Supporting Information for:

Lythrum salicaria Ellagitannins Stimulate IPEC-J2 Cells Monolayer Formation and Inhibit Enteropathogenic *Escherichia coli* Growth and Adhesion.

Sebastian Granica,[†,‡] Wilfried Vahjen,[§] Jürgen Zentek,[§] Matthias F. Melzig,[^] Karolina A. Pawłowska,[†‡] and Jakub P. Piwowarski*[†,‡,§,^]

[†]Department of Pharmacognosy and Molecular Basis of Phytotherapy, and [‡]Centre for Preclinical Studies, Medical University of Warsaw, Poland

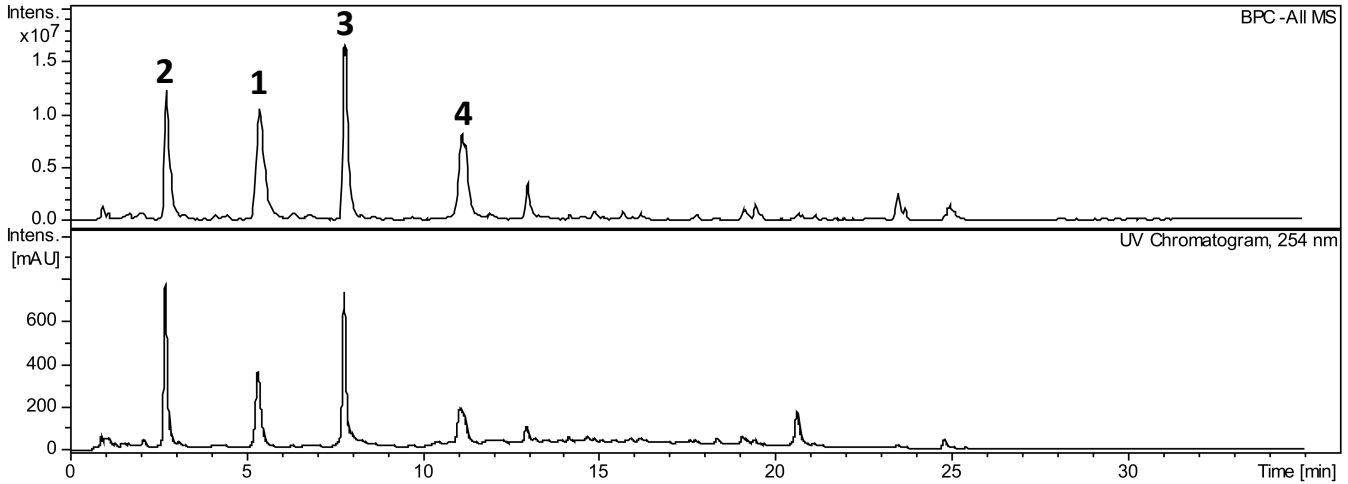
[§]Institute of Animal Nutrition, and [^]Department of Pharmaceutical Biology, Freie Universität Berlin, Germany

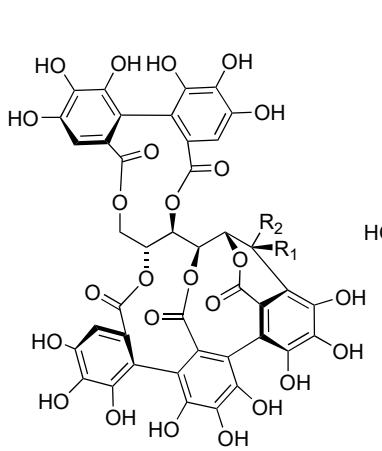
Corresponding Author

Jakub P. Piwowarski − Department of Pharmacognosy and Molecular Basis of Phytotherapy, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, Warsaw 02-097, Poland; Institute of Animal Nutrition, and Department of Pharmaceutical Biology, Freie Universität Berlin, Germany; orcid.org/0000-0002-5011-0983; Phone: +48 225720953; Email: jpiwowarski@wum.edu.pl

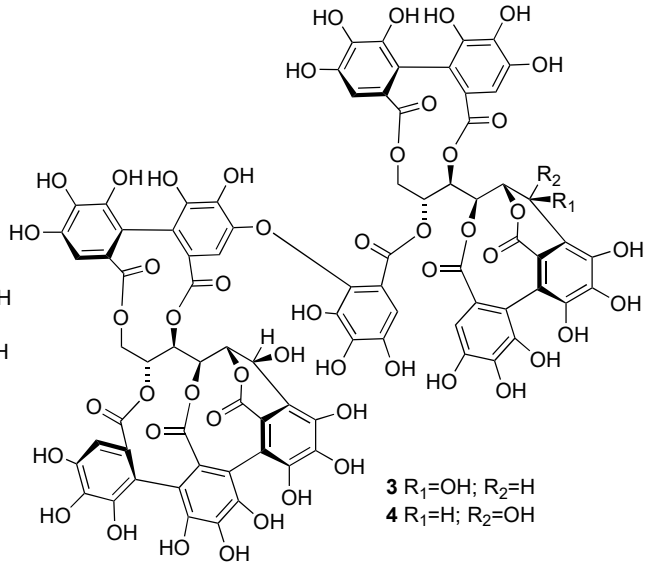
Figure S1. UPLC-DAD-MS chromatograms of *Lythrum salicaria* L. aqueous extract (LSH) recorded at 254 nm and structures of dominating ellagitannins: castalagin (**1**), vescalagin (**2**), salicarinin A (**3**) and salicarinin B (**4**).

UPLC-DAD-MS analysis conditions:
Reversed-phase Kinetex C8 analytical column (100mm×2.1mm×1.7µm), Phenomenex (Torrance, CA, USA). The column temperature was 25°C. Mobile phase A was 0.1% HCOOH in water (v/v) and mobile phase B was 0.1% HCOOH in MeCN (v/v). A multistep gradient solvent system used: 0–5 min 0% B, 5–35 min 0–35% B. The flow rate was 0.2 mL/min.





1 R_1=H; R_2=OH
2 R_1=OH; R_2=H



3 R_1=OH; R_2=H
4 R_1=H; R_2=OH

Figure S2. Structures of tested gut microbiota metabolites: urolithin C (**5**), urolithin A (**6**) and its phase II conjugate-mixture of urolithin A 3-*O*-glucuronide and 8-*O*-glucuronide urolithin A glucuronide (**7**).

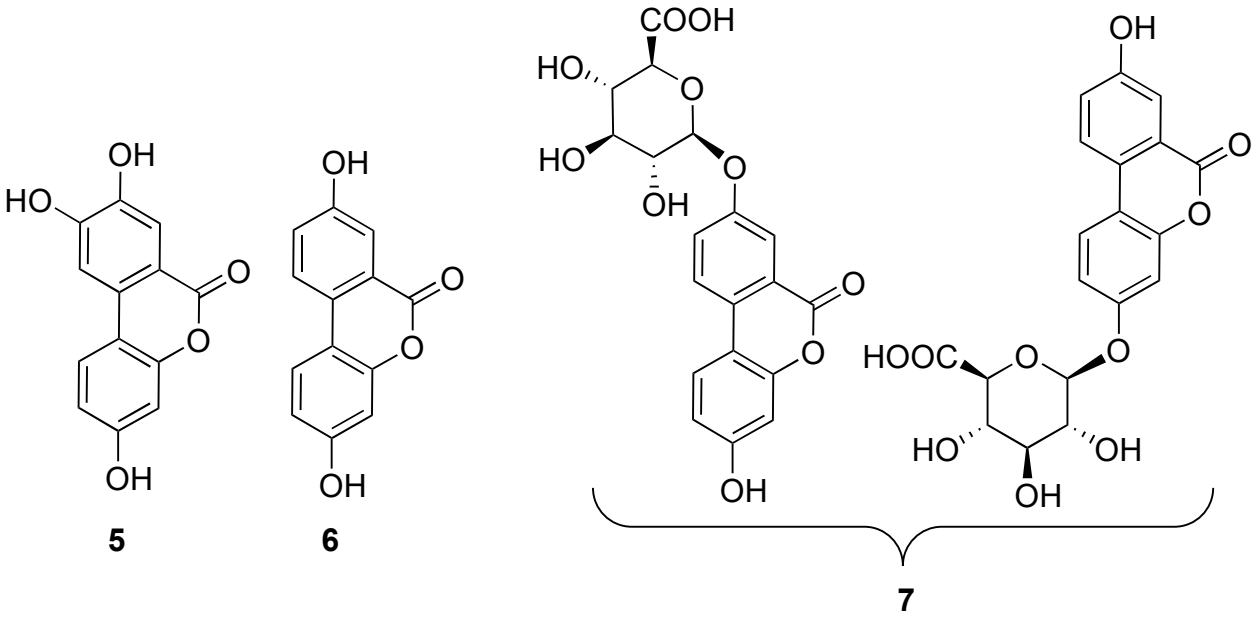


Figure S3A. Growth curves of enteropathogenic *E. coli* strain: IMT O147:K89:K88 (Abbottstown) (A and B) and control strain: DSM 2840 (C and D) incubated with *Lythrum salicaria* extract (LSH) (A and C) or castalagin (**1**) (B and D). The data are representative for three independent experiments assayed in triplicate. Doubling time (G) and lag time (λ) values are

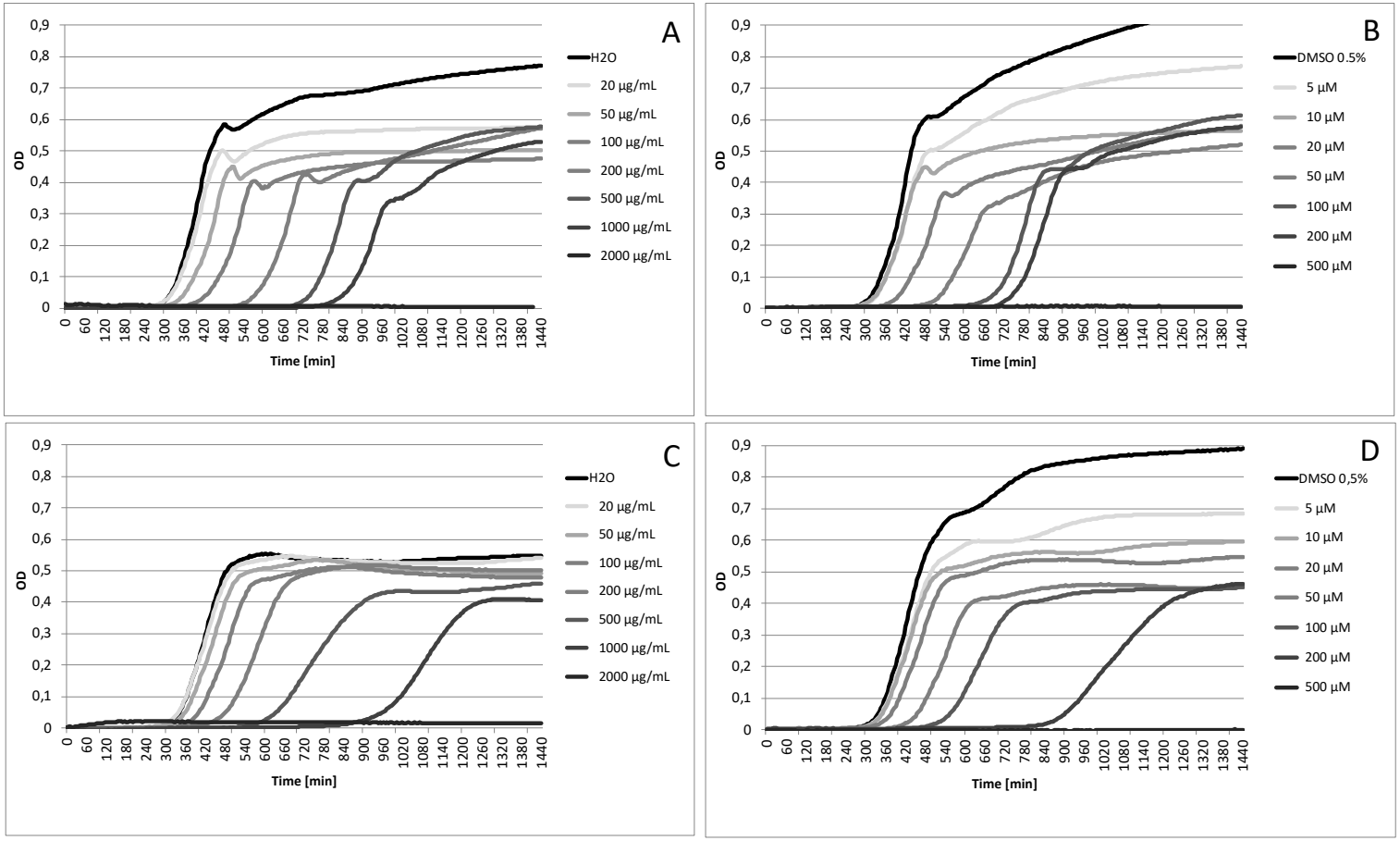
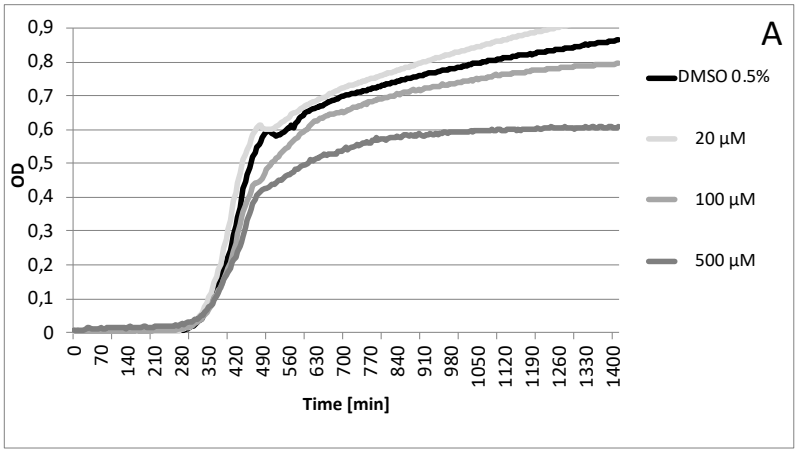
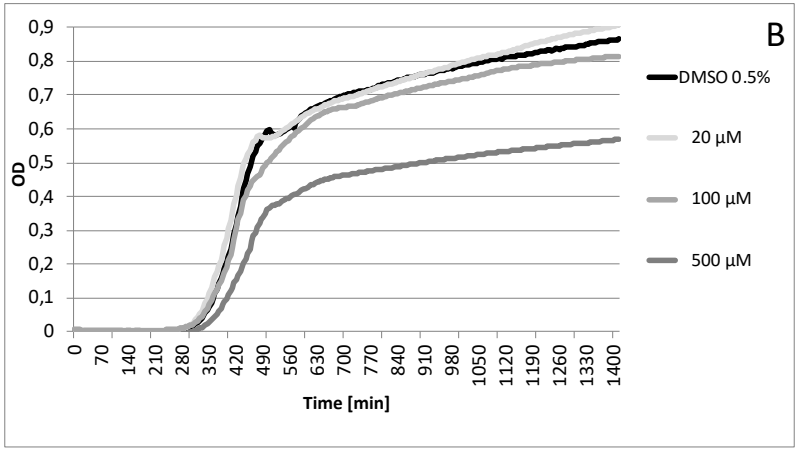


Figure S3B. Growth curves of enteropathogenic *E. coli* strain IMT O147:K89:K88 (Abbottstown) incubated with urolithin C (**5**) (A), urolithin A (**6**) (B) and with its phase II conjugate- mixture of urolithin A 3-O-glucuronide and 8-O-glucuronide urolithin A glucuronide (**7**) (C).





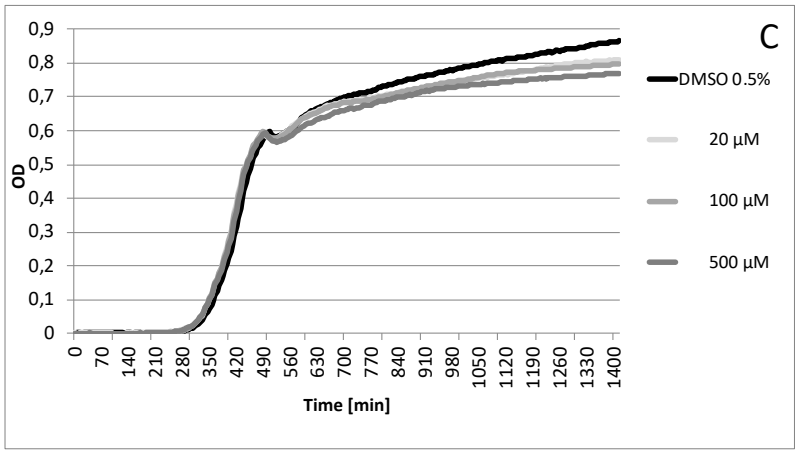


Figure S3C. Growth curves of enteropathogenic *E. coli* strain IMT O147:K89:K88 (Abbottstown) (A) and control strain DSM 2840 (B) incubated with positive control- ciprofloxacin.

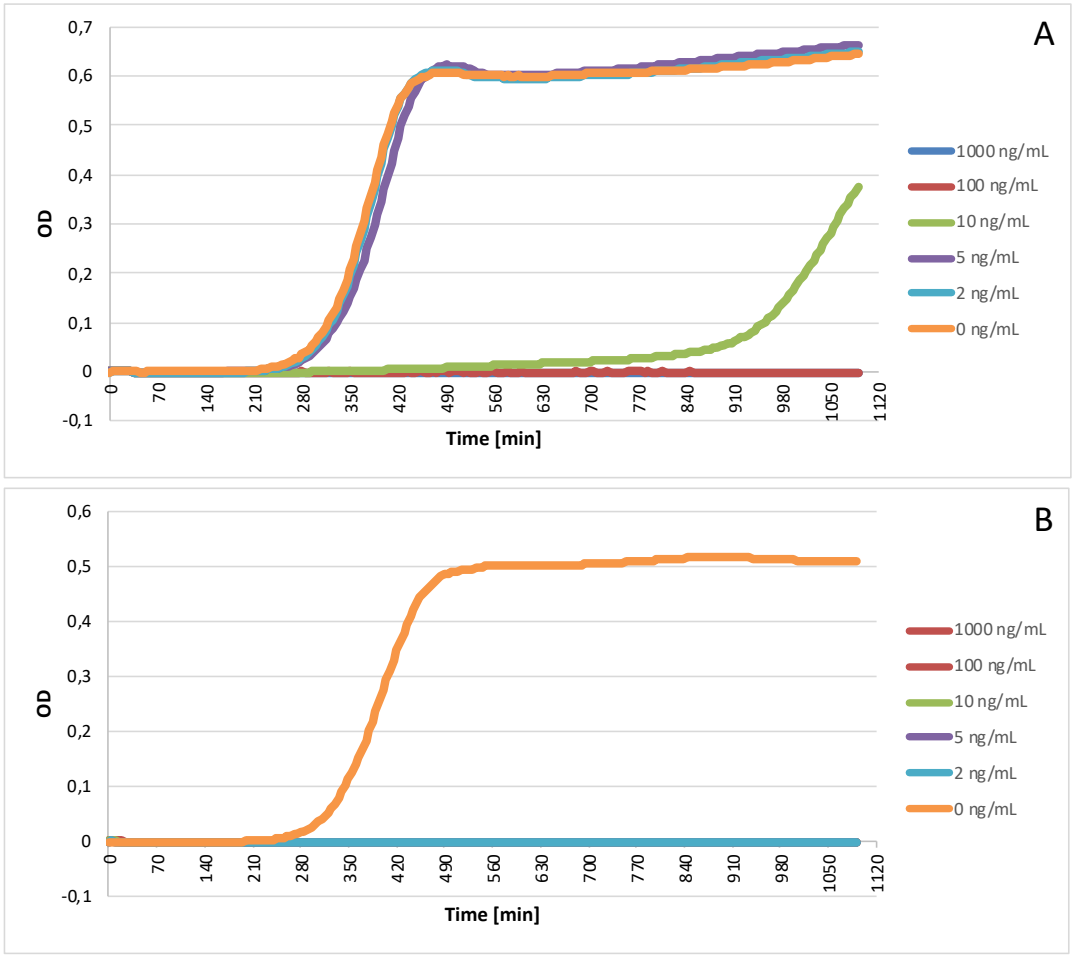


Figure S4. Growth curves of enteropathogenic *E. coli* strain: IMT O147:K89:K88 in piglets fecal samples monitored by qPCR determination of fimbrial *fae* gene copies. Growth curves for increasing *E. coli* inoculum (10^3- 10^6 CFU/mL). pc- pure E. coli culture (BHI), M-fecal sample.

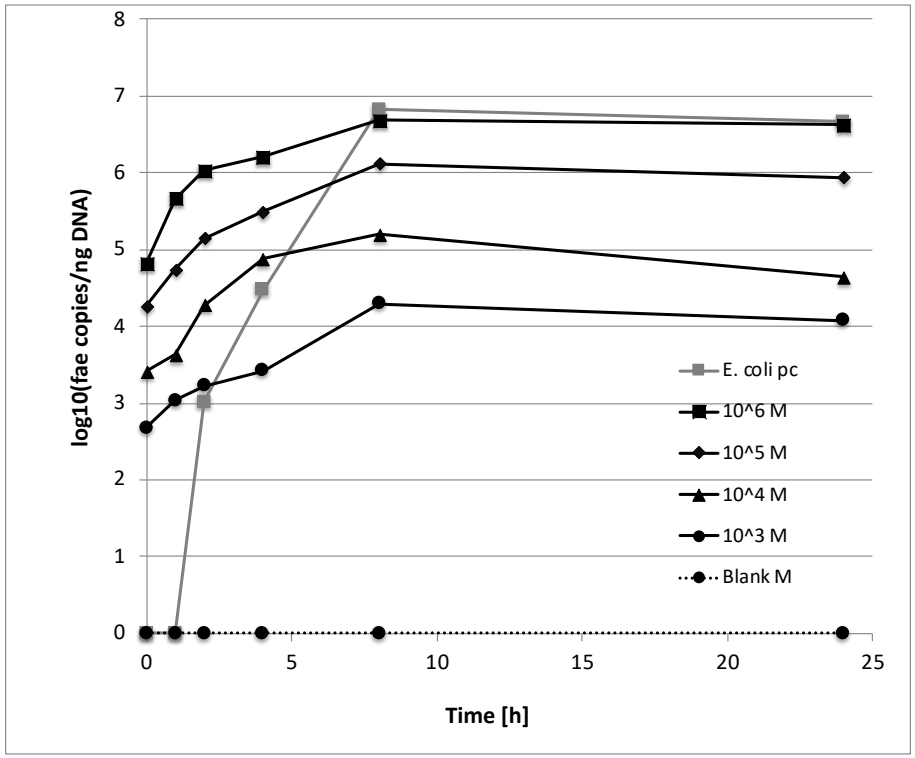


Figure S5. Influence of LSH and castalagin (**1**) on IPEC-J2 cell viability determined by propidium iodide (PI) staining followed by FACS analysis. Statistical significance *p<0.05, **p < 0.01 increase versus PBS control (Dunnett's post-hoc test).

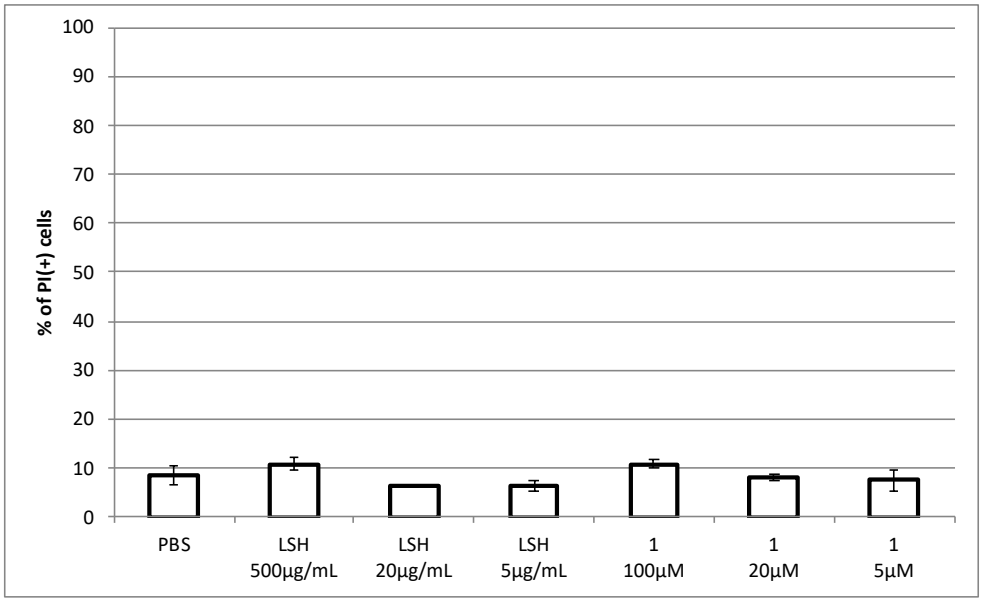


Figure S6. Changes in TEER of IPEC-J2 cells monolayers incubated from day 7 with medium containing urolithin C (**5**), urolithin A (**6**) and its phase II conjugate- mixture of urolithin A 3-*O*-glucuronide and 8-*O*-glucuronide urolithin A glucuronide (**7**) at the concentration of 50 µM.

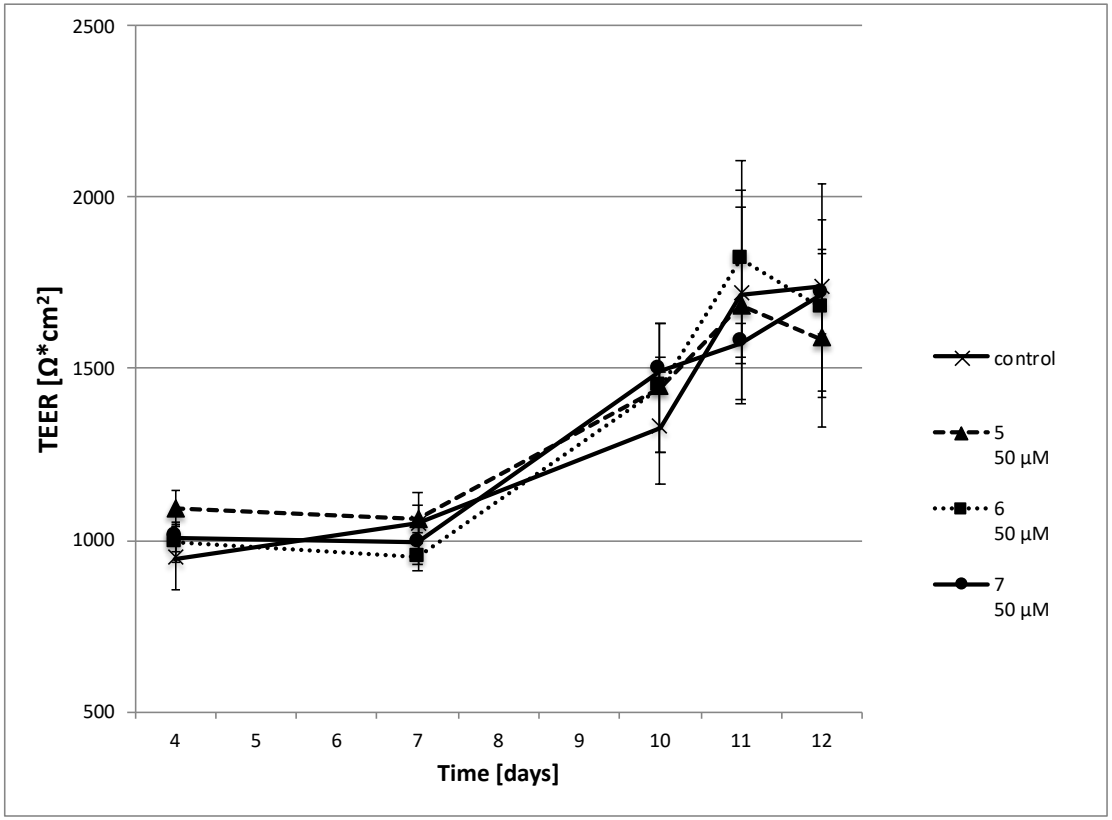


Figure S7. UPLC-DAD-MS analysis of different *Lythrum salicaria* L. extracts recorded at 254 nm with DAD device UPLC-DAD-MS analysis conditions: Reversed-phase Kinetex C8 analytical column (150mm×2.1mm×1.7µm), Phenomenex (Torrance, CA, USA). The column temperature was 25°C. Mobile phase A was 0.1% HCOOH in water (v/v) and mobile phase B was 0.1% HCOOH in MeCN (v/v). A multistep gradient solvent system used: 0–5 min 0% B, 5–35 min 0–35% B. The flow rate was 0.3 mL/min.