Supplementary data to:

Original article:

THERAPEUTIC POTENTIAL OF BIOACTIVE COMPOUNDS FROM PUNICA GRANATUM EXTRACTS AGAINST AGING AND COMPLICITY OF FOXO ORTHOLOGUE DAF-16 IN CAENORHABDITIS ELEGANS

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**Supplementary Table 1:**  

**A)** The standard curve of tannic acid was prepared to obtain the total phenol content (TPC) from samples; **B)** The OD of replicates for all the samples, its average, standard deviation, and the TPC obtained are mentioned. (Raw data to Figure 3a in the main document)

### A)

| Aliquots conc. (µg) | OD at 760 nm  |
|---------------------|---------------|
|                     | 1  | 2  | 3  | AVG | SD       |
| 40                  | 0.165 | 0.156 | 0.162 | 0.161 | 0.004583 |
| 80                  | 0.227 | 0.218 | 0.221 | 0.222 | 0.004583 |
| 120                 | 0.398 | 0.386 | 0.389 | 0.391 | 0.006245 |
| 160                 | 0.464 | 0.46  | 0.462 | 0.462 | 0.002    |
| 200                 | 0.54  | 0.547 | 0.548 | 0.545 | 0.004359 |
| 240                 | 0.61  | 0.62  | 0.615 | 0.615 | 0.005    |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation

Standard Tannic Acid (1 mg/ml)
Sample (1 mg/ml) and volume (50 µl)
Standard curve equation obtained was: \( R^2=0.978; Y=0.0024X + 0.0683 \)

### B)

| Samples           | OD of replicates | AVG    | SD       | TPC (mg TAE/gm of extract) |
|-------------------|------------------|--------|----------|---------------------------|
|                   | 1    | 2    | 3    |       |                         |
| Outer peel        | 0.516 | 0.527 | 0.534 | 0.525666667 | 0.009073772 | 190.55 |
| Inner peel        | 0.088 | 0.095 | 0.082 | 0.0883333333 | 0.006506407 | 8.33  |
| Juice             | 0.137 | 0.128 | 0.14  | 0.135 | 0.006244998 | 27.79 |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation

The total phenol content (TPC) was obtained using the following equation:

\[ TPC = C \times \frac{V}{W} \]

Where, 
- \( C \): Conc. From std. curve (mg/ml)  
- \( V \): Vol. of sample used in experiment in ml  
- \( W \): Weight of sample in gm
Supplementary Table 2: A) The standard curve of quercetin was prepared to obtain the total flavonoid content (TFC) from samples; B) The OD of replicates for all the samples, its average, standard deviation, and the TFC obtained are mentioned. (Raw data to Figure 3b in the main document)

### A)

| Aliquots conc. (µg) | OD at 510 nm |       |       |       |       |
|---------------------|--------------|-------|-------|-------|-------|
|                     | 1            | 2     | 3     | AVG   | SD    |
| 10                  | 0.143        | 0.146 | 0.149 | 0.146 | 0.003 |
| 20                  | 0.185        | 0.178 | 0.18  | 0.181 | 0.003606 |
| 30                  | 0.221        | 0.224 | 0.212 | 0.219 | 0.006245 |
| 40                  | 0.281        | 0.28  | 0.276 | 0.279 | 0.002646 |
| 50                  | 0.304        | 0.306 | 0.296 | 0.302 | 0.005292 |
| 60                  | 0.347        | 0.339 | 0.349 | 0.345 | 0.005292 |
| 70                  | 0.379        | 0.378 | 0.374 | 0.377 | 0.002646 |
| 80                  | 0.442        | 0.444 | 0.437 | 0.441 | 0.003606 |
| 90                  | 0.491        | 0.495 | 0.487 | 0.491 | 0.004 |
| 100                 | 0.537        | 0.532 | 0.539 | 0.536 | 0.003606 |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation
Standard Quercetin (1 mg/ml)
Sample (1 mg/ml) and volume (30 µl)
Standard curve equation obtained was: $R^2=0.995; Y=0.0043X + 0.0941$

### B)

| Samples          | OD of replicates | AVG       | SD         | (TFC) mg QE/gm of extract |
|------------------|------------------|-----------|------------|---------------------------|
|                  | 1                | 2         | 3          |                           |
| Outer peel       | 0.1051           | 0.1101    | 0.0982     | 0.1044666667              | 0.0059752 | 2.39 |
| Inner peel       | 0.122            | 0.1131    | 0.109      | 0.1147                    | 0.0066461 | 4.8  |
| Juice            | 0.089            | 0.1022    | 0.0931     | 0.0947666667              | 0.006756  | 0.154 |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation
The total flavonoid content (TFC) was obtained using the following equation:
$TFC=C\times V/W$
Where, $C =$ Conc. From std. curve (mg/ml)
$V =$ Vol. of sample used in experiment in ml
$W =$ Weight of sample in gm
**Supplementary Table 3:**

A) The standard curve of quercetin was prepared to obtain the total vitamin C content (TVC) from samples; B) The OD of replicates for all the samples, its average, standard deviation, and the TVC obtained are mentioned. (Raw data to Figure 3c in the main document)

| Aliquots conc. (µg) | OD at 540 nm |  |  | AVG | SD |
|---------------------|-------------|---|---|-----|----|
| Blank               | 0           | 0 | 0 | 0   | 0  |
| 10                  | 0.007       | 0.009 | 0.005 | 0.007 | 0.002 |
| 20                  | 0.08        | 0.12 | 0.07 | 0.09  | 0.026458 |
| 30                  | 0.131       | 0.133 | 0.126 | 0.13  | 0.003606 |
| 40                  | 0.152       | 0.151 | 0.147 | 0.15  | 0.002646 |
| 50                  | 0.179       | 0.185 | 0.176 | 0.18  | 0.004583 |
| 60                  | 0.221       | 0.224 | 0.215 | 0.22  | 0.004583 |
| 70                  | 0.229       | 0.234 | 0.227 | 0.23  | 0.003606 |
| 80                  | 0.252       | 0.252 | 0.246 | 0.25  | 0.003464 |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation

Standard Ascorbic acid (1 mg/ml) and volume (200 µl)
Standard curve equation obtained was: R²=0.933; Y=0.0034X

| Samples          | OD of replicates | AVG         | SD            | TVC (mg AAE/gm of extract) |
|------------------|------------------|-------------|---------------|---------------------------|
| Outer peel       | 0.00181          | 0.00189     | 0.001886667   | 7.5056E-05               | 0.555 |
| Inner peel       | 0.0041           | 0.0037      | 0.004266667   | 0.000668583             | 1.25  |
| Juice            | 0.0051           | 0.0059      | 0.005733333   | 0.00056862              | 1.685 |

1, 2, 3: Replicate number; OD: Optical density; AVG: Average; SD: Standard Deviation

The total vitamin C content (TVC) was obtained using the following equation:

\[ \text{TVC} = \frac{C \times V}{W} \]

Where, \( C = \) Conc. From std. curve (mg/ml)  
\( V = \) Vol. of sample used in experiment in ml  
\( W = \) Weight of sample in gm
**Supplementary Table 4:** The antioxidant activity of outer peel, inner peel and juice analyzed by *in vitro* DPPH scavenging assay. (Raw data to Figure 8a in the main document)

| Samples   | OD of replicates | AVG   | SD     | % Inhibition |
|-----------|------------------|-------|--------|--------------|
|           | 1                | 2     | 3      |              |
| Outer Peel| 0.076            | 0.073 | 0.077  | 0.075333     | 0.002082  | 66.51    |
| Inner Peel| 0.117            | 0.105 | 0.11   | 0.110667     | 0.006028  | 50.89    |
| Juice     | 0.135            | 0.145 | 0.14   | 0.14         | 0.005     | 37.5     |

1, 2, 3: Replicate number

**Supplementary Table 5:**

A) Standard curve of FeSO$_4$ to obtain the FRAP value; B) The antioxidant activity of outer peel, inner peel and juice analyzed by *in vitro* Ferric Reducing Antioxidant Power Assay (FRAP assay). (Raw data to Figure 8b in the main document)

**A)**

| Aliquots conc. (µM) | OD at 593 nm  | AVG | SD   |
|---------------------|---------------|-----|------|
|                     | 1 | 2 | 3 |
| 100                 | 0.076 | 0.071 | 0.078   | 0.075 | 0.003606 |
| 200                 | 0.142 | 0.145 | 0.133   | 0.14  | 0.006245 |
| 300                 | 0.209 | 0.213 | 0.208   | 0.21  | 0.002646 |
| 400                 | 0.267 | 0.26  | 0.262   | 0.263 | 0.003606 |
| 500                 | 0.313 | 0.316 | 0.304   | 0.311 | 0.006245 |
| 600                 | 0.37  | 0.369 | 0.365   | 0.368 | 0.002646 |
| 700                 | 0.418 | 0.411 | 0.419   | 0.416 | 0.004359 |
| 800                 | 0.496 | 0.498 | 0.488   | 0.494 | 0.005292 |
| 900                 | 0.512 | 0.516 | 0.505   | 0.511 | 0.005568 |
| 1000                | 0.56  | 0.561 | 0.568   | 0.563 | 0.004359 |

1, 2, 3: Replicate number

Standard curve equation obtained was: $R^2=0.981; Y=0.0006X$

**B)**

| Samples       | OD of replicates | AVG  | SD    | mM Fe$^{2+}$ equivalents |
|---------------|------------------|------|-------|--------------------------|
|               | 1 | 2  | 3     |                            |
| Outer peel    | 0.867 | 0.854 | 0.859 | 0.86  | 0.006557 | 1.433 |
| Inner Peel    | 0.808 | 0.817 | 0.805 | 0.81  | 0.006245 | 1.35  |
| Juice         | 0.602 | 0.61  | 0.6    | 0.604 | 0.005292 | 1.006 |

1, 2, 3: Replicate number
Supplementary Table 6: The antioxidant activity of outer peel, inner peel and juice analyzed by *in vitro* H$_2$O$_2$ scavenging assay. (Raw data to Figure 8c in the main document)

| Samples      | OD of replicates | AVG   | SD     | Percentage scavenging of H$_2$O$_2$ (%) |
|--------------|------------------|-------|--------|-----------------------------------------|
| Control      | 0.1174 0.1025 0.1317 | 0.1172 | 0.014601 | -                                       |
| Outer peel   | 0.03953 0.04377 0.04164 | 0.041647 | 0.00212 | 64.5                                   |
| Inner Peel   | 0.06288 0.04182 0.0508 | 0.051833 | 0.010568 | 55.8                                   |
| Juice        | 0.05032 0.060702 0.055814 | 0.055612 | 0.005194 | 52.55                                  |

1, 2, 3: Replicate number

Supplementary Table 7: The antioxidant activity of outer peel, inner peel and juice analyzed by *in vitro* Reducing Power Assay. (Raw data to Figure 8d in the main document)

| Samples      | OD of replicates | AVG   | SD     | % Increase in RPA |
|--------------|------------------|-------|--------|-------------------|
| Control      | 0.318 0.323 0.31 | 0.317 | 0.006557439 | -                 |
| Outer peel   | 0.562 0.552 0.557 | 0.557 | 0.005 | 75.7              |
| Inner peel   | 0.59 0.569 0.579 | 0.579333 | 0.010503968 | 82.64          |
| Juice        | 0.476 0.514 0.495 | 0.495 | 0.019 | 56.15             |

1, 2, 3: Replicate number
**Supplementary Table 8:** Number of dead worms over a time period of days maintained at A) 20 °C and B) 25 °C under the treatment of outer peel of *P. granatum*, and the fraction of survival was calculated using prism software. (A) Raw data to Figure 9a and B) Figure 9d in the main document)

### A) Raw data to Figure 9a

| Days | Control | 10 µg | 20 µg |
|------|---------|-------|-------|
|      | Fraction of survival | Fraction of survival | Fraction of survival |
| 0    | 1 | 1 | 1 |
| 2    | 1 | 1 | 1 |
| 4    | 1 | 1 | 1 |
| 6    | 0.857143 | 1 | 0.933333 |
| 8    | 0.642857 | 0.928571 | 0.733333 |
| 10   | 0.5 | 0.714286 | 0.6 |
| 12   | 0.214286 | 0.571429 | 0.466667 |
| 14   | 0.214286 | 0.428571 | 0.333333 |
| 16   | 0.214286 | 0.428571 | 0.333333 |
| 18   | 0.071429 | 0.357143 | 0.266667 |
| 20   | 0 | 0.285714 | 0.2 |
| 22   | 0 | 0 | 0.2 |
| 24   | 0 | 0.214286 | 0.066667 |
| 26   | 0 | 0 | 0 |
| 28   | 0 | 0 | 0 |

1, 2, 3: Replicate number; AVG: Average

### B) Figure 9d

| Days | Control | 10 µg | 20 µg |
|------|---------|-------|-------|
|      | Fraction of survival | Fraction of survival | Fraction of survival |
| 0    | 1 | 1 | 1 |
| 2    | 1 | 1 | 1 |
| 4    | 1 | 1 | 1 |
| 6    | 0.333333 | 0.684211 | 0.416666667 |
| 8    | 0.333333 | 0.684211 | 0.416666667 |
| 10   | 0.263158 | 0.416666667 |
| 12   | 0.210526 | 0.416666667 |
| 14   | 0.105263 | 0.105263 | 0.105263 |
| 16   | 0.243 | 0.25 |
| 18   | 0 | 0 | 0 |

1, 2, 3: Replicate number; AVG: Average
Supplementary Table 9: Number of dead worms over a time period of days maintained at A. 20°C and B. 25°C under the treatment of inner peel of *P. granatum*, and the fraction of survival was calculated using prism software. (A) Raw data to Figure 9b and B) Figure 9e in the main document

### A)

| Days | Control | 10 µg | 20 µg |
|------|---------|-------|-------|
|      | 1 2 3 AVG | 1 2 3 AVG | 1 2 3 AVG |
| 0    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 2    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 4    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 6    | 2 2 2 2 0.857143 | 1 0 2 1 0.9 | 0 0 0 0 1 |
| 8    | 2 3 4 3 0.642857 | 3 2 1 2 0.7 | 2 0 1 1 0.9 |
| 10   | 4 1 1 2 0.5 | 0 0 0 0 0.7 | 4 3 2 3 0.6 |
| 12   | 5 3 4 4 0.214286 | 2 1 3 2 0.5 | 1 0 2 1 0.5 |
| 14   | 0 0 0 0 0.214286 | 0 0 0 0 0.5 | 0 2 1 1 0.4 |
| 16   | 0 0 0 0 0.214286 | 0 0 0 0 0.5 | 0 0 0 0 0.4 |
| 18   | 2 2 2 2 0.071429 | 2 3 1 2 0.3 | 0 0 0 0 0.4 |
| 20   | 0 1 2 1 0 | 2 1 3 2 0.1 | 1 0 2 1 0.3 |
| 22   | 0 0 0 0 0 | 1 0 2 1 0 | 0 0 0 0 0.3 |
| 24   | 0 0 0 0 0 | 0 0 0 0 0 | 1 0 2 1 0.2 |
| 26   | 0 0 0 0 0 | 1 0 2 1 0 | 0 0 0 0 0.3 |
| 28   | 0 0 0 0 0 | 2 1 3 2 0 | 0 0 0 0 0 |

1, 2, 3: Replicate number; AVG: Average

### B)

| Days | Control | 10 µg | 20 µg |
|------|---------|-------|-------|
|      | 1 2 3 AVG | 1 2 3 AVG | 1 2 3 AVG |
| 0    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 2    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 4    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 6    | 5 3 4 4 0.428571 | 0 0 0 0 1 | 0 0 0 0 1 |
| 8    | 4 3 2 3 0 | 0 0 0 0 1 | 0 0 0 0 1 |
| 10   | 0 0 0 0 0 | 3 4 5 4 0.428571 | 2 3 4 3 0.727273 |
| 12   | 3 2 1 2 0.142857 | 3 5 4 3 0.363636 |
| 14   | 0 2 1 1 0 | 2 0 1 1 0.272727 |
| 16   | 0 0 0 0 | 1 3 2 2 0.090909 |
| 18   | 0 0 0 0 | 1 0 2 1 0 |
| 20   | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |

1, 2, 3: Replicate number; AVG: Average
**Supplementary Table 10:** Number of dead worms over a time period of days maintained at **A)** 20 °C and **B)** 25 °C under the treatment of juice of *P. granatum*, and the fraction of survival was calculated using prism software. **(A)** Raw data to Figure 9c and **B)** Figure 9f in the main document.

**A)**

| Days | Control | 10 μg | 20 μg |
|------|---------|-------|-------|
|      | Fraction of survival | Fraction of survival | Fraction of survival |
| 0    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 2    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 4    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 6    | 2 3 1 2 | 0 0 0 0 | 0 0 0 0 |
| 8    | 3 2 4 3 | 0 0 0 0 | 0 0 0 0 |
| 10   | 1 3 2 2 | 0 2 1 1 | 4 2 3 3 |
| 12   | 4 3 5 4 | 2 1 3 2 | 1 4 1 2 |
| 14   | 0 0 0 0 | 0 0 0 0 | 1 2 0 1 |
| 16   | 0 0 0 0 | 0 0 0 0 | 2 0 1 1 |
| 18   | 3 1 2 2 | 0 0 1 0 | 4 2 3 3 |
| 20   | 2 1 0 1 | 6 9 6 7 | 1 2 3 2 |
| 22   | 0 0 0 0 | 2 2 2 2 | 2 2 2 2 |
| 24   | 4 3 2 3 | 0 0 0 0 | 0 0 0 0 |
| 26   | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |

1, 2, 3: Replicate number; AVG: Average.

**B)**

| Days | Control | 10 μg | 20 μg |
|------|---------|-------|-------|
|      | Fraction of survival | Fraction of survival | Fraction of survival |
| 0    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 2    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 4    | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 6    | 4 5 3 4 | 0 0 0 0 | 0 0 0 0 |
| 8    | 2 4 3 3 | 0 0 0 0 | 0 0 0 0 |
| 10   | 0 0 0 0 | 2 4 6 4 | 8 7 6 7 |
| 12   | 1 3 2 2 | 0 0 0 0 | 0 2 1 1 |
| 14   | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 16   | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| 18   | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |

1, 2, 3: Replicate number; AVG: Average.
Supplementary Table 11: % survival of treated and control *C. elegans* under the oxidative stress produced by different concentrations of H$_2$O$_2$ (Raw data to Figure 10 in the main document)

| Conc. of H$_2$O$_2$ | 10 mM | 15 mM | 20 mM |
|-------------------|------|------|------|
|                   | C    | OP   | IP   | J    | C    | OP   | IP   | J    | C    | OP   | IP   | J    |
| 1                 | 3.168| 5.386| 4.872| 7.3  | 2.836| 4.948| 4.665| 6.25 | 2.66 | 4.628| 4.3  | 4.26 |
| 2                 | 3.17 | 5.372| 4.894| 7.19 | 2.839| 4.954| 4.66 | 6.13 | 2.671| 4.633| 5   | 4.16 |
| 3                 | 3.16 | 5.394| 4.874| 7.26 | 2.824| 4.941| 4.655| 6.17 | 2.662| 4.614| 4.2  | 4.14 |
| AVG               | 3.166| 5.384| 4.88 | 7.25 | 2.833| 4.947| 4.66 | 6.183| 2.664| 4.625| 4.5  | 4.186|
| SD                | 0.005292| 0.011136| 0.012166| 0.055678| 0.007937| 0.006506| 0.005| 0.061101| 0.005859| 0.009849| 0.43589| 0.064291|

C: Control; OP: Outer Peel; IP: Inner Peel; J: Juice; AVG: Average; SD: Standard Deviation

1, 2, 3: replicate numbers