

DESIGNER Extracts as Tools to Balance Estrogenic and Chemopreventive Activities of Botanicals for Women's Health

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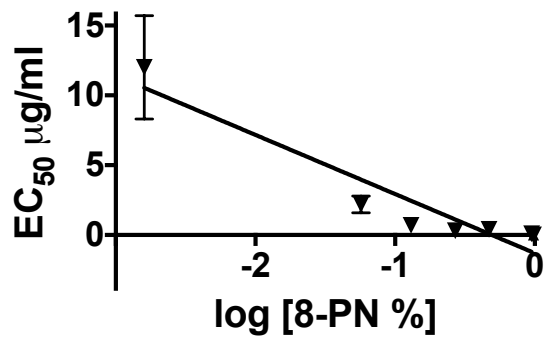
■ Supporting Information

■ Table of Contents

| | |
|------------------------------------------------------------------------------------------------------------------------------------|----------|
| S1. The Estrogenic Activity of the DESIGNER Hop Extracts Correlates with the 8-Prenylnaringenin (8-PN) Concentration. | 3 |
| S2. The Cytotoxicity of the Hop DESIGNER Extracts Correlates with the Xanthohumol (XH) Concentration. | 4 |
| S3. Cytotoxicity of the Hop DESIGNER Extracts is Mainly, But Not Only Dependent on the Xanthohumol (XH) Concentration. | 5 |
| S4. Xanthohumol (XH) Depicts Anti-estrogenic Activities Before It Shows Cytotoxicity. | 6 |
| S5. Names and Codes of DESIGNER Extracts with Their Deposited Specimen Codes. | 7 |

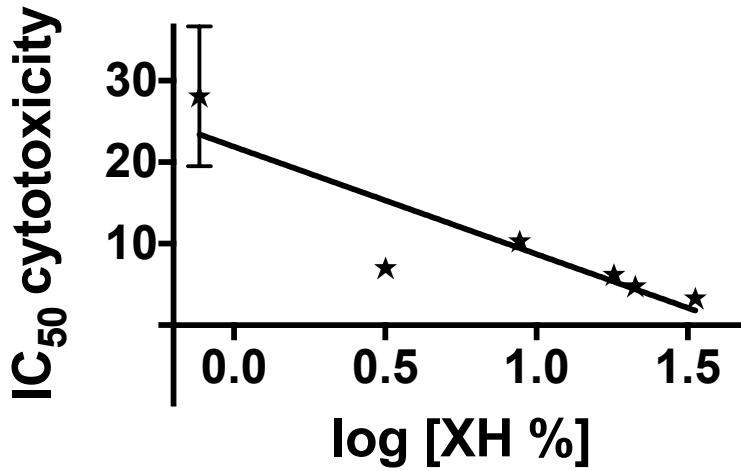
S1. The Estrogenic Activity of the DESIGNER Hop Extracts Correlates with the 8-Prenylnaringenin (8-PN) Concentration.

Linear regression of the EC₅₀'s generated in Fig. 2B (Table 2) and the corresponding log 8-PN% concentration (Table 1) performed with GraphPad Prism 6 ($r^2 = 0.91$). According to the Pearson correlation, the EC₅₀'s of the DESIGNER hop extracts significantly correlate ($r = -0.95$, $p < 0.0034$) with the log 8-PN% concentration. Ex5 and Ex7 were not active in the AP assay (Table 2); therefore, an EC₅₀ could not be determined.



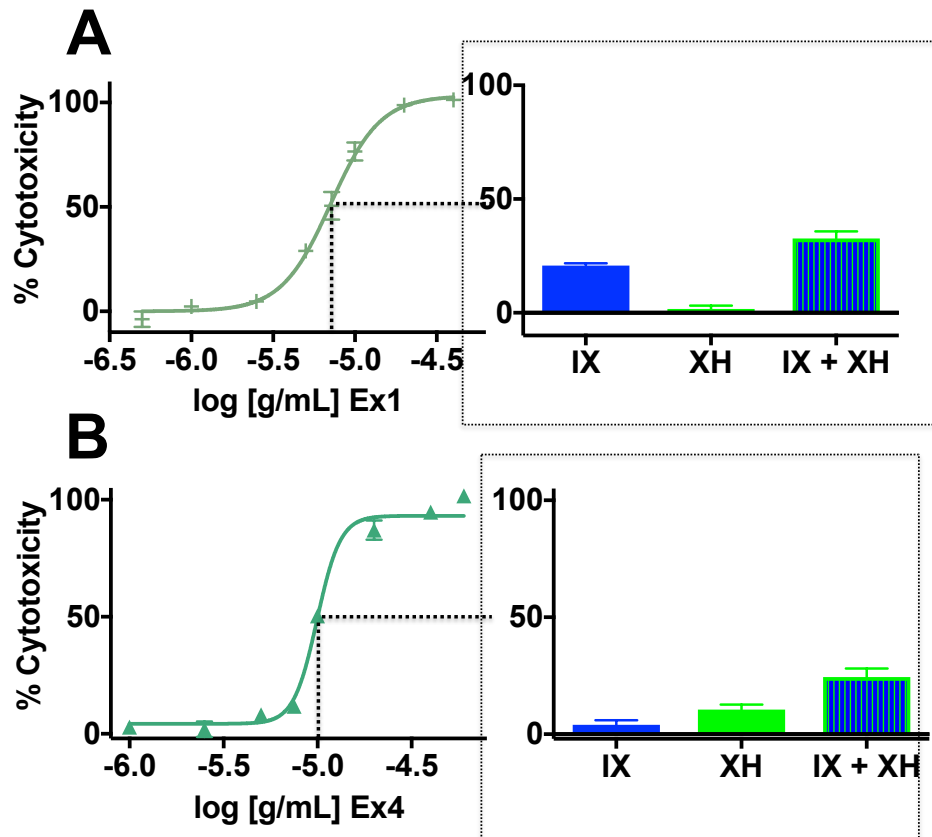
S2. The Cytotoxicity of the Hop DESIGNER Extracts Correlates with the Xanthohumol (XH) Concentration.

Linear regression of the IC_{50} 's generated in Fig. 4B (Table 2) and the corresponding log XH% concentration performed with GraphPad Prism 6 ($r^2 = 0.78$). According to the Pearson correlation, the IC_{50} 's of the DESIGNER hop extracts significantly correlate ($r = -0.88$, $p < 0.021$) with the log XH% concentration. Ex6 and Ex8 were not active in this assay; therefore an IC_{50} could not be determined.



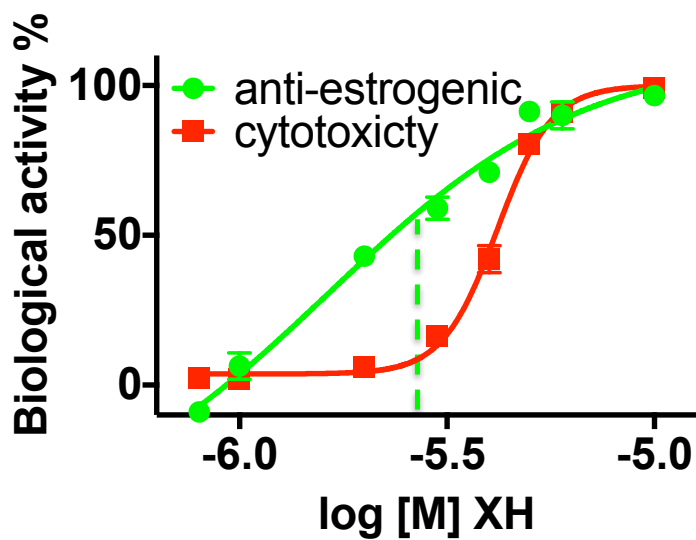
S3. Cytotoxicity of the Hop DESIGNER Extracts is Mainly, But Not Only Dependent on the Xanthohumol (XH) Concentration.

In IX rich extracts IX can significantly contribute to hops cytotoxicity. Cytotoxicity was performed in parallel to the AP assay with the SRB assay in Ishikawa cells. Cytotoxicity of the DESIGNER hop extracts, (A) the IX rich extract, Ex1 (7.2 $\mu\text{g}/\text{mL}$), and the equivalent concentrations of XH (0.64 μM), IX (12.7 μM), and their combinations and (B) Ex4 (10.3 $\mu\text{g}/\text{mL}$) and the equivalent concentrations of XH (2.6 μM), IX (0.31 μM) and their combinations. Dose-response curves were generated by non-linear regression analysis using GraphPad Prism 6.



S4. Xanthohumol (XH) Depicts Anti-estrogenic Activities Before It Shows Cytotoxicity.

Inhibition of estradiol-induced activity of alkaline phosphatase in Ishikawa cells by XH. In parallel, cytotoxicity was determined with the SRB assay. Results are the means \pm SEM of at least three independent determinations in triplicate. Dose-response curves were generated by non-linear regression analysis using GraphPad Prism 6.



S5. Names and Codes of DESIGNER Extracts with Their Deposited Specimen Codes.

| DESIGNER extract names | code based on [8-PN] | code for deposited specimens |
|---------------------------------------|----------------------|------------------------------|
| <i>specialized extracts</i> | | |
| Isoxanthoflav | Ex1 | BC691 |
| Isoxantho | Ex2 | BC690 |
| Clinical | Ex3 | BC402 |
| Xantho | Ex4 | BC692 |
| <i>“Knock-out (KO)”-type extracts</i> | | |
| IX-KOE | Ex5 | |
| Multi-KOE | Ex6 | |
| 8-PN/6-PN-KOE | Ex7 | |
| XH-KOE | Ex8 | |