	283, 313, 337, 367, 379, 397, 415, 433, 579
Flavonoid *C*- hexoside	Luteolin –*C*-hexoside	6.077	449	$C_{21}H_{20}O_{11}$	

(*Continued*)

Table 3 (*Continued*)

Class	Name	Rt (min)	$[M+H]^{+}$	Molecular formula	Fragments (m/z)
					299, 311, 325, 329, 339, 353, 395, 413, 431
	Apigenin –*C*-hexoside	7.490	433	$C_{21}H_{20}O_{10}$	283, 309, 313, 323, 337, 349, 367, 379, 397, 415, 433
	Orientin (luteolin 8-*C*-hexoside)	7.589	449	$C_{21}H_{20}O_{11}$	299, 329, 413
Flavonoid-dihexoside	Quercetin 3-*O*-neohesperidoside 7-*O*-hexoside	7.998	757	$C_{33}H_{40}O_{20}$	302, 611,449
Anthocyanidin-*O*-hexoside	Cyanidin –*O*-rutinoside	6.732	595	$C_{27}H_{31}O_{15}$	287, 449, 595
	Cyanidin –*O*- hexoside	7.668	499	$C_{21}H_{21}O_{11}$	287, 499
	Malvidin -*O*-hexoside	8.066	493	$C_{23}H_{25}O_{12}$	287, 315, 332
Catechin	Catechin	4.737	291	$C_{15}H_{14}O_6$	123, 139, 147, 165, 291
	Pentahydroxyflavan	5.5687	291	$C_{15}H_{14}O_6$	123, 139, 147, 165, 207, 291
Coumarin	Dimethoxy-methyl coumarin	1.315	221	$C_{12}H_{12}O_4$	121, 135, 163, 178
Anthraquinone	Hydroxyanthraquinone	13.780	225	$C_{14}H_8O_3$	105, 153, 197
Phenolic acids	Caffeic acid	13.054	181	$C_9H_8O_4$	117, 145, 163

Table 4 Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS) results of the principal phytoconstituents in Zaghloul leaf extract

Class	Name	Rt (min)	$[M+H]^{+}$	Molecular formula	Fragments (m/z)
Flavonols	Quercetin	7.017	303	$C_{15}H_{10}O_7$	137, 153, 201, 229, 303
	Methoxy-trihydroxyflavonol	7.400	317	$C_{16}H_{12}O_7$	153,179, 215, 273, 302, 317
Flavanonol	Taxifolin (dihydroquercetin)	1.809	305	$C_{15}H_{12}O_7$	176, 184, 215 305,358
Flavanones	3', 4', 5, 7-tetrahydroxyflavanone	1.815	289	$C_{15}H_{12}O_6$	174, 289
Flavones	3, 3', 4', 5-tetrahydroxy-7-methoxyflavone	1.5063	317	$C_{16}H_{12}O_7$	179, 215, 317
	Luteolin	7.264	287	$C_{15}H_{10}O_6$	153, 287
	Trihydroxy-methoxyflavone	10.92133	301	$C_{16}H_{12}O_6$	72,137,143, 301
Flavonoid-*O*-hexoside	Kaempferol 3-*O*-rutinoside	6.070	595	$C_{27}H_{30}O_{15}$	299, 329, 353, 383, 395, 413, 431, 449, 595
	Isorhamnetin 3-*O*-hexoside	7.770	479	$C_{22}H_{22}O_{12}$	317, 479
	Baicalin (baicalein 7-*O*-glucuronide)	1.888	447	$C_{21}H_{18}O_{11}$	271, 447
	Linarin (acacetin 7-*O*-rutinoside)	3.877	593	$C_{28}H_{32}O_{14}$	147, 285, 593
	Gossypin (gossypetin 8-*O*-hexoside)	5.813	481	$C_{21}H_{20}O_{13}$	244, 260, 317, 318, 319, 481
	Rutin (quercetin 3-*O*-rutinoside)	6.641	611	$C_{27}H_{30}O_{16}$	303, 354, 503, 465
	Diosmin (diosmetin 7-*O*-rutinoside)	6.864	609	$C_{28}H_{32}O_{15}$	313, 343, 367, 397, 409, 427, 445, 609
	Rhoifolin (apigenin 7-*O*-rhamnohexoside)	7.580	579	$C_{27}H_{30}O_{14}$	271, 579
	Vitexin –*O*-hexoside	8.587	579	$C_{27}H_{30}O_{14}$	283, 313, 337, 379, 397, 415, 433, 579
	Luteolin –*O*-hexoside	9.271	449	$C_{21}H_{20}O_{11}$	153, 180, 207, 223, 287, 329, 431, 449
	Quercetin –*O*-hexoside	11.795	465	$C_{21}H_{20}O_{12}$	344, 448, 465
Flavonoid-*C*- hexoside	Luteolin –*C*-hexoside	6.044	449	$C_{21}H_{20}O_{11}$	299, 325, 329, 339, 353, 365, 377, 383, 395, 413, 431, 449
	Hyperoside (quercetin-*O*-hexoside)	7.017	465	$C_{21}H_{20}O_{12}$	303, 465
	Apigenin –*C*-hexoside	6.801	433	$C_{21}H_{20}O_{10}$	283, 309, 313, 323, 349, 361, 397, 415
	Luteolin-*C*-hexoside	10.177	449	$C_{21}H_{20}O_{11}$	299, 383, 449
Flavonoid-di hexoside	Apigenin –*C*-hexoside –*O*-hexoside	9.927	595	$C_{27}H_{30}O_{15}$	191, 211, 413, 457, 577, 595
Anthocyanidin-*O*-hexoside	Cyanidin –*O*-rutinoside	6.915	595	$C_{27}H_{31}O_{15}$	287, 449, 595
	Malvidin –*O*-hexoside	7.311	493	$C_{23}H_{25}O_{12}$	331, 493
	Petunidin –*O*- hexoside	7.342	479	$C_{22}H_{23}O_{12}$	208, 210, 317, 479
	Cyanidin –*O*-hexoside	7.639	449	$C_{21}H_{21}O_{11}$	287, 449
	Cyanidin di-*O*-hexoside	5.955	611	$C_{27}H_{31}O_{16}$	287, 299, 329, 431, 449, 611
Coumarins	Scopoletin	9.94	193	$C_{10}H_8O_{44}$	193
	Esculin	6.314	341	$C_{15}H_{16}O_9$	135, 340

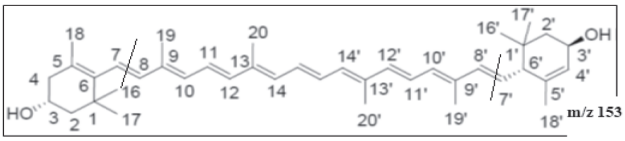
(*Continued*)

Table 4 (*Continued*)

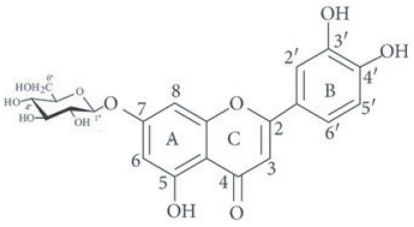
Class	Name	Rt (min)	$[M+H]^+$	Molecular formula	Fragments (m/z)
Isoflavonoid *C*- hexoside	Daidzein –*C*-hexoside	1.455	417	$C_{21}H_{20}O_9$	155,167, 181, 417
Methyl isoflavones	Formononetin	6.915	269	$C_{16}H_{12}O_4$	269
Methylated flavonoids	Dihydroxy-methoxyflavone	1.429	287	$C_{16}H_{14}O_5$	125, 166, 286
Alkaloids	Harmaline	2.049	215	$C_{13}H_{14}N_2O$	214, 215
	Methylxanthine	4.430	167	$C_6H_6N_4O_2$	123, 143, 149, 167, 287
	Caffeine	4.922	195	$C_8H_{10}N_4O_2$	136, 195
Stilbenes	Resveratrol	6.301	229	$C_{14}H_{12}O_3$	95, 131, 229, 229
Bicyclic monoterpenoids	Sabinene	2.223	137	$C_{10}H_{16}$	66, 81, 122, 137
Phenolics	*p*-Coumaric acid (4'-Hydroxycinnamic acid)	7.868	165	$C_9H_8O_3$	78, 83, 117, 120, 149, 165
	Sinapyl aldehyde	8.482	209	$C_{11}H_{12}O_4$	55, 78, 89, 103, 110, 121, 130, 139, 149, 162, 191

system [29] as illustrated in Fig. S1, supplementary material. ^{1}H-NMR (400 MHz, $CDCl_3$, δ) 6.60–6.55 (m, 4H, H-11, 11', 15, 15'), 6.31 (d, 2H, H-12, 12'), 6.24 (m, 2H, H-14, 14'), 6.09–5.98 (m, 5H, H-7, 8, 8', 10, 10'), 5.55 (s,1H, H-4'), 5.41 (dd,1H, H-7'), 4.20 (m, 1H, H-3'), 4.00 (m, 1H, H-3), 2.42 (d,1H, H-6'), 2.34–2.27 (m, 2H, H-4), 1.95–1.92 (s,6H, H-19, 19'), 1.90 (br s, 6H, H-20, 20'), 1.83 (dd, 1H, H-2'), 1.71–1.68 (s, 6H, H-18, 18'), 1.47 (m, 1H, H-2), 1.41–1.35 (dd, 2H, H-2'), 0.82 (s, 3H, H-17'). ^{13}C-NMR (125 MHz, $CDCl_3$, δ):139.1 (C-8), 138.2 (C-8'), 137.5 (C-5'), 136.9 (C-6),136.6 (C-12, 12',), 136.3 (C-13, 13'), 136.1 (C-9, 9'), 133.5 (C-14, 14'), 132.0 (C-10), 130.9 (C-10'), 130.0 (C-15, 15'), 127.2 (C-7'), 126.4 (C-5), 125.8 (C-7), 122.5 (C-4'), 124.1 (C-11), 123.8 (C-11'), 66.1 (C-3'), 65.6 (C-3), 55.4 (C-6'), 48.7 (C-2), 43.9 (C-2'), 42.7 (C-4), 38.1 (C-1), 34.3 (C-1'), 30.8 (C-17), 29.7 (C-16'), 27.8 (C-16), 23.3 (C-17'), 22.6 (C-18'), 22.1 (C-18), 13.5 (C-19,19'), 12.5 (C-20, 20')

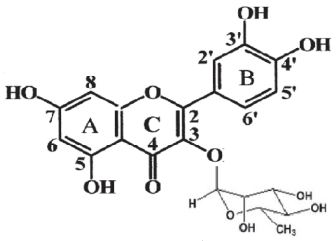
Figure S1



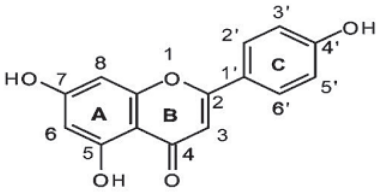
Compound 1 (Lutein)



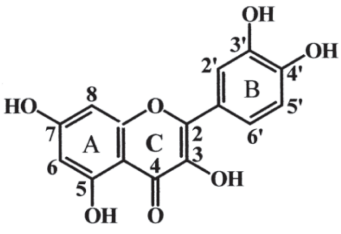
Compound 2 (luteolin 7-O-glucoside)



Compound 3 (Quercetrin)



Compound 4 (Apigenin)



Compound 5 (Quercetin)

Structures of isolated compounds.

Compound 2 (luteolin 7-O-glucoside): was obtained as a yellow amorphous powder from fraction (CH_3Cl: EA, 40:60). Its melting point is 257°C. R_f=0.66. IR (KBr, cm^{-1}) showed one broad peak at 3370 (OH group) and at 1655 (C=O), beside to two bands at 1580 and 1561 (C=C). ^{1}H-NMR (400 MHz, CD_3OD, δ) 12.72 (s, 1H, OH-5), 7.38 (dd,1H, H-6′), 7.30 (d, 1H, H-2′), 6.89 (d,1H, H-5′), 6.78 (d,1H, H-8), 6.70 (s, 1H, H-3), 6.43 (d,1H, H-6), 5.00 (d, 1H, H-1′′), 3.70-3.19 attributable to a sugar moiety. ^{13}C-NMR (125 MHz, CD_3OD, δ) 180.5 (C4), 165.2 (C-2), 160.3 (C-7), 159.0 (C-5), 155.3 (C-9), 150.2 (C-4′), 144.5 (C-3′), 120.4 (C-1′), 119.2 (C-6′), 116.2 (C-5′), 111.5 (C-2′), 105.8 (C-10), 102.7 (C-3), 98.8 (C-1′′), 97.1 (C6), 95.5 (C-8), 74.0 (C-5′′), 76.5 (C-3′′), 72.0 (C-2′′), 68.2 (C-4′′), 59.7 (C-6′′). The above spectral analyses confirmed the structure of luteolin 7-*O*-hexoside by comparing with the literature [30].

Compound 3 (Quercetrin; Quercetin 3-O- rhamnoside): was isolated as a yellow powder from fraction (CH_3Cl: EA, 40:60), melting point 182°C. R_f=0.60. IR (KBr, cm^{-1}) showed strong absorption band at 3420 (OH group) and at 2975, (C-H), 1725(C=O) and peak at 1470 (C=C), ^{1}H-NMR (400 MHz, CD_3OD, δ) 7.64 (dd, 1H, H-6′), 7.53 (d, 1H, H-2′), 6.61 (d,1H, H-5′), 6.30 (d,1H, H-8), 6.23 (d,1H, H-6), 5.12 (d, 1H, H-10′), 3.94–3.18 (attributable to a sugar moiety), 0.99 (d,3H, H-6′′).^{13}C-NMR (125 MHz, CD_3OD, δ) 178.3 (C-4), 166.4 (C-7), 161.2 (C-5), 157.8 (C-9), 154.7 (C-2), 151.3 (C-4′), 144.27 (C-3′), 135.2 (C-3), 121.5 (C-1′), 120.5 (C-6′), 118.3 (C-2′), 114.3 (C-5′), 104.9 (C-10), 102.7 (C-1′′), 98.5 (C6), 94.1 (C-8), 78.1 (C-5′′), 77.4 (C-2′′), 72.1 (C-3′′), 69.3 (C-4′′), 67.5 (C-6′′). The above spectral analyses confirmed the structure of quercetin-3-*O*-hexoside by comparing it with the literature [31,32].

Additionally, by comparing the chromatographic profile of compounds 4 and 5 with available authentic and reviewing published data, they were identified as apigenin (R_f 0.87), and quercetin (R_f 0.55) in $CHCl_3$: CH_3OH (9:1 v/v) [33]. It is to be mentioned that compound 1 was previously isolated from *P. dactylifera* fruits by [34] and identified in *P. dactylifera* leaf using LC–MS by [35] while, compounds 2 and 3 were isolated for the first time from *P. dactylifera* leaf in this study. compounds 4 and 5 were previously isolated from *P. dactylifera* seeds by [36].

Biological investigation

In vitro antioxidant assay

DPPH inhibition activity assay revealed that the three total leaf extracts proved significant antioxidant effect in a dose dependent manner. However, Zaghloul leaf extract exhibited relatively higher antioxidant activity (72.73±0.21% at concentration 0.05 µg/ml) compared with Vitamin C as demonstrated in Table 5. The highest antioxidant activity of Zaghloul total leaf extract could be accredited to the high abundance of phenolic and flavonoid contents (2231.304 mg GAE/g and 2185.00 mg CE/g), separately.

In vitro antidiabetic activity

In vitro antidiabetic activity of the three extracts has been tested using acarbose as standard antidiabetic drug. The results are illustrated in Table 6, where they showed considerable percentages of inhibitory activity of carbohydrate metabolizing enzymes compared with standard acrobase. However, Zaghloul and Hayani leaf extracts declared similar activity.

In vivo anti-diabetic activity

Rats showed a significant b.wt. increase after (HFD) and oral cholesterol regimen, when compared with a normally fed control group with a weight increase of 114%. However standard fed rats increased by 24.15%. Meanwhile, the introduction of the three total extracts along with HFD-STZ caused a significant reduction as shown in Table 7. Relatively, data from Table 8 represented fasting blood glucose (FBG) levels, wherever diabetic rats established FBG significant increase (276.91%) comparing to a normal control group. Treatment with different extracts in combination of glibenclamide and fenofibrate caused significant decreases by 64.81, 65.61, 69.61, and 66.87%, respectively.

Lipid profile parameters

Serum lipids profile showed that HFD-STZ diabetic group displayed a significant serum altitude in TC, LDL-C, TG and TL obtaining 177.75, 525.78, 311.100, and 240.23%), respectively, while serum HDL-C possessed significant decline by a value of

Table 5 DPPH scavenging activity of the three *P. dactylifera* leaf extracts

	Diphenyl-β-picrylhydrazyl scavenging activity percentage	
Total leaf extract	0.01 µg/ml	0.05 µg /ml
Samani	46.01±0.73	59.08±0.52
Hayani	43.43±2.21	69.19±3.63
Zaghloul	53.54±2.21	72.73±0.21
Vitamin C	81.00±0.82	89.00±0.10

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

Table 6 Inhibitory activity of carbohydrate metabolizing enzymes of the three *P. dactylifera* cultivars' leaf extracts

Groups	α-amylase				α-glucosidase				β- galactosidas			
Concentration	Acarbose	Samani	Hayani	Zaghloul	Acarbose	Samani	Hayani	Zaghloul	Acarbose	Samani	Hayani	Zaghloul
10 µg/ml	36.181±1.19^{a}	10.00±1.22^{b}	14.00±1.00^{c}	15.10±1.00^{c}	46.50±2.19^{d}	6.37±0.32^{b}	11.00±1.11^{b}	16.00±1.00^{c}	60.00±9.10^{f}	10.20±1.00^{b}	20.00±1.12^{g}	22.05±1.53^{g}
50 µg/ml	40.10± 2.66^{d}	12.15±1.13^{b}	16.10±1.22^{c}	17.13± 1.00^{c}	70.220±5.12^{h}	9.00±1.08^{b}	14.00±1.11^{c}	22.30±1.32^{g}	72.00±11.00^{h}	22.00±0.52^{g}	26.00±0.98^{g}	30.00±2.00^{a}
100 µg/ml	49.70±3.20^{d}	18.00±1.00^{c}	30.00±2.00^{a}	34.10± 1.20^{a}	82.23±9.11^{j}	13.00±0.21^{c}	23.00±1.23^{g}	30.55±2.20^{a}	79.30±10.20^{j}	25.00±1.30^{g}	33. 0±2.00^{a}	37.20±2.22^{a}
500 µg/ml	59.54±3.29^{f}	22.19±2.00^{g}	36.00±2.30^{a}	40.00± 2.89^{d}	89.33±6.00^{l}	20.00±1.34^{g}	30.10±2.23^{a}	33.50±2.01^{a}	85.20±9.67^{l}	30.20±2.51^{a}	35.65±2.00^{a}	39.00±2.11^{a}
1000 µg/ml	75.00±4.54^{h}	26.00±2.00^{a}	41.33±3.34^{d}	46.07± 3.51^{d}	93.10±8.11^{l}	25.10±2.27^{g}	34.00±3.20^{a}	42.00±3.12^{d}	90.40±11.98^{l}	33.10±1.23^{a}	42.12±2.10^{d}	47.00±2.45^{d}
LSD 5%	5.22	5.18	5.80	5.90	5.90	6.1	5.93	5.57	5.90	5.80	6.1	5.93

Enzymes are expressed as %. Data are mean±SD of 3 replicates. Statistical analysis is carried out using one way analysis of variance (ANOVA), combined with post hoc and Co-Stat computer programs, where different superscript letters between groups are significant differences at *P* values less than 0.05. Enzymes are expressed as %. Data are mean±SD of 3 replicates. Statistical analysis is carried out using one way analysis of variance (ANOVA), combined with post hoc and Co-Stat computer program.

Table 7 Effect of three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on body weight (g)

Groups	Initial weight	Diseased group post 12 weeks	Treatment group post 4 weeks
Control	128.7 ±3.30^{a}	159.78±5.00^{b}	171.60±2.89^{b}
Normal/ Samani	130.0 ±2.98^{a}	162.00±4.09^{b}	169.22±4.61^{b}
Normal/ Hayani	125.7 ±4.30^{a}	163.18±6.00^{b}	169.67±5.92^{b}
Normal/ Zaghloul	126.0 ±4.43^{a}	157.67±3.32^{b}	168.98±8.90 ^{b}
HFD-STZ	130.45 ±2.30^{a}	278.80±11. 45^{c}	285.45±6.89^{c}
HFD-STZ/ Samani	127.0 ±1.00^{a}	275.90±10.00^{c}	189.12±11.87^{b}
HFD-STZ/ Hayani	127.67 ±2.20^{a}	276.32±9.89^{c}	180.22±10.87^{b}
HFD-STZ/ Zaghloul	129.0 ±3.00^{a}	274.87±12.76^{c}	176.98±9.12^{b}
HFD-STZ/ Standard drug	127.90 ±2.00^{a}	276.80±11.21^{c}	176.00±88^{b}

Values are expressed as mean±SD, (*n*=6). Statistical analysis is carried out using one way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at *P* less than or equal to 0.05.

54.33% (Table 9). Treatment of diabetic rats with Samani, Hayani, and Zaghloul extracts triggered significant reduction in serum values by 47.10, 39.00, 50.00, and 56.80%, correspondingly, for Samani; 52.39, 41.00, 53.70, and 59.10%, respectively, for Hayani extract and 60.33, 51.20, 66.87, and 60.43% when compared with the untreated diseased group. Concerning HDL-c, Zaghloul extract recorded marked elevation of 71.65%. Relatively, reference treatment combination (glibenclamide and fenofibrate) produced a trivial increment in serum HDL (42.74%).

Table 8 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on fasting blood glucose level (mg/dl)

Groups	72 Hours post induction	Post 4 weeks of treatment
Control	89.00±1.30^{a}	92.09±6.20^{a}
Normal/Samani	90.0±2.00^{a}	89.50±8.00^{a}
Normal/Hayani	102.70±2.00^{a}	98.20±5.43^{a}
Normal/Zaghloul	100.0±7.00^{a}	97.21±6.30^{a}
HFD-STZ	335.45±3.00^{b}	378.00±12. 00 ^{c}
HFD-STZ/Samani	327.0±4.66^{b}	133.00±9.20^{c}
HFD-STZ/Hayani	321.60±6.20^{b}	130.60±8.30^{c}
HFD-STZ/Zaghloul	335.0±9.00^{b}	114.87±10.11^{c}
HFD-STZ/Standard drug	334.90±11.00^{b}	125.22±9.20^{c}

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at *P* less than or equal to 0.05.

Table 9 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on lipid profile level

Groups	Total cholesterol (TC, mg/dl)	Low-density lipoprotein (LDL-C, mg/dl)	High-density lipoprotein HDL-C (mg/dl)	Triglycerides (TG, mg/dl)	Total lipids (TL, mg/dl)
Control	87.13±4.11[a]	15.98±1.77[a]	75.00±3.55[a]	29.30±1.09[a]	200.10±9.80[a]
Normal/Samani	85.77±3.00[a]	17.00±1.32[a]	74.00±3.22[a]	28.13±1.80[a]	198.20±11.30[a]
Normal/Hayani	82.99±4.99[a]	17.90±2.00[a]	72.52±4.00[a]	26.22±2.00[a]	190.66±8.90[a]
Normal/Zaghloul	88.76±6.21[a]	17.00±1.30[a]	72.89±6.00[a]	24.10±2.87[a]	185.90±12.90[a]
HFD-STZ	242.00±9.77[a]	100.0±11.80[b]	34.25±2.12[b]	120.44±4.88 [b]	680.80±20.32[b]
HFD-STZ/Samani	128.00±4.32[b]	61.00±4.52[c]	46.79±3.00[c]	60.22±3.21[c]	289.87±13.89[c]
HFD-STZ/Hayani	115.20±2.00[b]	59.00±3.21[c]	41.42±2.90[c]	56.00±4.65[c]	278.90±12.11[c]
HFD-STZ/Zaghloul	96.0±9.00[b]	48.80±6.10[c]	58.79±3.00[d]	39.90±2.80[a]	269.90±10.89[c]
HFD-STZ/Standard drug	95.00±10.00[b]	42.90±4.00[d]	48.89±2.14[c]	48.65±4.90[a]	258.00±9.87[c]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

Table 10 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on liver function enzymes

Groups	Serum aspartate AST (U/L)	Alanine aminotransferases ALT (U/L)	Alkaline phosphatase ALP (U/L)	Alkaline phosphatase GGT (U/L)	Total bilirubin (mg/dl)
Control	32.12±1.48[a]	39.88±4.60[a]	30.22±2.11[a]	9.31±1.00[a]	0.53±0.04[a]
Normal/Samani	34.10±1.87 [a]	40.22±4.00[a]	33.20±1.00[a]	8.39±1.20[a]	0.55±0.03[a]
Normal/Hayani	33.00±2.09[a]	34.60±2.90[a]	25.87±1.10[a]	9.00±1.06[a]	0.59±0.05[a]
Normal/Zaghloul	32.32±2.11[a]	37.87±3.87[a]	24.89±1.70[a]	9.31±1.00[a]	0.50±0.03[a]
HFD-STZ	98.40±3.21[b]	66.81±5.00[b]	89.70±3.22[b]	45.00±4.22[b]	1.55±0.22[b]
HFD-STZ/ Samani	65.10±1.99[b]	56.00±3.22[c]	47.00±3.22[c]	29.30±1.00[c]	0.82±0.09[c]
HFD-STZ/Hayani	64.17±5.90[c]	52.88±4.60[c]	46.20±2.44[c]	26.22±1.33[c]	0.74±0.05[c]
HFD-STZ/ Zaghloul	57 13±3.55[c]	49.76±3.00[c]	43.00±2.32[c]	19.98±1.00[c]	0.70±0.11[c]
HFD-STZ/ Standard drug	48.22±3.00[c]	50.10±2.60[c]	41.20±3.00[c]	22.90±2.00[c]	0.66±0.07[c]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

Hepatic markers

Concerning hepatic assessment (Table 10), the HFD-STZ induced diabetic rats depicted a significant raising in hepatic function's parameters: ALT, AST, ALP, GGT, and total bilirubin up to 206.35, 67.53, 196.82, 383.35, and 192.45%, respectively, in contrast to the control levels. Treatment with Samani, Hayani and Zaghloul extracts deduced significant reduction in all values by fluctuated percentages of reduction, while standard drugs depicted (50.00, 25.01, 54.10, 49.11, and 57.42%), respectively.

Kidney function markers

Diabetic rats showed substantial elevation in serum urea and creatinine levels recording 303.13 and 210.00%, respectively, in contrast to normal negative control. Treatment with different extracts caused significant decrease in tested values, the highest reduction was recorded for Zaghloul extract (59.100 and 60.65%) for urea and creatinine, respectively, compared with standard drug (Table 11).

Pro-inflammatory markers

In regard to the anti-inflammatory effect of the three *P. dactylifera* cultivars' extracts, Tables 12 and 13, HFD-STZ rats displayed a significant increment in TNF-α and IL-6; in percentages 147.47 and 92.86%, respectively, as compared with normal control values. HFD-STZ different extracts conducting caused serum TNF-α and IL-6 significant fall, Zaghloul extract showed the best achievement (49.55 and 40.26%, for TNF-α and IL-6) regarding the drugs.

Table 11 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on kidney function level

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	32.00±2.40[a]	0.50±0.02[a]
Normal/Samani	30.00±3.00[a]	0.43±0.02[a]
Normal/Hayani	28.88±2.76[a]	0.45±0.07[a]
Normal/Zaghloul	28.00±2.20[a]	0.55±0.06[a]
HFD-STZ	129.00±3.40[b]	1.55±0.53[b]
HFD-STZ/Samani	62.99±4.00[c]	0.82±0.12[c]
HFD-STZ/Hayani	58.22±3.22[c]	0.65±0.11[a]
HFD-STZ/Zaghloul	52.76±4.12[c]	0.61±0.04[a]
HFD-STZ/Standard drug	48.90±3.87[c]	0.62±0.05[a]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

Table 12 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on Pro-inflammatory markers

Groups	Serum tumor necrosis factor-alpha (TNF-α, Pg/ml)	Interleukin-6 (IL-6, Pg/ml)
Control	88.90±9.80[a]	98.00±8.98[a]
Normal/ Samani	80.44±6.90[a]	90.11±6.41[a]
Normal/Hayani	83.00±5.88[a]	92.30±4.43[a]
Normal/ Zaghloul	90.00±5.21[a]	89.00±6.78[a]
HFD-STZ	220.00±11.98[b]	189.00±11.90[b]
HFD-STZ/ Samani	132.00±6.76[c]	122.60±7.61[c]
HFD-STZ/ Hayani	120.00±10.45[c]	115.00±9.90[a]
HFD-STZ/ Zaghloul	111.00±8.65[a]	112.90±8.90[a]
HFD-STZ/ Standard drug	102.00±6.81[a]	110.20±10.00[a]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

HFD-STZ rats showed a significant rise in sICAM-1 and sVCAM-1 with percentages of 233.33 and 85.57%, respectively. Samani, Hayani, and Zaghloul extracts administration led to a significant alleviation in sICAM-1 and sVCAM-1 levels (31.82 and 27.28%), correspondingly. Also, standard drugs showed percentages of reduction in adhesion molecule levels reached 40.91% and 38.89%, separately, for sICAM-1 and sVCAM-1. Hence, Zaghloul extract treatment presented the superlative results, followed by Hayani and Samani related to standard drugs.

Table 13 Effect of the three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on adhesion molecules

Groups	Serum soluble intercellular adhesion molecule-1, (sICAM-1, Pg/mL)	Vascular cell adhesion molecule-1(sVCAM-1, Pg/mL)
Control	330.00±16.99[a]	9700±230.00[a]
Normal/ Samani	322.00±10.00[a]	9500±230.00[a]
Normal/ Hayani	320.00±9.00[a]	9330±220.00[a]
Normal/ Zaghloul	320.00±11.00[a]	9600±255.00[a]
HFD-STZ	1100.00±14.90[b]	18000±500.00[b]
HFD-STZ/ Samani	750.23±22.80[c]	13000±550.00[c]
HFD-STZ/ Hayani	723.00±30.21[c]	11680±430.00[a]
HFD-STZ/ Zaghloul	680.00±21.00[c]	11500±449.00[a]
HFD-STZ/ Standard drug	650.00±20.11[c]	11000±440.00[a]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

Oxidative stress markers

Additionally, the *in vivo* antioxidant study revealed that the untreated hyperlipidemic-diabetic rats depicted a significant escalation in hepatic MDA (430. 83%). However, a significant incline in hepatic GSH occurred in percentage of 68.49%. Administrating of HFD-STZ rats with different extracts caused a significant incline in MDA while noticeable elevation in GSH levels, with the preeminent results of Zaghloul extract (76.40 and 203.19%, for MDA and GSH, respectively), in respect to standard drug results; MDA (77.64%) and GSH (194.53%, Table 14).

Histopathological results

Hepatic histopathological investigation

Rat liver of control, displayed normal histological hepatocytes. Normal rats treated with different extracts showing the same observations (Photomicrographs –4). While, HFD-STZ induced rats showed infiltration of the portal area with inflammatory cells and severe sinusoidal dilatation (Photomicrographs 5–7). Treatment of HFD-STZ rats with Hayani and Samani extracts showed moderate vacuolar degeneration of hepatocytes (Photomicrographs 8,9). However, Zaghloul extract and standard drug treated to HFD-STZ rats showed nearly normal hepatic cells (Photomicrographs 10, 11, respectively), Fig. 1.

Table 14 Effect of three *P. dactylifera* cultivars' leaf extracts and different therapeutic groups on oxidative stress assessment levels

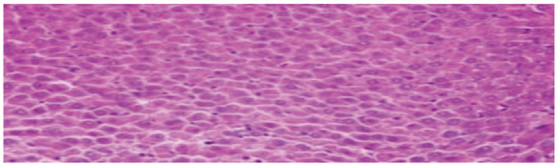
Groups	Malonaldehyde (MDA, nmol/g. tissue)	Glutathione (GSH, nmol/g. tissue)
Control	60.66±4.90[a]	730.00±31.90[a]
Normal/Samani	61.90±3.22[a]	1030.00±22.10[b]
Normal/Hayani	63.31±2.90[a]	1090 .00±50.90[b]
Normal/ Zaghloul	57.95±4.80[a]	1130.00±65.91[b]
HFD-STZ	322.00±8.98[b]	230.88±10.12[c]
HFD-STZ/ Samani	99 .00±5.00[c]	488.00±12.00[d]
HFD-STZ/ Hayani	79.00±2.87[a]	590.00±20.00[d]
HFD-STZ/ Zaghloul	76.00±3.21[a]	700.00±22.00[a]
HFD-STZ/ Standard drugs	72.00±2.90[a]	680.00±23.00[a]

Values are expressed as mean±SD, (n=6). Statistical analysis is carried out using one-way analysis of variance (ANOVA) coupled with co-state computer program, where different letters are significant and similar letters are not significant at P less than or equal to 0.05.

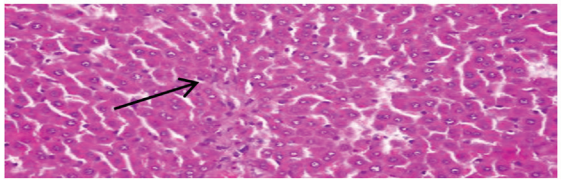
Pancreatic histopathological investigation

Photomicrographs 13–15 showed that rat pancreas treated with different extracts, showing normal size of islets of Langerhans with normal exocrine pancreas compared with control pancreas (photomicrograph 12). HFD-STZ rats showed vacuolar degeneration and epithelial cells necrosis in addition to deformation and atrophy of islets of Langerhans (Photomicrographs 16, 17). Treatment of HFD-STZ rats with Hayani and Samani extracts showed moderate size of Langerhans islets with few cells necrosis (Photomicrographs 18, 19, respectively). However, Zaghloul extract and standard drug showed the nearly normal pattern of Langerhans islets cells (Photomicrographs 20, 21). Table 15, indicated Scoring of histopathological changes of all tested groups, Fig. 2.

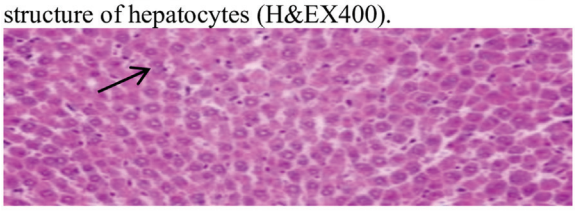
Figure 1



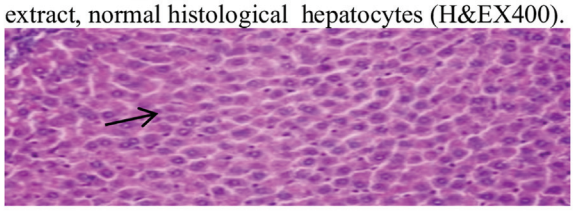
Photomicrograph 1. Rat liver, normal histological structure of hepatocytes (H&EX400).



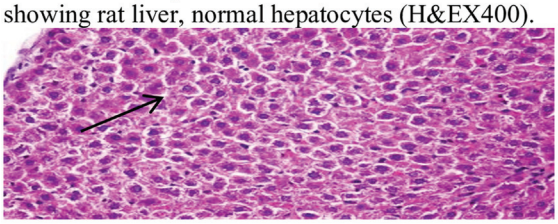
Photomicrograph 2. Rat liver treated with Hayani extract, normal histological hepatocytes (H&EX400).



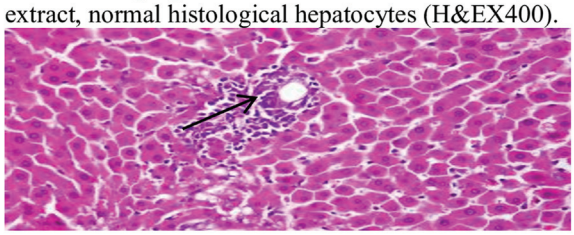
Photomicrograph 3. Rat treated Samani extract showing rat liver, normal hepatocytes (H&EX400).



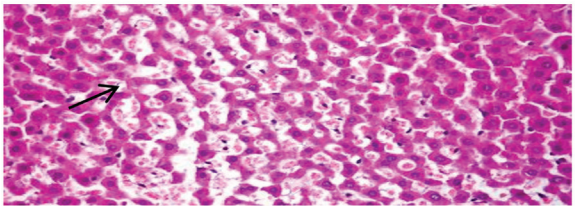
Photomicrograph 4. Rat liver treated with Zaghloul extract, normal histological hepatocytes (H&EX400).



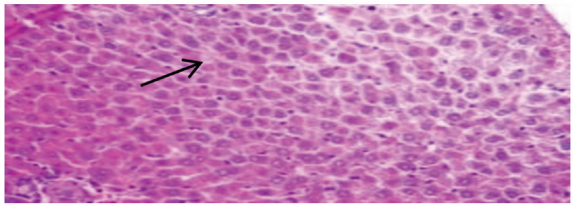
Photomicrograph 5. Rat liver of HFD –STZ, showing diffuse vacuolar degeneration of hepatocytes (H&EX400).



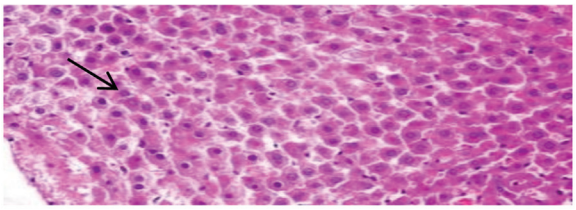
Photomicrograph 6. Rat liver of HFD-STZ, showing infiltration of the portal area with mononuclear inflammatory cells (H&EX400).



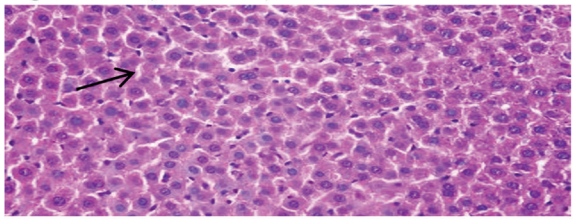
Photomicrograph 7. Rat liver of HFD-STZ , severe sinusoidal dilatation (H&EX400)



Photomicrograph 8. Rat liver of HFD-STZ treated with Hayani extract**,** moderate vacuolar hepatocytes degeneration (H&EX400).



Photomicrograph 9. Rat liver of HFD-STZ treated with Samani extract**,** moderate vacuolar hepatocytes degeneration with sinusoidal dilatation (H&EX400).



Photomicrograph 10. Rat liver of HFD-STZ treated with Zaghloul extract, nearly normal hepatocytes (H&EX400).

Hepatic histopathological investigation for all tested group.

Table 15 Histopathological changes scoring in liver and pancreas of all tested groups

Lesions	Group 5	Group 6	Group 7	Group 8	Group 9	Groups (1–4)
Liver						
Vacuolar hepatocytes degeneration	3	2	2	1	1	0
Congestion of central vein	3	1	2	1	1	0
Sinusoidal congestion	3	2	2	0	1	0
Mononuclear inflammatory cell infiltration in portal area						
Pancreas	2	0	1	0	0	0
Distortion and atrophy of Langerhans islets	3	1	1	0	0	0
Degeneration of exocrine pancreas	3	1	2	1	0	0

The score system was designed as: score 0=absence of the lesion in all rats of the group (n=5), score 1= (<30%), score 2= (<30% – 50%), score 3= (>50%).

This study compared the bioactivities of 3 most common date cultivars growing in Egypt: Samani, Hayani and Zaghloul 80% methanol leaf extracts. However, UPLC-QTOF-MS/MS analysis (Tables 2–4) revealed the presence of variable flavonoid classes such as flavonols, flavones, flavonoid-hexosides, anthocyanidin/and coumarins. Moreover, flavonoids pharmacological properties are typically structure related. For instance, their antioxidant activity *in vivo* are credited to free radical transfer, scavenging enzymes stimulation, and suppression of oxidases enzymes [37,38]. The crucial antidiabetic potential role related to flavonoids is achieved by controlling blood sugar levels through insulin secretion augmenting, stimulating pancreatic β-cells propagation, and decreasing inflammatory and oxidative stress parameters [39]. On the other hand, Xu [40] proved that presence of di hydroxyl substitution on C-3' and C-4' catechol system creates an essential lead in α- glucosidase inhibition through affecting the electron cloud distribution on B ring forming hydrogen bonds with α- glucosidase bonding-site. Nonetheless, isoflavones possess anti-diabetic effects, particularly, by direct effect on β-cell proliferation and regulating insulin secretion [39].

Additionally, cyanidins and their glycosides has been depicted intestinal α-glucosidase and pancreatic α-amylase inhibition besides, reversal of degenerative β-cells variations in in STZ-induced diabetes [41]. Regarding flavonol classes one of the major identified from the analysis, numerous studies reported their mechanism of action in diabetes, including lipid peroxidation decreases, antioxidant enzymes: (MDA and GSH) levels increment, and intestinal glucose uptake reduction [42,43].

Nevertheless, the current research work simulated metabolic syndrome through hyperlipidemia accompanied with hyperglycemia (T2DM) which was compatible with those previously stated by [44]. Upon administrating different extracts, FBG levels were considerably lessened, even surpassing the hypoglycemic effect observed following glibenclamide administration. Even though fenofibrate has been shown to increase the hypoglycemic impact of glibenclamide [9].

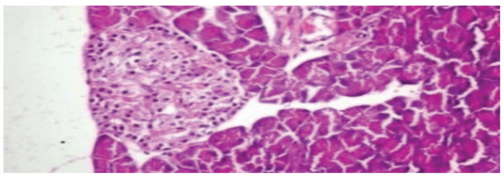
According to the current research, the Zaghloul extract has the highest activity against oxidative stress markers, ALT, AST, and ALP enzyme levels, followed by Hayani and Samani extracts. The existence of distinct types of phenolic compounds including variant subclasses, which were detected in the three extracts, may be correlated to the activity. The mechanism of action could involve detoxifying reactive oxygen species (ROS), hence lowering hyperlipidemia [45,46].

A study reported that date palm leaf exhibited anti-diabetic activity due to phenolics presence as well as many chemical classes such as anthocyanins, and flavonoids which could act synergistically as glucose lowering agents, which agrees with our current study results [47,48].

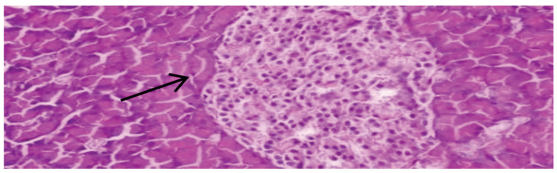
It is noteworthy that dietary manipulation caused a notorious body weight gain together with hyperlipidaemia in the current study [49]. The tested leaf extracts significantly reduced body weight as well as blood lipid profile markers. This could be related to flavonoids dominance in the extracts that led to *in vivo* hypocholesterolemic impact [50]. A recent study estimated that 75% of diabetes people have fatty liver and that matched the results of the current study [51].

Nephropathy is considered as the prevalent diabetes impediment [52]. Treatment with the three extracts resulted in significant incline in serum urea and creatinine levels. Nevertheless, concerning oxidative stress state triggered an increase in ROS produced

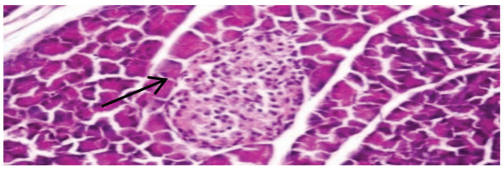
Figure 2



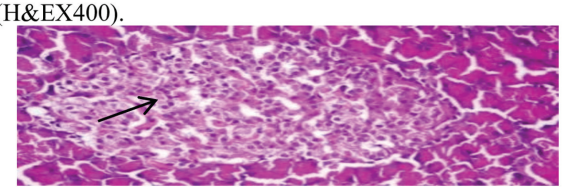
Photomicrograph 12. Rat pancreas showing normal size of islets of Langerhans (H&EX400).



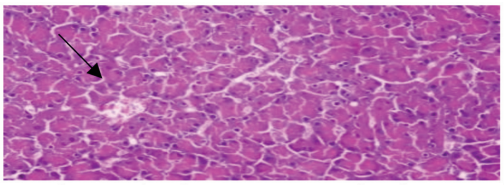
Photomicrograph 13. Rat pancreas treated with Hayani leaf extract showing normal size of islets of Langerhans (H&EX400).



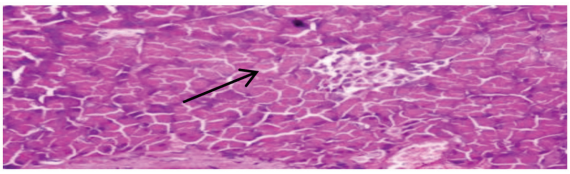
Photomicrograph 14. Rat pancreas treated with Samani leaf extract, showing normal size of islets of Langerhans (H&EX400).



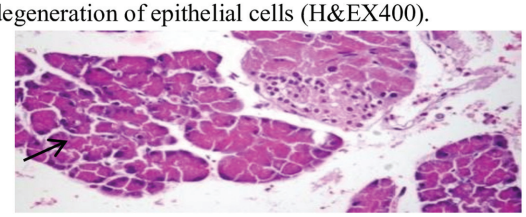
Photomicrograph 15. Rat pancreas treated with Zaghloul leaf extract, showing normal size of islets of Langerhans (H&EX400).



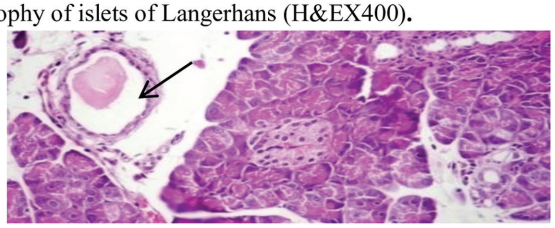
Photomicrograph 16. Rat pancreas, showing vacuolar degeneration of epithelial cells (H&EX400).



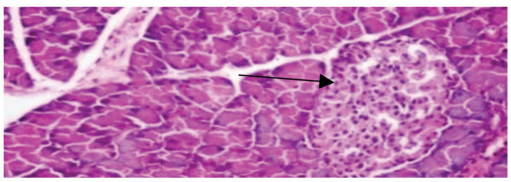
Photomicrograph 17. Rat pancreas showing distortion and atrophy of islets of Langerhans (H&EX400).



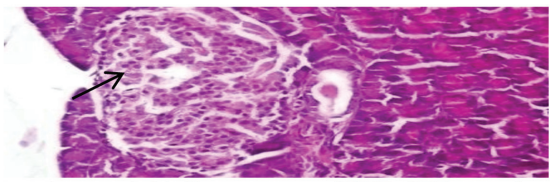
Photomicrograph 18. Rat pancreas of HFD-STZ treated with Hayani leaf extract showing moderate size of islets of Langerhans with necrosis of few cells of exocrine pancreas (H&EX400).



Photomicrograph 19. Rat pancreas of HFD-STZ, treated with Samani leaf extract showing moderately sized islets of Langerhans with necrosis of few cells of exocrine pancreas (H&EX400).



Photomicrograph 20. Rat pancreas of HFD-STZ treated with Zaghloul leaf extract showing nearly normal size of islets of Langerhans with normal exocrine pancreas (H&EX400).



Photomicrograph 21. Rat pancreas of HFD-STZ treated with standard drug , showing nearly normal size of islets of Langerhans with normal exocrine pancreas (H&EX400).

Pancreatic histopathological investigation for all tested groups.

by CYP2E1, a key element of insulin resistance in diabetes [51,53,54].

Moreover, insulin resistance is an inflammatory status that are connected to raised levels of pro-inflammatory mediators and cytokines [55]. TNF- and IL-6 were significantly elevated in HFD-STZ rats in the current investigation. Meanwhile, treatment with various palm leaf extracts reversed these alterations, suggesting that the anti-inflammatory effect [56]. Also, one devastating consequence of type 2 DM has been recognized as cardiovascular issues [57].

The diabetic group results revealed higher adhesion molecules due to enhanced phospholipase activity. However, treatment with different extracts triggered a reduction in adhesion molecules readings. These conclusions matched those of [58].

Conclusion

The current investigation found that Samani, Hayani, and Zaghloul leaf extracts have very encouraging anti-diabetic, anti-inflammatory, and antioxidant properties, which might be attributable to the incidence of a diversity of phytochemicals, particularly those of the flavonoid class. The tested leaf extracts exhibited amelioration in pathologic conditions of HFD-STZ-induced rats in all tested parameters. These findings were consistent with histological studies of liver and pancreas tissues. However, more adequate clinical research studies are suggested to support the use of palm leaf extracts as a prospective nutraceutical anti-diabetic agent as an effective food supplement that could effectively help many diabetic patients. Furthermore, it would be beneficial to spot the light on some future study perspectives regarding the role of the extracts on some vital outcomes, such as insulin sensitivity, oxidative stress markers, lipid profile as the extracts proved promising effects on dyslipidemia, as well as some crucial factors regarding duration of treatment, dose-response relationships, and long-term safety profiles would provide a comprehensive evaluation of the antidiabetic effects of the extracts.

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Ethics approval and consent to participate: The experiment was performed on 54 adult male albino of the Wistar strain rats, supplied from the animal house of the NRC (Dokki, Cairo, Egypt). The study was approved by the Medical Research Ethics Committee of the NRC approval number (19288) following the Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011, published by The National Academies Press, 2101 Constitution Ave.

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Conflicts of interest

The authors declare that they have no known competing interests.

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