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## Data Article

Data on the effects of *Glochidion zeylanicum* leaf extracts in *Caenorhabditis elegans*

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## ABSTRACT

The present article contains the data on the effects of *Glochidion zeylanicum* leaf extracts in *C. elegans*, which is related to the article " *Glochidion zeylanicum* leaf extracts exhibit lifespan extending and oxidative stress resistance properties in *Caenorhabditis elegans* via DAF-16/FoxO and SKN-1/Nrf-2 signaling pathways" Chatrawee et al., 2019. This dataset was generated to better understand the antioxidant and anti-aging properties of *G. zeylanicum* leaf extracts in *C. elegans*. The bioactive compounds of the extracts were analyzed using GLC-MS, LC-MS, and RP-HPLC. The antioxidant properties were determined using phenolics, flavonoids, ABTS and DPPH assays. The *in vivo* antioxidant properties were performed using the intracellular ROS accumulation and the survival rate under oxidative stress condition assays. The brood size, body

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length and life-span were determined regarding anti-aging properties in this data.

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#### Specifications Table

Subject	Natural Products Research
Specific subject area	Biochemistry, Biology, Ageing
Type of data	Table and Figure
How data were acquired	Screening bioactive compounds of <i>G. zeylanicum</i> leaf extracts.
Data format	Conducting of antioxidant and anti-aging activities with <i>G. zeylanicum</i> leaf extracts in <i>C. elegans</i> . Raw and analyzed data
Parameters for data collection	Leaf of <i>G. zeylanicum</i> was extracted in hexane and methanol solvents to investigate the bioactive compounds, antioxidant and anti-aging effects in <i>C. elegans</i> .
Description of data collection	The bioactive compounds of the extracts were analyzed using GLC-MS, LC-MS, and RP-HPLC. The antioxidant properties were determined using phenolics, flavonoids, ABTS and DPPH assays. The <i>in vivo</i> antioxidant properties were performed using the intracellular ROS accumulation and the survival rate under oxidative stress condition assays. The anti-aging properties were conducted using the brood size, body length and life-span assays.
Data source location	<i>G. zeylanicum</i> was collected from Jana, Songkhla, Thailand (6.8447° N, 100.6647° E). Plant extraction was done at Chulalongkorn University, Bangkok, Thailand (13.7323° N, 100.5368° E). GLC-MS and LC-MS were performed at University Kebangsaan Malaysia, Bangi, Malaysia (2.9300° N, 101.7774° E). Antioxidant and anti-aging properties in <i>C. elegans</i> were performed at Heidelberg University, Heidelberg, Germany (49.4191° N, 8.6702° E).
Data accessibility	Data are available within this article
Related research article	Chatrawee Duangjan, Panthakarn Rangsinth, Xiaojie Gu, Shaoxiong Zhang, Michael Wink, and Tewin Tencomnao, <i>Glochidion zeylanicum</i> leaf extracts exhibit lifespan extending and oxidative stress resistance properties in <i>Caenorhabditis elegans</i> via DAF-16/FoxO and SKN-1/Nrf-2 signaling pathways, <i>Phytomedicine: international journal of phytotherapy and phytopharmacology</i> , 2019; 64:153061, <a href="https://doi.org/10.1016/j.phymed.2019.153061">https://doi.org/10.1016/j.phymed.2019.153061</a> .

#### Value of the data

- This data provides novel bioactivity for medicinally important plant and supplement drugs against oxidative stress.
- Data proved the natural plant leaf extracts can effectively exert both oxidative stress resistance and anti-aging properties in the *C. elegans* model and may lead to new agents to benefit humans in the near future.
- This data provides information to researchers of herbal medicine for the discovery of new therapeutic applications of compounds from *G. zeylanicum*.
- This data will help in further research to identify bioactive molecules from the crude extracts that possessed antioxidant and anti-aging activities.

## 1. Data

The dataset in this study shows the potential of *Glochidion zeylanicum* leaf extracts (hexane and methanol) on lifespan extending and oxidative stress resistance properties in *Caenorhabditis elegans*. Bioactive compounds in GZ hexane and methanol extracts were analyzed by GLC-MS, LC-MS and HPLC (Tables 1–3). *G. zeylanicum* leaf extracts showed antioxidant activities *in vitro* (Table 4, Fig. 1) and *in vivo* (Figs. 2 and 3). Moreover, *G. zeylanicum* leaf extracts have oxidative stress resistance properties (Figs. 4 and 6) and anti-aging in *C. elegans* (Tables 5 and 6 and Fig. 5) [1].

Bioactive compounds in GZ hexane extract was analyzed by GLC-MS, The secondary metabolites from GZ hexane extract contains pentadecanoic acid, n-hexadecanoic acid, phytol, octadecatrienoic acid, octadecanoic acid, hexanedioic acid and benzoic acid.

**Table 1**

Proposed phytochemical constituents in the GZ hexane extract using GLC-MS.

Peak No.	Rt (min)	Area (%)	Concentration <sup>a</sup>	Proposed compound	Match	Prob.
14	30.9820	1.0160	–	Pentadecanoic acid	755	68.30
20	33.1280	48.3180	3727.26	n-Hexadecanoic acid or Palmitic acid	897	71.50
26	34.7690	0.6850	–	Phytol	798	42.30
29	35.9420	11.0570	429.55	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- or $\alpha$ -Linolenic acid	812	9.90
31	36.2630	10.9150	–	Octadecanoic acid or Stearic acid	900	89.60
38	39.3530	1.6730	–	Hexanedioic acid, mono(2-ethylhexyl)ester or Adipic acid	808	79.00
39	40.3620	1.0370	–	Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	591	607.00

Library: MAINLIB.

<sup>a</sup> mg/100 g of crude extract.**Table 2**

Proposed phytochemical constituents in the GZ methanol extract using LC-MS.

Peak No.	Rt (min)	[M + H] <sup>+</sup> (m/z)	Area (%)	Proposed compound	Theoretical mass	Mass error (ppm)
11	1.8	116.0723	13.9908	L-Proline	115.0633	5
15	2.1	242.1015	4.68035	Resveratrol 4'-methyl ether	219.1107	6
17	2.3	193.0700	1.34899	Quinic acid	192.0634	3
29	6.4	171.0290	1.72094	Gallic acid	170.0215	1
41	8.7	449.1092	3.81108	Quercitrin or Kaempferol 3- $\alpha$ /beta-D-galactoside or Kaempferol 3- $\alpha$ /beta-D-glucoside	448.1006	3
47	9.1	447.1272	1.73585	Ginkgolide B	424.1383	2
48	9.1	447.1294	3.08348	Glycitin	446.1213	1
74	13.4	290.8464	18.7542	Catechin	290.0790	1

Database: METLIN (CA, USA) and KNApSACk Keyword Search Web Version 1.000.01.

**Table 3**

Individual phytochemical constituents in the GZ methanol extract using HPLC.

Peak No.	Rt (min)	Compound	Concentration <sup>a</sup>
1	11.7	Gallic acid	2998.63
2	21.1	Catechin	36714.74
3	37.3	Oxyresveratrol	2.17
4	41.8	Quercetin	8.33

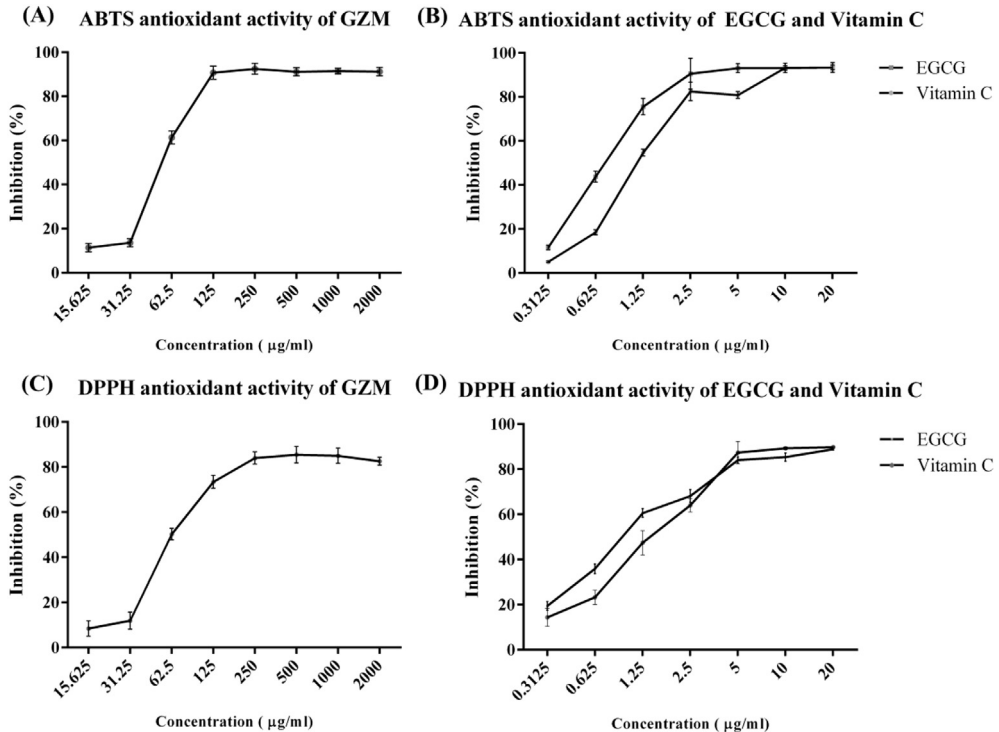
<sup>a</sup> mg/100 g of crude extract.**Table 4**

Total phenolic content, total flavonoid content and free radical scavenging capacity of GZ extracts.

Extract	Total Phenolics mg GAE/g <sup>b</sup>	Total Flavonoids mg QE/g <sup>b</sup>	DPPH scavenging assay		ABTS scavenging assay	
			%Radical Scavenging activity <sup>a</sup>	EC <sub>50</sub> ( $\mu$ g/mL)	%Radical Scavenging activity <sup>a</sup>	EC <sub>50</sub> ( $\mu$ g/mL)
GZH	7.33 $\pm$ 2.29	3.77 $\pm$ 1.37	14.75 $\pm$ 3.02	–	21.64 $\pm$ 1.13	–
GZD	8.49 $\pm$ 0.62	0.35 $\pm$ 0.27	12.17 $\pm$ 2.56	–	22.50 $\pm$ 1.18	–
GZM	162.81 $\pm$ 3.64	46.96 $\pm$ 0.09	86.66 $\pm$ 0.44	65.27 $\pm$ 11.63	93.98 $\pm$ 0.05	63.45 $\pm$ 1.14
Vitamin C	–	–	–	1.28 $\pm$ 0.12	–	1.38 $\pm$ 0.02
EGCG	–	–	–	0.95 $\pm$ 0.08	–	0.95 $\pm$ 0.02

GZH; 1 mg/mL of GZ hexane extract, GZD; 1 mg/mL GZ dichloromethane extract, GZM; 1 mg/mL GZ methanol extract.

<sup>a</sup> Of 1 mg/mL extract.<sup>b</sup> Dry weight sample, Values are expressed as the mean  $\pm$  SD (n = 3).



**Fig. 1.** ABTS radical scavenging activity of GZM (15.625–2000 µg/mL) (A), ABTS radical scavenging activity of EGCG and vitamin C (0.3125–20 µg/mL) (B), DPPH radical scavenging activity of GZM (15.625–2000 µg/mL) (C), DPPH radical scavenging activity of EGCG and vitamin C (0.3125–20 µg/mL) (D). Values are expressed as the mean  $\pm$  SD (n = 3).

Bioactive compounds in GZ methanol extract was analyzed by LC-MS, The secondary metabolites from GZ methanol extract contains l-proline, resveratrol 4'-methyl ether, quinic acid, gallic acid, quercitrin/kaempferol 3- $\alpha$ -d-glucoside, ginkgolide b, glycitin, and catechin.

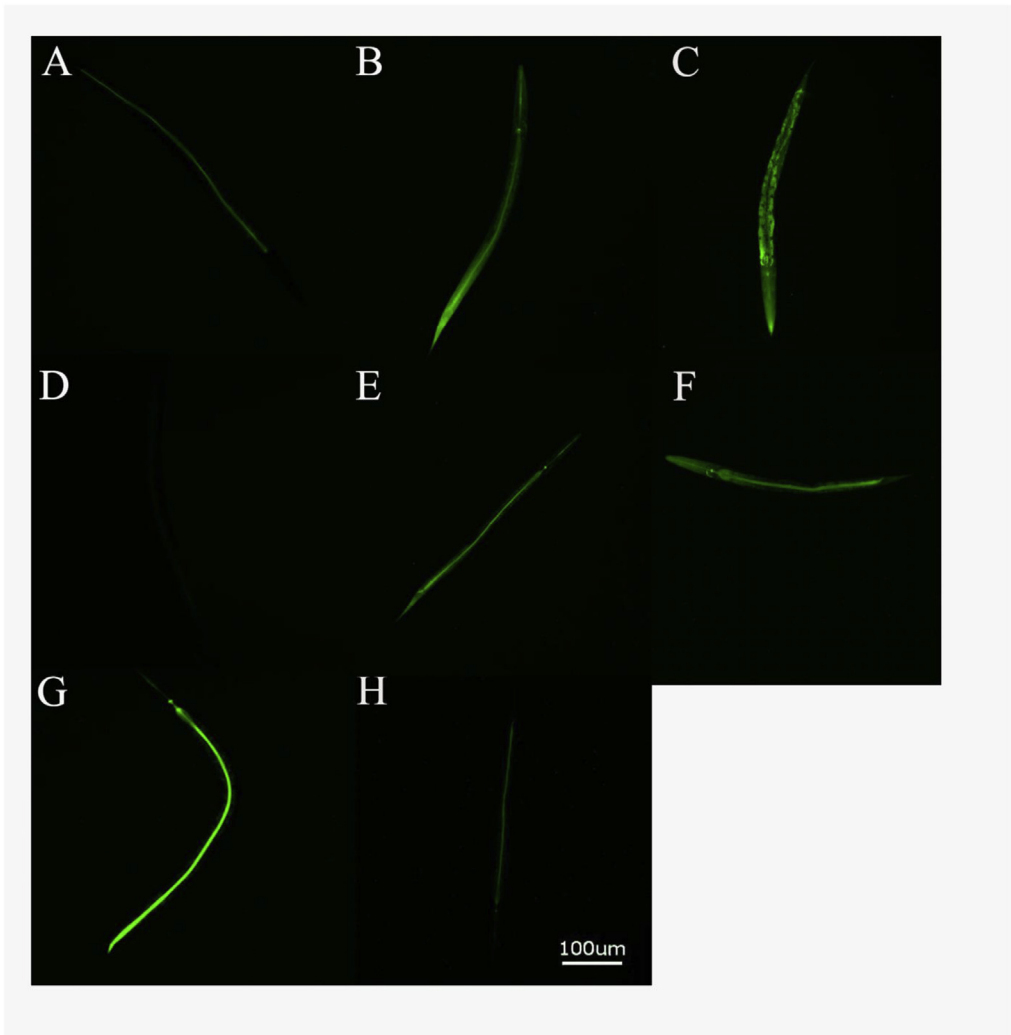
The GZ methanol extract exhibited powerful antioxidant activity. When tested in the DPPH and ABTS assays, GZ methanol extract effectively scavenged the radical by 86.66% (EC<sub>50</sub> = 65.27 µg/mL) and 93.98% (EC<sub>50</sub> = 1.38 µg/mL), respectively. In accordance with the antioxidant activities, high phenolic and flavonoid contents of 162.81 GAE/g dry weight sample and 46.96 QE/g dry weight sample were recorded from the methanol extract.

GZ extracts showed lower levels of ROS in N2 worms when compared to the DMSO control group. DMSO and EGCG were used as a solvent control and positive control group, respectively.

GZ extracts failed to decrease the level of ROS in CF1038 and EU1 worms. DMSO and EGCG were used as the solvent control and positive control groups, respectively. Data are presented as the mean  $\pm$  SEM (n = 80, replicated three times). Treatment groups are compared to the DMSO control by one-way ANOVA following Bonferroni's method (posthoc).

GZ extracts failed to increase the survival rate in CF1038 and EU1 worms under oxidative stress condition. Data are presented as the mean  $\pm$  SEM (n = 80, replicated three times). Treatment groups are compared to the DMSO control by one-way ANOVA following Bonferroni's method (posthoc).

GZ extracts at low concentrations (5–10 µg/mL GZM and 0.25–0.5 µg/mL GZM) failed to neither decrease the level of ROS nor increase the survival rate in wild-type worms. DMSO was used as the solvent control. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 and \*\*\*\*p < 0.0001, compared to the DMSO control by one-way ANOVA following Bonferroni's method (posthoc). Data are presented as the mean  $\pm$  SEM (n = 80, replicated three times).



**Fig. 2.** Representative pictures of DCFDA fluorescence in worms treated with 25 µg/mL GZH (A); 50 µg/mL GZH (B); 100 µg/mL GZH (C); 1 µg/mL GZM (D); 2.5 µg/mL GZM (E); 5 µg/mL GZM (F); DMSO solvent control (G); and 25 µg/mL EGCG (H).

The lifespan assay was carried out with wild-type (N2) and *mev-1(kn-1)* worms at 20 °C. P-value log rank as compared to the control worms; the mean lifespan in days is the average number of days the worms survived in each group. Each treatment was compared to the control by the non-parametric log rank (Mantel–Cox) tests.

## 2. Experimental design, materials, and methods

### 2.1. Qualitative phytochemical screening

For GLC-MS, chromatographic separation was carried out on a Clarus 600 GC-MS system (Perkin Elmer, Shelton, CT, USA) separated with a 30 m × 0.25 mm × 0.25 µm Elite-5MS column (Perkin Elmer,

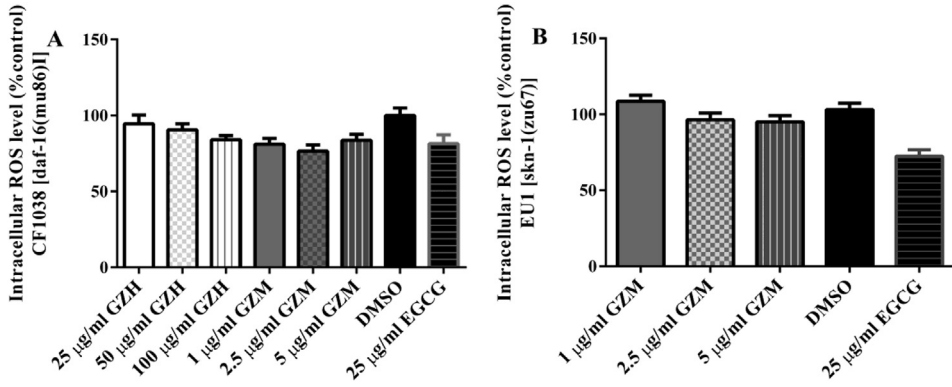


Fig. 3. Effect of GZ extracts on the intracellular ROS of CF1038 (A) and EU1 (B) worms.

USA). Plant extracts were incubated in MeOX and pyridine at 40 °C for 90min at 40 °C. Then, the MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) were added to the sample and incubated for 90min at 40 °C. The temperature of the oven was set at 40 °C and was increased by 5 °C/min until it reached 250 °C, and the carrier gas was helium at a constant flow of 1 mL/min. The MS parameters used were electron impact mode (EI) following an ionization voltage of 70 eV, an ion source temperature of 200 °C and a scan range of 40–600 Da.

The National Institute of Standards and Technology (NIST, version 2.0, Gaithersburg, MD, USA) database was used for the identification of top ten compounds by exceeding the signal-to-noise ratio (S/N) of 100 and comparing the volatile information based on the compound name. Match and reverse match values below 800 were filtered.

For LC-MS, chromatographic separation was carried out on a Dionex™ UltiMate 3000 UHPLC system (Thermo Scientific) equipped with an Acclaim™ Polar Advantage II C18 column (3 × 150 mm, 3 µm particle size) (Thermo Scientific, USA) by using a 1 µL injection volume. The mobile phase comprised 0.1% formic acid in water (solvent A) and 100% acetonitrile (solvent B), which had a flow rate of 400 µL/min for 22 min. At 0–3 min, 3–10 min, 10–15 min, and 15–22 min; 5% B, 80% B, 80% B, and 5% B were used for the gradient elution, respectively. High-resolution MS analysis was carried out in the positive electrospray ionization mode using a MicroTOF-Q III (Bruker Daltonik GmbH, Bremen, Germany). A capillary voltage of 4500 V, drying gas flow of 8 L/min, an ion source temperature of 200 °C, a nebulizer

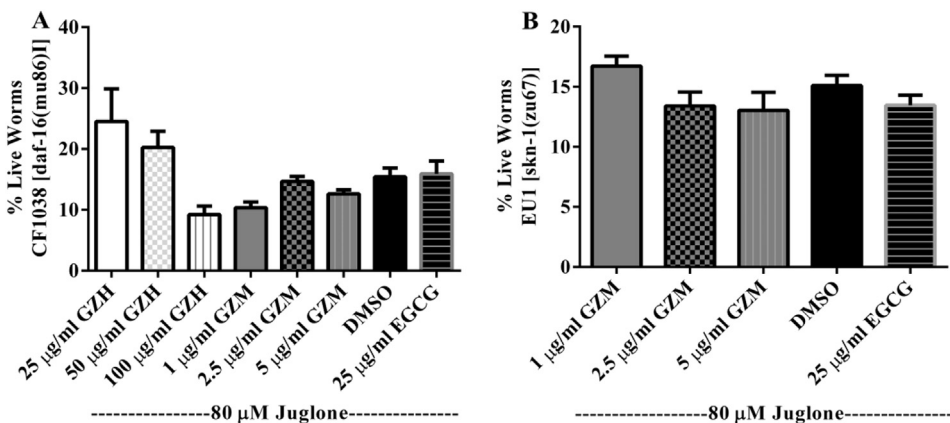


Fig. 4. Effect of GZ extracts on the survival rate of CF1038 (A) and EU1 (B) worms under oxidative stress induced by juglone.

**Table 5**  
Effect of GZ extracts on markers of aging and development in *C. elegans*.

Extract	Brood size(mean egg lay)	Body length (mean length ( $\mu\text{m}$ ))
DMSO reagent control	245.5 $\pm$ 33.50	1517 $\pm$ 18.97
25 $\mu\text{g}/\text{mL}$ GZH	273.3 $\pm$ 22.87	1417 $\pm$ 19.93
50 $\mu\text{g}/\text{mL}$ GZH	251.3 $\pm$ 15.12	1444 $\pm$ 27.13
100 $\mu\text{g}/\text{mL}$ GZH	288.8 $\pm$ 2.496	1460 $\pm$ 37.37
25 $\mu\text{g}/\text{mL}$ GZD	272.8 $\pm$ 15.19	1444 $\pm$ 36.78
50 $\mu\text{g}/\text{mL}$ GZD	274.3 $\pm$ 15.88	1889 $\pm$ 434.9
100 $\mu\text{g}/\text{mL}$ GZD	247.3 $\pm$ 9.322	1500 $\pm$ 15.30
1.0 $\mu\text{g}/\text{mL}$ GZM	228.0 $\pm$ 13.45	1417 $\pm$ 18.38
2.5 $\mu\text{g}/\text{mL}$ GZM	222.0 $\pm$ 11.00	1542 $\pm$ 18.55
5 $\mu\text{g}/\text{mL}$ GZM	231.3 $\pm$ 8.159	1442 $\pm$ 47.51

(Results are the means  $\pm$  SEM).

GZH; GZ hexane extract, GZD; GZ dichloromethane extract, GZM; GZ methanol extract.

pressure of 1.2 bar, an end plate offset of  $-500$  V, and a scan range from  $m/z$  50 to 1000 were used as parameters for the instrument. The METLIN and KnapSack databases were used for identification of top ten compounds by comparing the observed  $m/z$  values with the calculated mass values from previously published data. The abundance of individual compounds was calculated from the percentage of peak area relative to the total area of all peaks in the chromatograms.

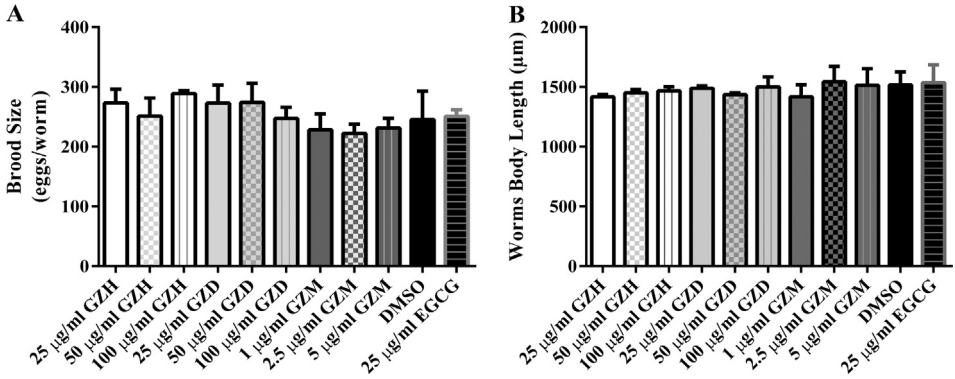
For HPLC, the chromatography was carried out on SHIMADZU LC-10 HPLC equipped with an analytical C18 reversed-phase column (ODS3 C18,  $4.6 \times 250$  mm i.d.,  $5\text{-}\mu\text{m}$  particle size) and UV detector (best condition at 220 nm). The mobile phase consists of 0.02 M sodium acetate, buffered to a pH

**Table 6**  
Results and statistical analyses of GZ extracts treated *C. elegans* in lifespan assay.

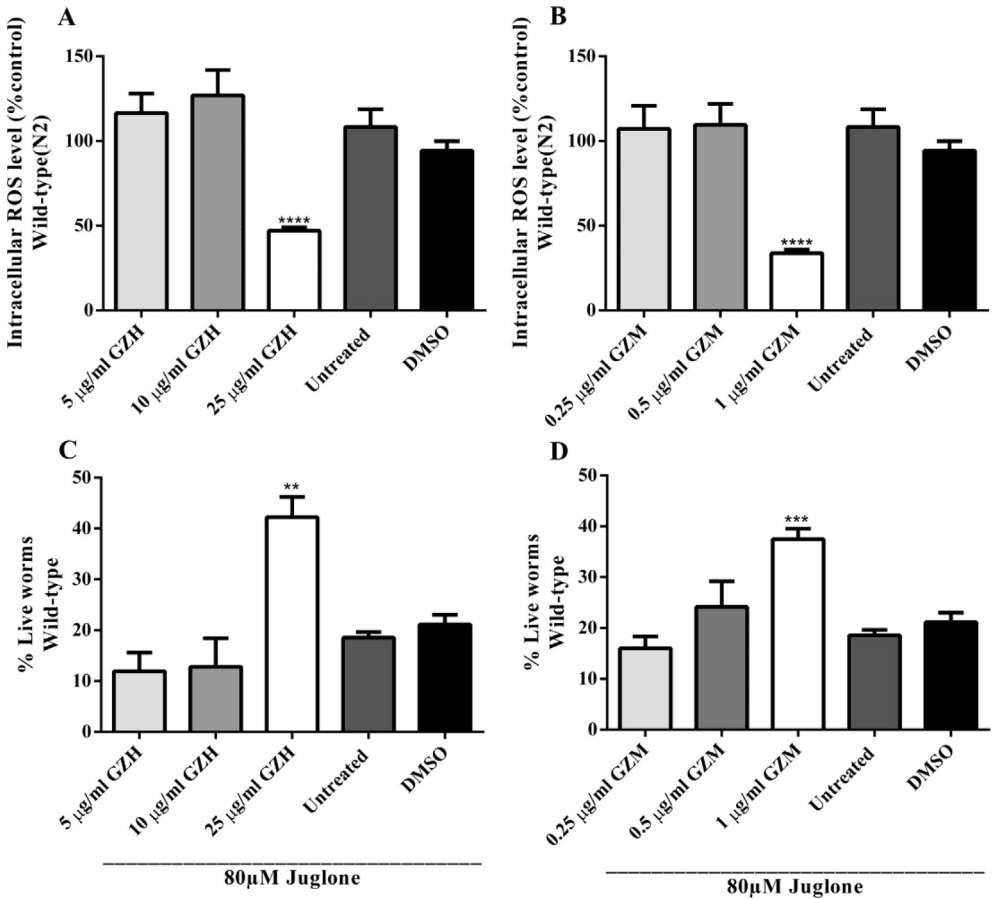
Strain	Treatment	Mean lifespan (day) $\pm$ SEM	Maximum lifespan (days)	Percentage of increased lifespan (vs control)	P value (vs control)	P value summary	Number of worms
N2	DMSO control	14.28 $\pm$ 0.3599	21				N = 120
N2	25 $\mu\text{g}/\text{mL}$ GZH	13.93 $\pm$ 0.3507	24	-2.45098	0.901	ns	N = 121
N2	50 $\mu\text{g}/\text{mL}$ GZH	14.21 $\pm$ 0.3805	24	-0.490196	0.833	ns	N = 105
N2	100 $\mu\text{g}/\text{mL}$ GZH	15.71 $\pm$ 0.5486	32	10.014006	0.0056	**	N = 132
N2	1.0 $\mu\text{g}/\text{mL}$ GZM	15.42 $\pm$ 0.4908	29	7.9831933	0.0139	*	N = 127
N2	2.5 $\mu\text{g}/\text{mL}$ GZM	12.55 $\pm$ 0.4489	29	-12.11485	0.7191	ns	N = 112
N2	5 $\mu\text{g}/\text{mL}$ GZM	15.53 $\pm$ 0.4635	34	8.7535014	0.0066	**	N = 132
TK22	DMSO control	9.667 $\pm$ 0.4144	15				N = 33
TK22	25 $\mu\text{g}/\text{mL}$ GZH	9.429 $\pm$ 0.4126	16	-2.46198407	0.6491	ns	N = 35
TK22	50 $\mu\text{g}/\text{mL}$ GZH	9.581 $\pm$ 0.3525	15	-0.889624496	0.816	ns	N = 31
TK22	100 $\mu\text{g}/\text{mL}$ GZH	10.37 $\pm$ 0.5288	17	7.272163029	0.4833	ns	N = 27
TK22	1.0 $\mu\text{g}/\text{mL}$ GZM	11.54 $\pm$ 0.5245	17	19.37519396	0.1071	ns	N = 39
TK22	2.5 $\mu\text{g}/\text{mL}$ GZM	11.31 $\pm$ 0.5236	18	16.99596566	0.1798	ns	N = 39
TK22	5 $\mu\text{g}/\text{mL}$ GZM	10.88 $\pm$ 0.5015	17	12.54784318	0.4237	ns	N = 41

N2; Wild-type, TK22; *mev-1(kn1)*.

GZH; GZ hexane extract, GZM; GZ methanol extract.



**Fig. 5.** (A) Brood size and (B) body length of N2 (wt) worms after GZ extracts treatment. GZ extracts have no effect on egg laying activity and body length. Data are presented as the mean ± SEM (n = 80, replicated three times). Treatment groups are compared to the DMSO control by one-way ANOVA following Bonferroni's method (posthoc).



**Fig. 6.** Effect of GZ extracts at low concentrations on the intracellular ROS (A-B) and the survival rate (C-D) of wild-type (N2) worms.



of 4 with 0.0125 M citric acid, containing 0.042 M methanesulfonic acid and 0.1 mM EDTA. The flow rate was set at 1 mL/min. The working standard solutions were freshly prepared in 0.05 M perchloric acid containing 0.1 mM Na<sub>2</sub>EDTA on ice and stored at -20 °C before using. Peaks were identified by comparing the retention time of each peak in the sample solution, where each individual peak was further compared to the standard solution of gallic acid, catechin, oxyresveratrol, quercetin, octadecatrienoic acid (linolenic acid), and hexadecanoic (palmitic acid) (Sigma-Aldrich, USA) served as an internal standard. The calibration curves of internal standard compounds were constructed for quantification.

## 2.2. Assessment of resistance to oxidative stress

The wild-type (N2) and transgenic (*daf-16 (mu86)* mutant CF1038, *skn-1 (zu67)* mutant EU1) worms were used for analysis the survival rate under oxidative stress conditions, 80 worms at L1 larval stage were treated with plant extracts with different concentrations in S-medium for 48 h, each group contained 80 larvae. Each group was treated with 80 µM pro-oxidant juglone for 24 h. Then dead and live worms were counted.

## 2.3. Assessment of intracellular ROS

Worms were treated with plant extracts of different concentrations in S-medium for 48 h; each group comprised 50–100 larvae. After treatment, the worms were pelleted by centrifugation, added to 50 µM 2,7-dichlorodihydrofluorescein-diacetate (H<sub>2</sub>DCF-DA) solution and incubated in the dark at 20 °C for 1 h. After incubation, worms were paralyzed using 10 mM sodium azide and mounted on a microscopic glass slide; then, photos were taken randomly of 30 worms.

## 2.4. Expression of HSP-16.2, GST-4 and SOD-3

50–100 worms at L1 larval stage of transgenic worms were treated with different concentrations of plant extracts in S-medium at 20 °C for 72 h (TJ375 and CF1553 transgenic worms) and 48 h (CL166 transgenic worms). After treatment, TJ375 and CL166 transgenic worms were exposed to a nonlethal dose of 20 µM juglone for 24 h. The fluorescence intensity was measured by fluorescence microscopy as described above.

## 2.5. Subcellular DAF-16 and SKN-1 localization

50–100 worms at L1 larval stage of transgenic worms were treated with different concentrations of plant extracts in S-medium at 20 °C for 72 h. And the fluorescence intensity was measured by fluorescence microscopy as described above.

## 2.6. Assessment of auto fluorescent pigment

50–100 worms at L1 larval stage of BA17 transgenic worms were treated with different concentrations of plant extracts in S-medium at 25 °C. The media was changed every second day. On day 16, the worms were paralyzed with 10 mM sodium azide, mounted on a glass slide and photographed.

## 2.7. Assessment of pharyngeal pumping rate

Synchronized N2 worms at the L4 larval stage were sorted and placed one by one on individual NGM agar plates supplemented with plant extracts with different concentrations with an *E. coli* OP50 lawn as a food source at 20 °C for 24 h. (50 worms per each group). The adult worms were transferred to fresh medium every day during the reproductive phase to separate them from their progeny. After that, the adult worms were transferred to fresh medium with treatment every second day. Pharyngeal pumping was analyzed on days 6, 8, 10, and 12 by counting the pumping frequency of the terminal pharyngeal bulb of each single worm for 60 s. The dissection microscope was used to measure the

pumping rate of at least 20 worms from each group. When the worms were crawling on the *E. coli* OP50 lawn, the pumping frequency was recorded and represented as pumps  $\text{min}^{-1}$ .

### 2.8. Assessment of lifespan

The wild-type (N2) and transgenic (TK22) (A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and short lifespan) [2] worms were used for the lifespan assay. Synchronization and treatment were conducted as in the pharyngeal pumping rate assay. The worms were counted every day and documented as a percentage of surviving worms. Worms that failed to respond to a gentle touch with a platinum wire were scored as dead and excluded from the plates. The worms with internally hatched progeny or extruded gonads were scored as censors and discarded from the assay.

### 2.9. Assessment of antioxidant activity

The total phenolic and flavonoid content were examined by the Folin - Ciocalteu method and colorimetric aluminum chloride method as described previously [3]. The DPPH and ABTS radical scavenging assays were conducted according to the method followed in our previous work [3].

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## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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