# Supplementary Information

# Antihyperglycemic properties of extracts and isolated compounds from Australian *Acacia saligna* on 3T3-L1 adipocytes

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**Table S1.** The extraction results from dried (250g) flowers, leaves, or bark of *A. saligna*.

| Type of Extract | ID, Mass of Extract (g) | | |
|---|---|---|---|
| | **Dried Flowers (FL)** | **Dried Leaves (LF)** | **Dried Barks (BK)** |
| Hexane (hex) | FL-hex, 1.71 | LF-hex, 3.08 | BK-hex, 0.68 |
| Dichloromethane (DCM) | FL-DCM, 1.79 | LF-DCM, 4.98 | BK-DCM, 2.12 |
| Methanol (MeOH) | FL-MeOH, 26.16 | LF-MeOH, 25.37 | BK-MeOH, 18.26 |
| Water ($H_2O$) | FL-$H_2O$, 36.31 | LF-$H_2O$, 13.32 | BK-$H_2O$, 4.34 |

**Table S2.** Viable 3T3-L1 adipocytes treated with isolates for 24, 48, and 72 h.

| Sample | Incubation (h) | Viable adipocytes (%) at concentrations of (μM) | | | |
|---|---|---|---|---|---|
| | | 15.63 | 31.25 | 62.5 | 125 |
| Vehicle (treatment-free) | 24 | 100 ± 1.81 | | | |
| | 48 | 100 ± 2.52 | | | |
| | 72 | 100 ± 1.36 | | | |
| Naringenin 1 | 24 | 98.67 ± 3.21 | 99.29 ± 3.58 | 97.92 ± 3.91 | 93.54 ± 1.92 |
| | 48 | 91.82 ± 8.36 | 98.59 ± 4.79 | 98.18 ± 4.63 | 94.33 ± 3.55 |
| | 72 | 92.65 ± 5.58 | 84.54 ± 6.28 | 87.11 ± 7.49 | 78.31 ± 2.28** |
| Naringenin-7O-$\alpha$-L-arabinopyranose 2 | 24 | 100.6 ± 1.39 | 97.51 ± 1.27 | 97.53 ± 2.69 | 96.47 ± 3.10 |
| | 48 | 96.53 ± 8.24 | 94.4 ± 6.88 | 91.69 ± 6.60 | 90.01 ± 10.17 |
| | 72 | 78.09 ± 4.08* | 78.99 ± 5.77* | 89.79 ± 5.81 | 90.06 ± 4.65 |
| Isosalipurposide 3 | 24 | 93.75 ± 0.57* | 96.26 ± 1.82 | 96.65 ± 2.25 | 96.92 ± 1.87 |
| | 48 | 93.21 ± 0.10 | 93.78 ± 2.31 | 95.64 ± 3.49 | 95.71 ± 1.44 |
| | 72 | 100.2 ± 4.14 | 101 ± 3.92 | 101 ± 4.02 | 101.3 ± 3.07 |
| Quercitrin 4 | 24 | 97.81 ± 1.83 | 99.16 ± 1.23 | 99.1 ± 0.98 | 98.46 ± 1.56 |
| | 48 | 95.74 ± 1.42 | 96.33 ± 0.94 | 98.02 ± 1.59 | 96.99 ± 2.34 |
| | 72 | 98.54 ± 1.3 | 98.76 ± 1.56 | 99.32 ± 1.59 | 99.92 ± 2.12 |
| D-(+)-pinitol 5a | 24 | 93.58 ± 3.19 | 92.6 ± 2.69 | 93.65 ± 3.39 | 95.3 ± 3.63 |
| | 48 | 94.42 ± 4.16 | 97.64 ± 3.42 | 95.06 ± 3.84 | 95.32 ± 4.72 |
| | 72 | 97.92 ± 4.66 | 98.62 ± 4.07 | 98.19 ± 3.04 | 97.54 ± 3.11 |
| (–)-Pinitol 5b | 24 | 91.36 ± 2.37 | 91.89 ± 2.19 | 91.36 ± 1.73 | 93.41 ± 2.65 |
| | 48 | 92.01 ± 3.03 | 93.7 ± 3.96 | 93.45 ± 3.92 | 93.35 ± 3.85 |
| | 72 | 94.38 ± 2.90 | 93.97 ± 4.30 | 93.58 ± 3.58 | 95.94 ± 3.92 |
| (–)-Epicatechin 6 | 24 | 98.17 ± 1.55 | 100.1 ± 0.94 | 100 ± 0.41 | 99.31 ± 0.77 |
| | 48 | 97.94 ± 3.33 | 97.78 ± 4.22 | 98.39 ± 4.32 | 97.73 ± 1.01 |
| | 72 | 88.74 ± 7.24 | 94.62 ± 2.01 | 92.28 ± 1.38 | 86.87 ± 6.90* |
| 2,4-Di-t-butylphenol 7 | 24 | 100.7 ± 1.05 | 98.31 ± 2.16 | 101.2 ± 1.52 | 101.5 ± 1.60 |
| | 48 | 97.8 ± 4.18 | 90.83 ± 1.07 | 101.4 ± 3.42 | 101.5 ± 4.69 |
| | 72 | 98.8 ± 1.35 | 82 ± 5.34* | 88.6 ± 10.96 | 90.2 ± 7.58 |
| Myricitrin 8 | 24 | 98.77 ± 0.29 | 97.99 ± 3.07 | 100 ± 0.70 | 100.4 ± 1.57 |
| | 48 | 95.91 ± 2.47 | 94.4 ± 3.99* | 95.05 ± 3.17 | 95.6 ± 1.52 |
| | 72 | 104.6 ± 5.07 | 105.4 ± 5.27 | 102.7 ± 2.84 | 104 ± 3.47 |
| 3-Hydroxy-5-(2-aminoethyl)dihydrofuran-2(3H)-one 9 | 24 | 100.2 ± 1.41 | 100.9 ± 1.80 | 97.23 ± 4.11 | 98.67 ± 1.84 |
| | 48 | 96.19 ± 5.80 | 96.76 ± 5.56 | 95.56 ± 6.77 | 96.84 ± 3.16 |
| | 72 | 82.7 ± 3.24* | 95.72 ± 3.18 | 89.75 ± 6.28 | 85.22 ± 4.60* |

* $p = 0.01$; ** $p = 0.003$, $p$ values were from indicated samples *vs* vehicle control ($n = 3$, one-way ANOVA, with Dunnett post hoc tests).

**Table S3.** The estimated ROS level of adipocytes exposed to isolated compounds for 48 h.

| Sample | Cellular ROS level (%) at the corresponding concentration (μM) | |
| --- | --- | --- |
| | **0.5** | **10** |
| **Vehicle** | 100 ± 1.87 | |
| **Naringenin 1** | 98.7 ± 2.89 | 75.82 ± 6.20* |
| **Naringenin-7-O-$\alpha$-L-arabinopyranoside 2** | 99.06 ± 10.95 | 76.64 ± 5.16 |
| **Isosalipurposide 3** | 98.33 ± 2.27 | 80.13 ± 7.52 |
| **2,4-Di-t-buylphenol 7** | 99.95 ± 0.93 | 87.94 ± 5.29 |
| **Quercitrin 4** | 102.9 ± 0.36 | 87.65 ± 0.72 |
| **Myricitrin 8** | 100.5 ± 7.66 | 78.64 ± 6.14 |
| **3-Hydroxy-5-(2-aminoethyl) dihydrofuran-2(3H)-one 9** | 99.6 ± 6.24 | 92.67 ± 3.20 |
| **(−)-Pinitol 5b** | 88.76 ± 2.96 | 79.57 ± 6.40 |
| **(−)-Epicatechin 6** | 105.5 ± 3.99 | 71.45 ± 4.82** |
| ***D*-(+)-pinitol 5a** | 89.84 ± 0.88 | 69.24 ± 3.90** |
| **NAC 5 mM** | 78.28 ± 2.83 | |
| **NAC 10 mM** | 64.74 ± 2.24*** | |
| **Undifferentiated cells** | 53.79 ± 5.41**** | |

$p = 0.05$, ** $p = 0.002$, *** $p = 0.0003$, and **** $p = 0.000003$ were from the ROS level of the indicated samples *vs* vehicle control ($n = 3$, one-way ANOVA, with Tukey post hoc tests).

**Table S4**. Observed data of glucose uptake simulation with the fluoroprobe 2-NBDG assay for methanolic extracts on the 3T3-L1 adipocytes.

| Sample | 2-NBDG uptake percentage (%) | |
| --- | --- | --- |
| | **12.5 μg/mL** | **50 μg/mL** |
| **Vehicle** | 100 ± 6.54 | |
| **FL-MeOH** | 141.5 ± 27.94 | 185.3 ± 41.52 ** |
| **LF-MeOH** | 113.5 ± 6.1 | 198 ± 42.61 ** |
| **BK-MeOH** | 118.3 ± 9.517 | 161.6 ± 10.76 |
| **Insulin 100 nM** | 140.6 ± 18.36 | |
| **Metformin 10 μM** | 138 ± 28.26 | |

** $p = 0.007$ for FL-MeOH and ** $p = 0.006$ for LF-MeOH compared to the vehicle control ($n = 3$, one-way ANOVA, with Dunnett post hoc tests).

**Table S5**. Observed data of glucose uptake simulation with 2-NBDG fluorescence assay for isolated compounds on the 3T3-L1 adipocytes.

| Sample | 2-NBDG uptake percentage (%) | |
|---|---|---|
| | 0.5 μM | 10 μM |
| **Vehicle** | 100 ± 6.54 | |
| **Naringenin 1** | 89.3 ± 9.47 | 127.3 ± 15 |
| **Naringenin-7-O-*α*-*L*-arabinopyranoside 2** | 107.6 ± 7.89 | 156.4 ± 22.26 |
| **Isosalipurposide 3** | 110.7 ± 13.26 | 161 ± 39.47 |
| **Quercitrin 4** | 101.6 ± 14.07 | 151 ± 10.03 |
| ***D*-(+)-pinitol 5a** | 108.5 ± 11.36 | 143.9 ± 12.56 |
| **(−)-Pinitol 5b** | 96.99 ± 3.25 | 125.6 ± 13.27 |
| **(−)-Epicatechin 6** | 108.3 ± 1.12 | 187.9 ± 41.95* |
| **2,4-Di-*t*-buylphenol 7** | 86.39 ± 10.81 | 131.2 ± 21.57 |
| **Myricitrin 8** | 122.7 ± 10.74 | 152.3 ± 24.02 |
| **3-Hydroxy-5-(2-aminoethyl) dihydrofuran-2(3H)-one 9** | 89.56 ± 7.20 | 96.64 ± 10.97 |
| **Insulin 100 nM** | 140.6 ± 18.36 | |
| **Metformin 10 μM** | 138 ± 28.26 | |

*$p$ = 0.01, $p$ value was from the indicated sample against the vehicle control ($n$ = 3, one-way ANOVA, with Dunnett post hoc tests).

**Table S6**. Quantitative data of the ratio of expressed p-AMPK-*α* to AMPK-*α* (%) by adipocytes exposed to methanolic extracts.

| Treatment | Concentration | Ratio of p-AMPK-*α* to AMPK-*α* (%) |
|---|---|---|
| **Vehicle (treatment-free)** | - | 100 ± 17.16 |
| **Metformin** | 10 μM | 166.4 ± 14.08 |
| **FL-MeOH** | 12.5 μg/mL | 128.1 ± 10.56 |
| | 50 μg/mL | 177 ± 16.98* |
| **LF-MeOH** | 12.5 μg/mL | 128.9 ± 12.97 |
| | 50 μg/mL | 158.5 ± 13.76 |
| **BK-MeOH** | 12.5 μg/mL | 129.7 ± 22.79 |
| | 50 μg/mL | 149.1 ± 25.85 |

* $p$ = 0.02, $p$ value was of the sample against the vehicle control ($n$ = 3, one-way ANOVA, with Tukey post hoc tests).

**Table S7.** Quantitative data of the ratio of expressed p-AMPK-$\alpha$ to AMPK-$\alpha$ (%) by adipocytes exposed to isolated compounds.

| Sample | Concentration (μM) | Ratio of p-AMPK-$\alpha$ to AMPK-$\alpha$ (%) |
|---|---|---|
| **Vehicle** | - | 100 ± 13.36 |
| **Metformin** | 10 | 191.8 ± 21.86** |
| **Naringenin 1** | 0.5 | 123.8 ± 2.34 |
| | 10 | 148.4 ± 13.56 |
| **Naringenin-7-O-$\alpha$-L-arabinopyranoside 2** | 0.5 | 139.1 ± 13.04 |
| | 10 | 211.8 ± 30.27*** |
| **Isosalipurposide 3** | 0.5 | 129.8 ± 14.7 |
| | 10 | 196.6 ± 20.33** |
| **Quercitrin 4** | 0.5 | 110.7 ± 11.33 |
| | 10 | 148.6 ± 12.2 |
| ***D*-(+)-pinitol 5a** | 0.5 | 91.72 ± 6.07 |
| | 10 | 98.61 ± 8.55 |
| **(−)-Pinitol 5b** | 0.5 | 94.05 ± 7.39 |
| | 10 | 102.7 ± 22.67 |
| **(−)-Epicatechin 6** | 0.5 | 99.62 ± 3.88 |
| | 10 | 143.2 ± 17.25 |
| **Myricitrin 8** | 0.5 | 109.4 ± 10.11 |
| | 10 | 156 ± 8.11 |

** $p = 0.003$, *** $p = 0.0002$, $p$ values were of samples against the vehicle control ($n = 3$, one-way ANOVA, with Tukey post hoc tests).

**Legend**
1: vehicle control
2: metformin 10 μM
3: FL-MeOH 12.5 μg/mL
4: FL-MeOH 50 μg/mL
5: LF-MeOH 12.5 μg/mL
6: LF-MeOH 50 μg/mL
7: BK-MeOH 12.5 μg/mL
8: BK-MeOH 50 μg/mL
9: compound **2** 0.5 μM
10: compound **2** 10 μM
11: isosalipurposide **3** 0.5 μM
12: isosalipurposide **3** 10 μM

**(a) p-AMPK-α**

**Figure S1a.** Original Western blot images of membrane 1 for the immunoblot analysis of p-AMPK.

**Legend**
1: vehicle control
2: metformin 10 μM
3: FL-MeOH 12.5 μg/mL
4: FL-MeOH 50 μg/mL
5: LF-MeOH 12.5 μg/mL
6: LF-MeOH 50 μg/mL
7: BK-MeOH 12.5 μg/mL
8: BK-MeOH 50 μg/mL
9: compound **2** 0.5 μM
10: compound **2** 10 μM
11: isosalipurposide **3** 0.5 μM
12: isosalipurposide **3** 10 μM

**(b) AMPK-α**

**Figure S1b.** Original Western blot images of membrane 1 for the immunoblot analysis of AMPK.

**Legend**
1: vehicle control
2: metformin 10 µM
3: FL-MeOH 12.5 µg/mL
4: FL-MeOH 50 µg/mL
5: LF-MeOH 12.5 µg/mL
6: LF-MeOH 50 µg/mL
7: BK-MeOH 12.5 µg/mL
8: BK-MeOH 50 µg/mL
9: compound **2** 0.5 µM
10: compound **2** 10 µM
11: isosalipurposide **3** 0.5 µM
12: isosalipurposide **3** 10 µM

**(c) α-tubulin**

**Figure S1c.** Original Western blot images of membrane 1 for the immunoblot analysis of α-tubulin

Legend
1: vehicle
2: metformin 10 μM
3: quercitrin **4** 0.5 μM
4: quercitrin **4** 10 μM
5: myricitrin **8** 0.5 μM
6: myricitrin **8** 10 μM
7: metformin 10 μM
8: (−)-epicatechin **6** 0.5 μM
9: (−)-epicatechin **6** 10 μM
10: *D*-(+)-pinitol **5a** 0.5 μM
11: *D*-(+)-pinitol **5a** 10 μM
12: *D*-(+)-pinitol **5a** 10 μM

**(a) p-AMPK-α**

**Figure S2a.** Original Western blot images of membrane 2 for the immunoblot analysis of p-AMPK

**Legend**
1: vehicle
2: metformin 10 μM
3: quercitrin **4** 0.5 μM
4: quercitrin **4** 10 μM
5: myricitrin **8** 0.5 μM
6: myricitrin **8** 10 μM
7: metformin 10 μM
8: (−)-epicatechin **6** 0.5 μM
9: (−)-epicatechin **6** 10 μM
10: *D*-(+)-pinitol **5a** 0.5 μM
11: *D*-(+)-pinitol **5a** 10 μM
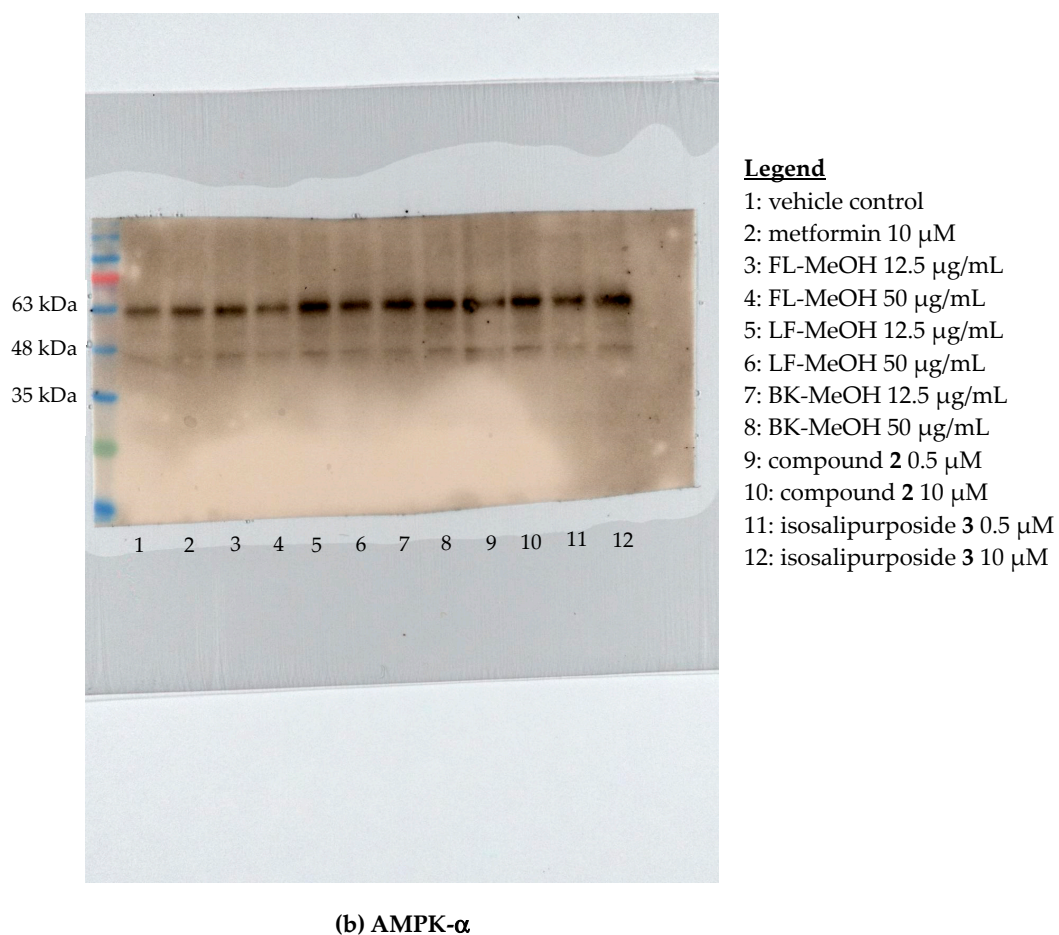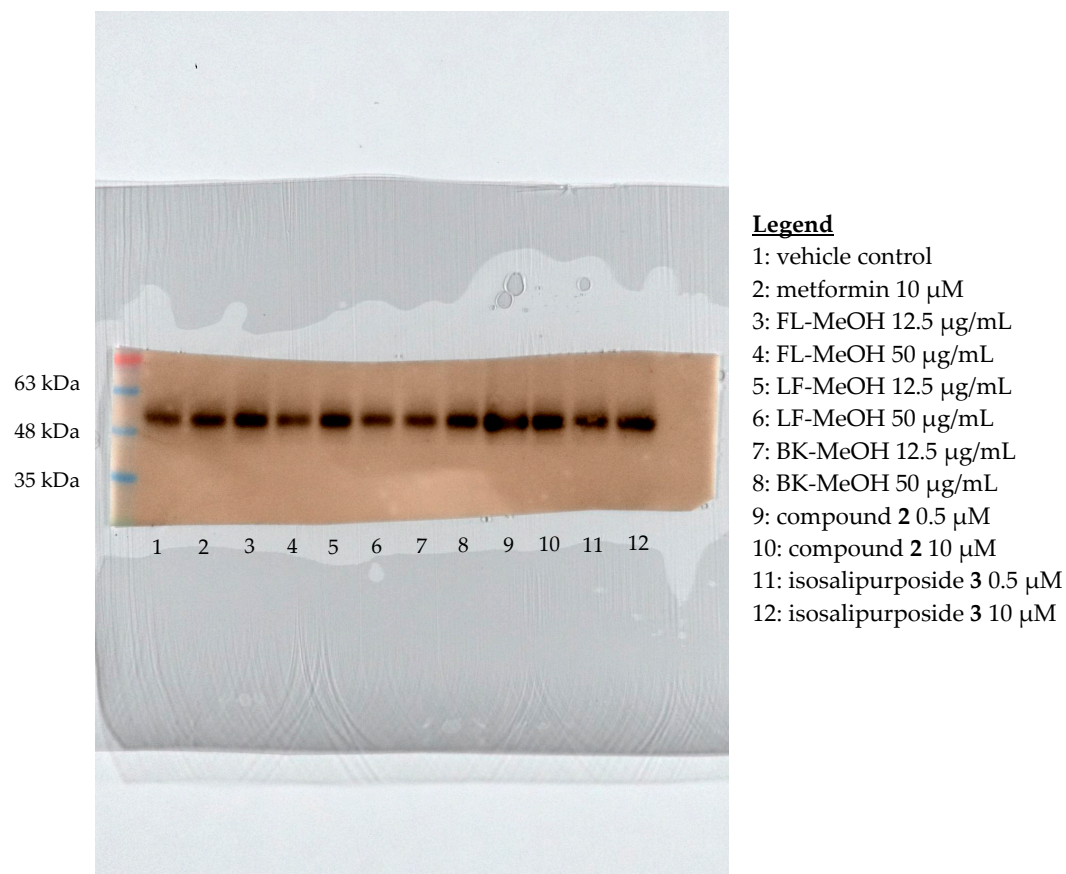12: *D*-(+)-pinitol **5a** 10 μM
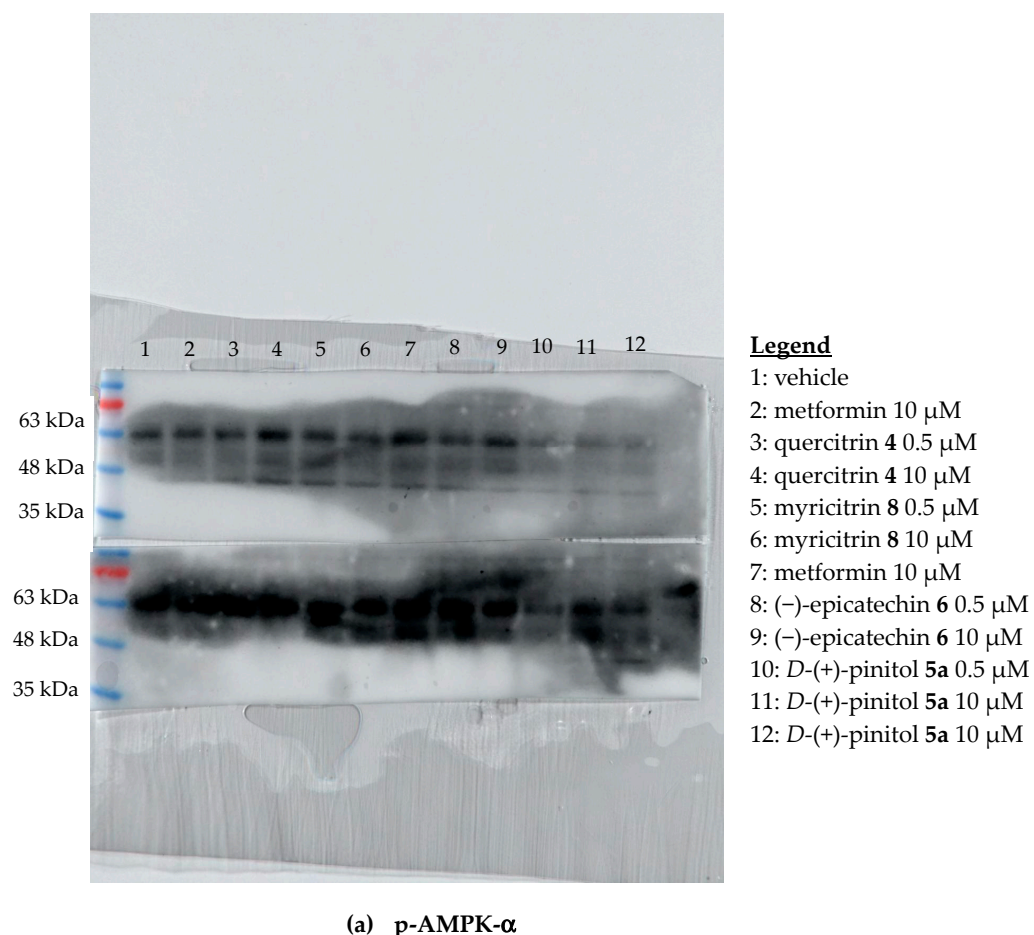
**(b) AMPK-α**

**Figure S2b.** Original Western blot images of membrane 2 for the immunoblot analysis of AMPK

**(c) α-tubulin**

**Figure S2c.** Original Western blot images of membrane 2 for the immunoblot analysis of α-tubulin
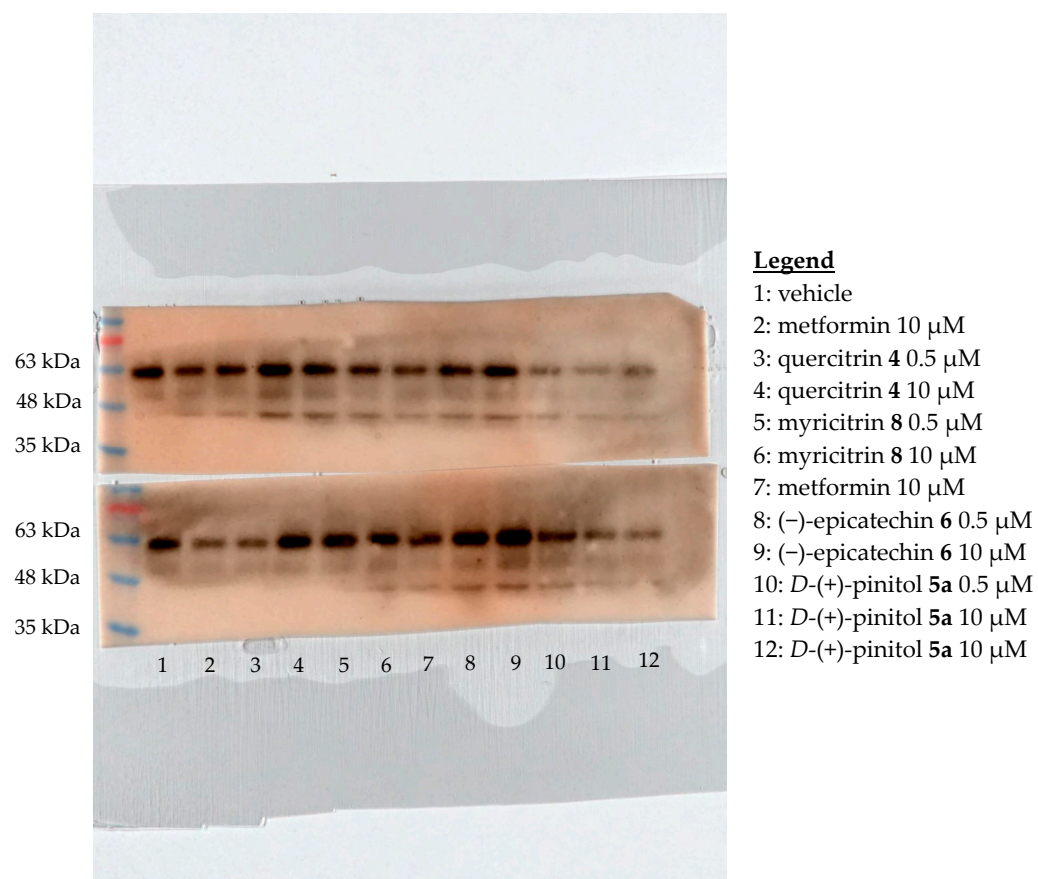
The blot shows molecular weight markers at 63 kDa, 48 kDa, and 35 kDa for both the upper and lower membranes, with lanes numbered 1 through 12.

**Legend**
1: vehicle
2: metformin 10 μM
3: quercitrin **4** 0.5 μM
4: quercitrin **4** 10 μM
5: myricitrin **8** 0.5 μM
6: myricitrin **8** 10 μM
7: metformin 10 μM
8: (−)-epicatechin **6** 0.5 μM
9: (−)-epicatechin **6** 10 μM
10: D-(+)-pinitol **5a** 0.5 μM
11: D-(+)-pinitol **5a** 10 μM
12: D-(+)-pinitol **5a** 10 μM

63 kDa
48 kDa
35 kDa

63 kDa
48 kDa
35 kDa

1  2  3  4  5  6  7  8  9  10  11  12

**Legend**
1: vehicle
2: metformin 10 μM
3: naringenin **1** 0.5 μM
4: naringenin **1** 10 μM
5: quercitrin **4** 0.5 μM
6: quercitrin **4** 10 μM
7: (−)-pinitol **5b** 0.5 μM
8: (−)-pinitol **5b** 10 μM
9: (−)-epicatechin **6** 0.5 μM
10: (−)-epicatechin **6** 10 μM
11: *D*-(+)-pinitol **5a** 0.5 μM
12: *D*-(+)-pinitol **5a** 10 μM
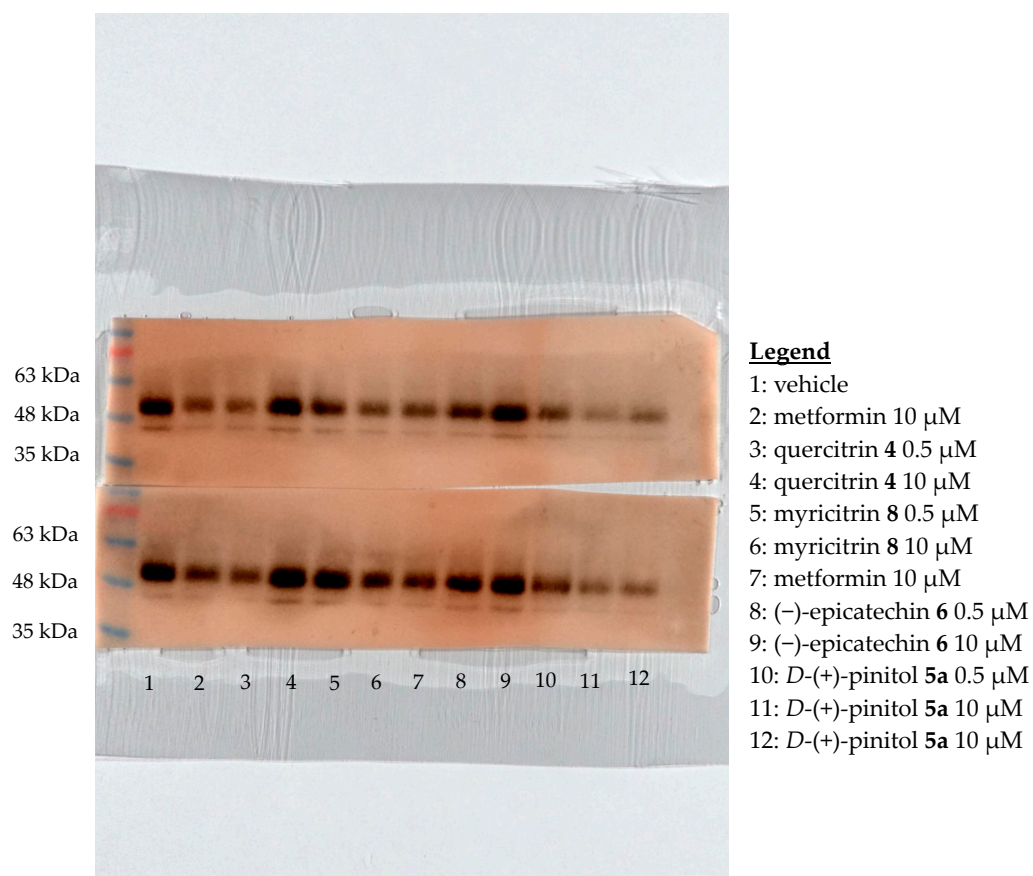
**(a) p-AMPK-α**

**Figure S3a.** Original Western blot images of membrane 3 for the immunoblot analysis of p-AMPK

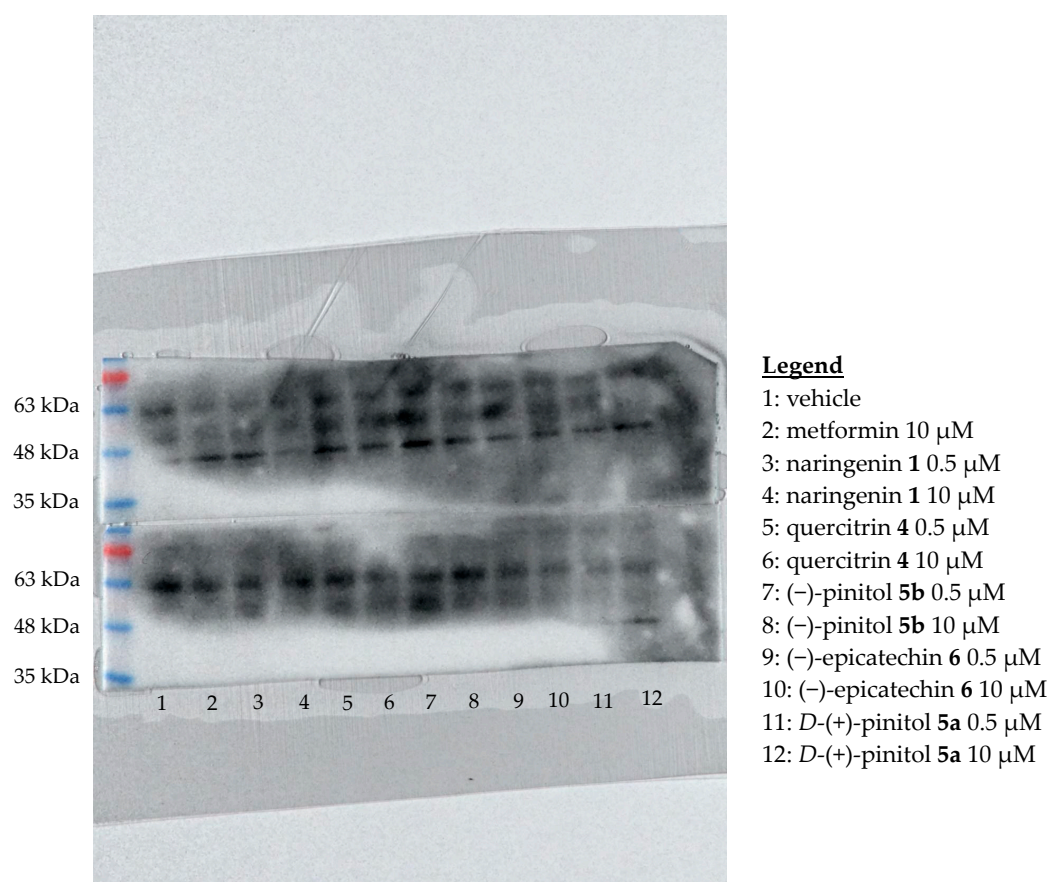**(b) AMPK-α**

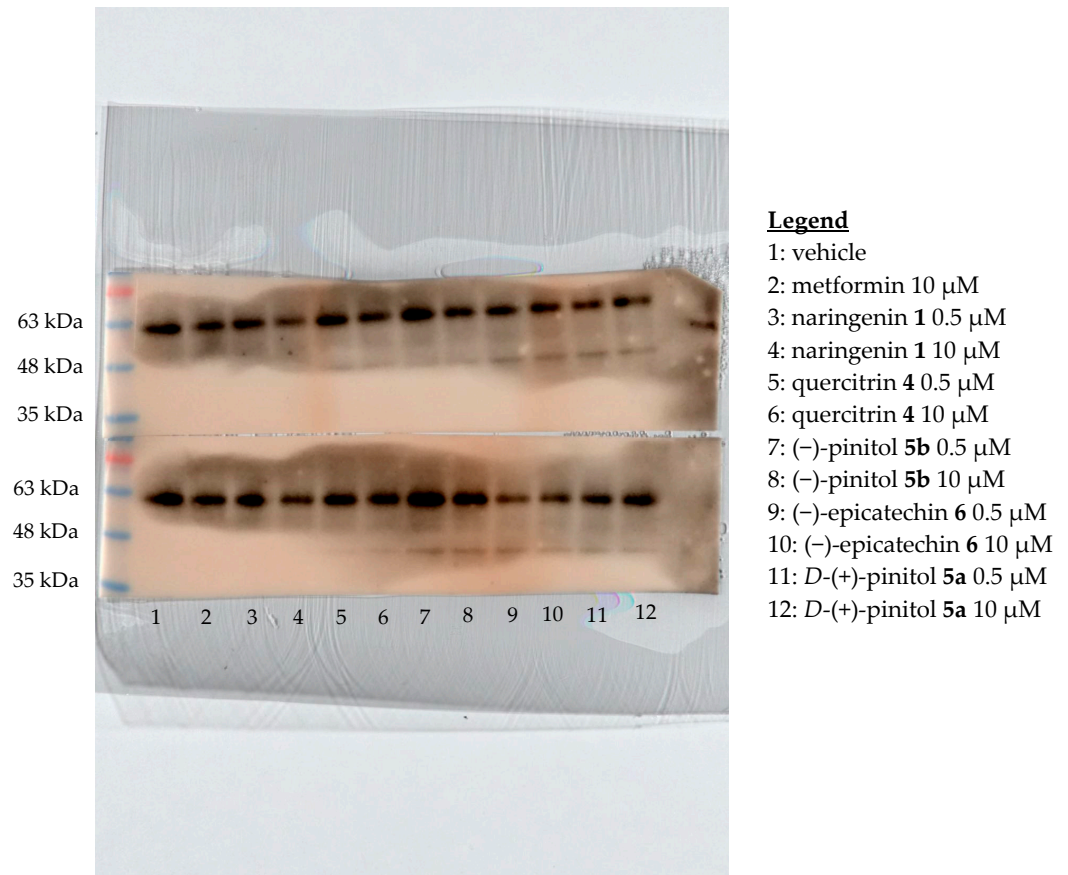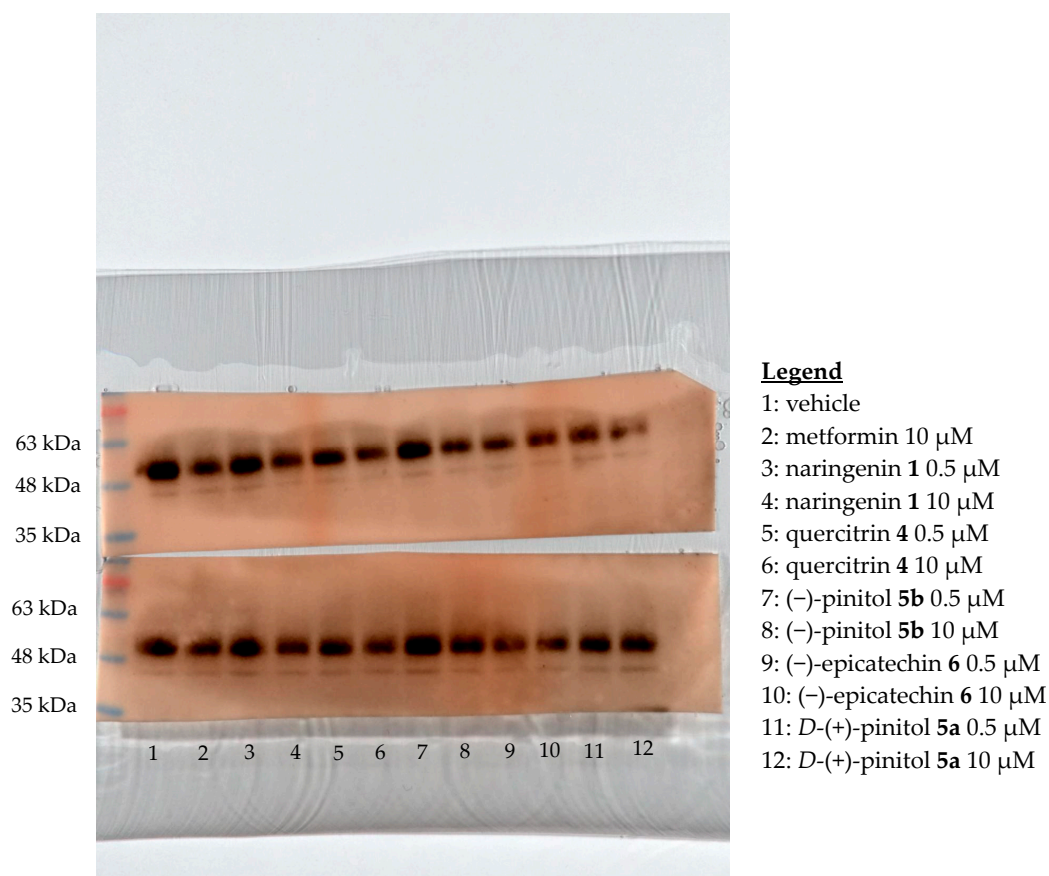**Figure S3b.** Original Western blot images of membrane 3 for the immunoblot analysis of AMPK

Legend for figure:

**Legend**
1: vehicle
2: metformin 10 μM
3: naringenin **1** 0.5 μM
4: naringenin **1** 10 μM
5: quercitrin **4** 0.5 μM
6: quercitrin **4** 10 μM
7: (−)-pinitol **5b** 0.5 μM
8: (−)-pinitol **5b** 10 μM
9: (−)-epicatechin **6** 0.5 μM
10: (−)-epicatechin **6** 10 μM
11: *D*-(+)-pinitol **5a** 0.5 μM
12: *D*-(+)-pinitol **5a** 10 μM

**Legend**
1: vehicle
2: metformin 10 μM
3: naringenin **1** 0.5 μM
4: naringenin **1** 10 μM
5: quercitrin **4** 0.5 μM
6: quercitrin **4** 10 μM
7: (−)-pinitol **5b** 0.5 μM
8: (−)-pinitol **5b** 10 μM
9: (−)-epicatechin **6** 0.5 μM
10: (−)-epicatechin **6** 10 μM
11: *D*-(+)-pinitol **5a** 0.5 μM
12: *D*-(+)-pinitol **5a** 10 μM

**(c) α-tubulin**

**Figure S3c.** Original Western blot images of membrane 3 for the immunoblot analysis of α-tubulin