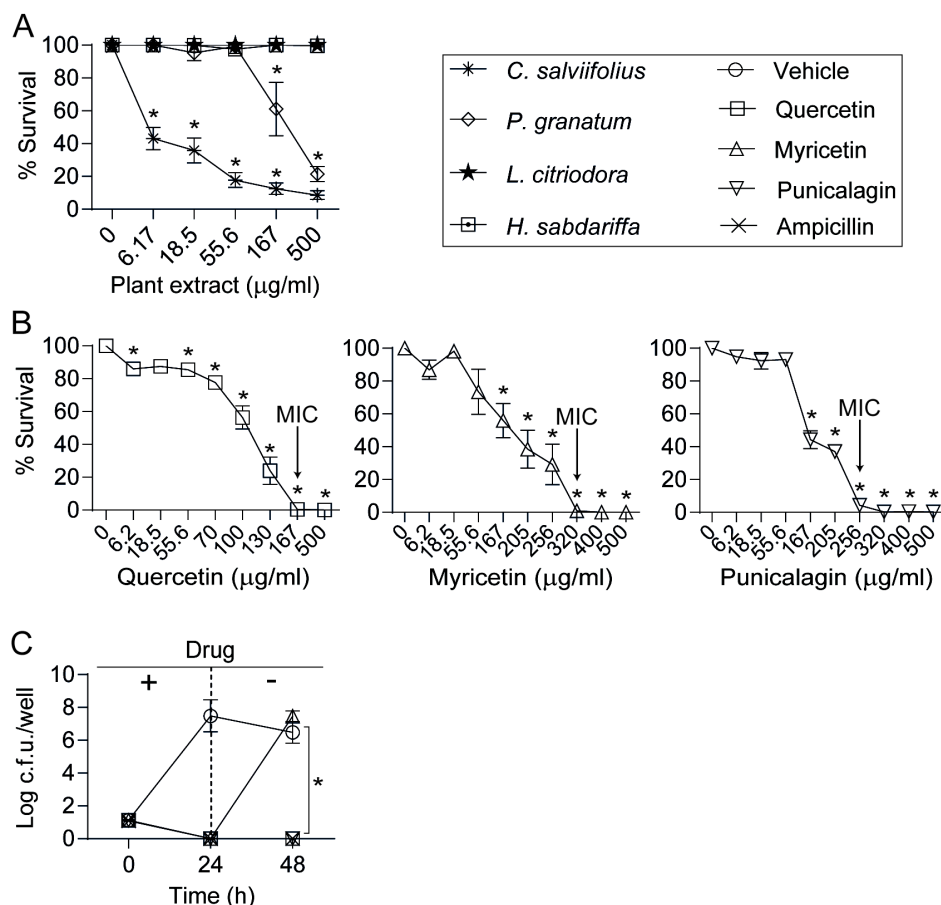


**Supplementary Material**

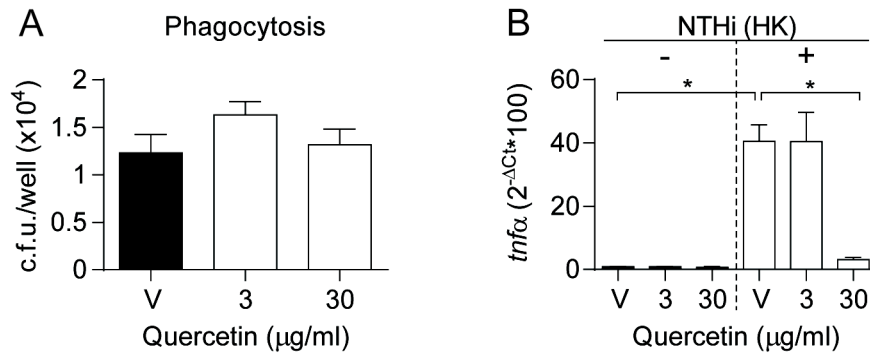
**Preclinical evaluation of the antimicrobial-immunomodulatory dual action of xenohormetic molecules against *Haemophilus influenzae* respiratory infection**

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**Figure S1. Antimicrobial effect of plant extracts and pure polyphenols on *H. influenzae* RdKW20.** (A) RdKW0 strain is susceptible to *C. salviifolius* and *P. granatum* extracts in a dose-dependent manner (\*  $P < 0.005$ ). (B) Polyphenols quercetin (\* $P < 0.005$ ), myricetin (\* $P < 0.0001$ ) and punicalagin (\* $P < 0.0001$ ), reduced RdKW20 survival in a dose-dependent manner. Survival percentage (mean  $\pm$  SEM) is shown (A and B). (C) Quercetin and punicalagin have a bactericidal effect on RdKW20 strain, when comparing bacterial counts (log c.f.u./well, mean  $\pm$  SD) after incubation with- and without polyphenol inhibitory concentrations. At 24 h, only bacteria incubated with vehicle control rendered counts, when compared with ampicillin-treated cultures (\* $P < 0.0001$ ). After polyphenol replacement by sBHI, 48 h bacterial cultures previously incubated with myricetin rendered significant counts, when compared with quercetin, punicalagin and ampicillin treated cultures (\* $P < 0.0001$ ). Statistical comparisons of the means were performed with two-way ANOVA (A and C) or one-way ANOVA (B), and Dunnett's multiple comparisons test.



**Figure S2. Quercetin has an anti-inflammatory effect on NTHi-infected alveolar macrophages.** (A) For phagocytosis assays, murine alveolar macrophages (MH-S) were pretreated with quercetin or DMSO (vehicle solution, V) for 4 h. Drugs were removed prior infection. M-HS cells were infected with NTHi375 strain for 1 h, and incubated with gentamicin for 1 h more. No significant differences were observed in NTHi375 phagocytosis between quercetin- and vehicle-treated cells. Results are shown as c.f.u./well (mean  $\pm$  SEM). (B) M-HS cells were pretreated with quercetin or DMSO for 4 h; next, heat killed (HK) bacteria were used as an inflammatory stimulus (NTHi HK) for 1 h (white bars). In parallel, non-stimulated cells were included as negative controls (black bars). Drugs were maintained throughout the assay. To monitor gene expression, relative quantity of murine *tnfa* was measured by qRT-PCR. Gene expression was significantly increased upon infection of vehicle-treated cells (\* $P$ <0.0001). Quercetin 30  $\mu$ g/mL significantly decreased *tnfa* expression level (\* $P$ <0.0001). Results are shown as the relative gene expression levels (mean  $\pm$  SEM). Statistical comparisons of the means were performed with one-way ANOVA and Bonferroni's multiple comparisons test.

51 **Table S1.** Primers used for qRT-PCR in this study.

| Primer name            | Primer ID | Sequence (5'-3')      | Organism | Reference  |
|------------------------|-----------|-----------------------|----------|------------|
| <i>il8</i> - qPCR-F    | 1241      | AGAGACAGCAGAGCACAC    | Human    | (1)        |
| <i>il8</i> - qPCR-R    | 1242      | AGTTCTTTAGCACTCCTTGG  | Human    | (1)        |
| <i>gapdh</i> -qPCR-F   | 1237      | GAAGGTGAAGGTCGGAGTC   | Human    | (1)        |
| <i>gapdh</i> -qPCR-R   | 1238      | GAAGATGGTGATGGGATTTC  | Human    | (1)        |
| <i>cycl1</i> - qPCR-F  | 1247      | GCAGGGAATTCACCCCAAGA  | Human    | (1)        |
| <i>cycl1</i> - qPCR-R  | 1248      | CTTCAGGAACAGCCACCAGT  | Human    | (1)        |
| <i>pde4b</i> - qPCR-F  | 1259      | GAGACAAAGAGCGGGAGAGG  | Human    | (2)        |
| <i>pde4b</i> - qPCR-R  | 1260      | GGTGGTGAGGGACTTTGAGG  | Human    | (2)        |
| <i>il6</i> - qPCR-F    | 1717      | TAGTGAGGAACAAGCCAGAGC | Human    | This study |
| <i>il6</i> - qPCR-R    | 1718      | TTGGGTCAGGGGTGGTTATTG | Human    | This study |
| <i>kc</i> - qPCR-F     | 1404      | GACAGACTGCTCTGATGGCA  | Mouse    | (2)        |
| <i>kc</i> - qPCR-R     | 1405      | TGCACTTCTTTTCGCACAAC  | Mouse    | (2)        |
| <i>mtnfa</i> - qPCR-F  | 1592      | AGGCACTCCCCCAAAAGATG  | Mouse    | (2)        |
| <i>mtnfa</i> - qPCR-R  | 1593      | GCTCCTCCACTTGGTGGTTT  | Mouse    | (2)        |
| <i>mpde4b</i> - qPCR-F | 1753      | TGGAAATCCTGGCTGCCAT   | Mouse    | This study |
| <i>mpde4b</i> - qPCR-R | 1754      | TCCACAGAAGCTGTGTGCT   | Mouse    | This study |
| <i>mgapdh</i> -qPCR-F  | 1430      | CCCACTAACATCAAATGGGG  | Mouse    | (3)        |
| <i>mgapdh</i> -qPCR-R  | 1431      | CCTTCCACAATGCCAAAGTT  | Mouse    | (3)        |

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**Table S2.** Minimal inhibitory concentration (MIC) of quercetin, myricetin and punicalagin against NTHi clinical isolates, measured by microdilution ( $\mu\text{g/mL}$ ).

| NTHi strain | MIC <sub>quercetin</sub> | MIC <sub>myricetin</sub> | MIC <sub>punicalagin</sub> |
|-------------|--------------------------|--------------------------|----------------------------|
| P650        | 76.0 $\pm$ 12.7          | 312.5 $\pm$ 21.3         | 125.0                      |
| P657        | 122.9 $\pm$ 50.7         | 165.3 $\pm$ 69.1         | 250.0                      |
| P665        | 90.6 $\pm$ 47.6          | 192.3 $\pm$ 19.6         | 62.5                       |

## References

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