

Article

In Vitro Evaluation of the Antioxidant Activity and Chemopreventive Potential in Human Breast Cancer Cell Lines of the Standardized Extract Obtained from the Aerial Parts of Zigzag Clover (*Trifolium medium* L.)

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Supplementary Materials

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Figure S1. A collection site of wild zigzag clover (in red frame) against the contour map of Poland and a fragment of enlarged map of the city of Lublin

Table S1. Mean retention times (t_R), linearity parameters for calibration curves ($y = ax + b$), and LOD and LOQ values ($\lambda = 260$ nm) obtained for reference isoflavones using RP-LC/PDA method

| Isoflavone | t_R (min) | a | b | R^2 | LOD (ng/ml) | LOQ (ng/ml) |
|--------------|-------------|----------|--------|---------|---------------|---------------|
| genistin | 36.31 | 49167.58 | -7.38 | 0.99994 | 86 | 287 |
| ononin | 43.57 | 36949.68 | 1.67 | 0.99996 | 123 | 410 |
| sissostrin | 54.64 | 51416.04 | -3.05 | 0.99998 | 97 | 323 |
| genistein | 64.01 | 76505.19 | 2.38 | 0.99998 | 71 | 237 |
| formononetin | 68.19 | 53013.74 | -4.72 | 0.99997 | 47 | 157 |
| biochanin A | 72.15 | 72150.31 | -12.47 | 0.99997 | 28 | 93 |

Abbreviations: a - slope; b - intercept; R^2 - regression coefficient; LOD - limit of detection; LOQ - limit of quantification

Table S2. Mean results ($\mu\text{g/g}$ dry wt) obtained for TML isoflavones in intra- and inter-day precision assays using the RP-LC/PDA method

| Inter-day ($n = 9$) | Intra-day ($n = 3$) | | | | | | | |
|-----------------------|-----------------------|-----------|---------------------|-----------|---------------------|-----------|-------|-----------|
| | 1 st day | | 2 nd day | | 3 rd day | | | |
| | Mean | RSD (%) | Mean | RSD (%) | Mean | RSD (%) | Mean | RSD (%) |
| genistein | 2001 | 1.2 | 2048 | 0.9 | 2037 | 1.4 | 2029 | 1.2 |
| genistin | 2247 | 1.3 | 2283 | 1.1 | 2299 | 1.2 | 2276 | 1.2 |
| biochanin A | 3525 | 1.9 | 3680 | 3.3 | 3603 | 1.1 | 3603 | 2.2 |
| sissostrin | 8360 | 1.2 | 8179 | 1.1 | 8278 | 0.9 | 8272 | 1.1 |
| sissostrin malonate | 20537 | 0.4 | 20618 | 0.2 | 20744 | 0.9 | 20633 | 0.5 |
| formononetin | 2963 | 2.7 | 2790 | 3.9 | 2989 | 4.6 | 2914 | 3.7 |
| ononin | 4409 | 2.3 | 4481 | 0.6 | 4337 | 1.9 | 4409 | 1.6 |
| ononin malonate | 7317 | 4.8 | 7012 | 3.4 | 7614 | 4.0 | 7314 | 4.1 |

Abbreviations: RSD – related standard deviation of the mean value (in %)