Genotoxic effect of *Lythrum salicaria* extract determined by the mussel micronucleus test

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Abstract

A wide range of aquatic plants have been proven to release allelochemicals, of them phenolics and tannin are considered rather widely distributed. Tannins, however, have been demonstrated to have genotoxic capacity. In our study genotoxic potential of *Lythrum salicaria* L. (Purple Loosestrife, family Lythraceae) was assessed by the mussel micronucleus test, using *Unio pictorum*. In parallel, total and hydrolysable tannin contents were determined. Results clearly show that the extract had a high hydrolysable tannin content and significant mutagenic effect. As *L. salicaria* has been long used in traditional medicine for chronic diarrhoea, dysentery, leucorrhoea and blood-spitting, genotoxic potential of the plant should be evaluated not only with regard to potential effects in the aquatic ecosystem, but also assessing its safe use as a medicinal herb.

keywords: *Lythrum salicaria*, tannin, genotoxicity, micronucleus test

A wide range of aquatic plants have been proven to release allelochemicals, of them phenolics and tannin are considered rather widely distributed [2]. The effect of these allelochemicals on algae has been widely studied, e.g. [12], concluding that allelopathy can be a competitive strategy. On the other hand, much less is known about what ecological effects these allelochemicals have on other elements of the aquatic ecosystem, though studies have reported the direct genotoxic effect of tannins such as delphinidin and procyanidin [10], tannic, ellagic and gallic acids [13]; [14].

In the present study, the genotoxic potential of *Lythrum salicaria* L. was assessed using the mussel micronucleus test (MNT). *L. salicaria* (Purple Loosestrife, family Lythraceae) is an aquatic species, having high tannin content: Piwowarski et al. [15] reported that tannin content of *L. salicaria* extract amounted to as much as 27.4%. Also, it is a traditional medicinal plant, which has been used as antihemorrhagic, cicatrizant, moderating

menstrual flow, antidiarrhoeal, typhoid, and astringent [17]. Phenolic compounds, especially, tannins and flavonoids were identified as being responsible for these actions, e.g. [16]; [5].

The mussel micronucleus test is a widely established, relatively easy-to-perform test. Micronuclei formation indicates chromosomal DNA damage occurring as a result of either chromosome breakage or chromosome mis-segregation during mitosis [1]. In our study, the freshwater bivalve *Unio pictorum* was selected as test organism. *Unio* species have already proven sensitive to tannins in genotoxicological studies [13]; [14].

L. salicaria sample was collected in the Kis-Balaton Water Protection System (west of Lake Balaton, Hungary). Aqueous extracts were prepared following the method given by Economou [7]. 30 grams of dried plant material (leaves and stems) were extracted with 500 ml of deionised water by shaking for 24 h at room temperature. As in the preliminary range-finding test even the 6 g/l concentration proved to be lethal, for further test series 1 g/l concentration was selected.

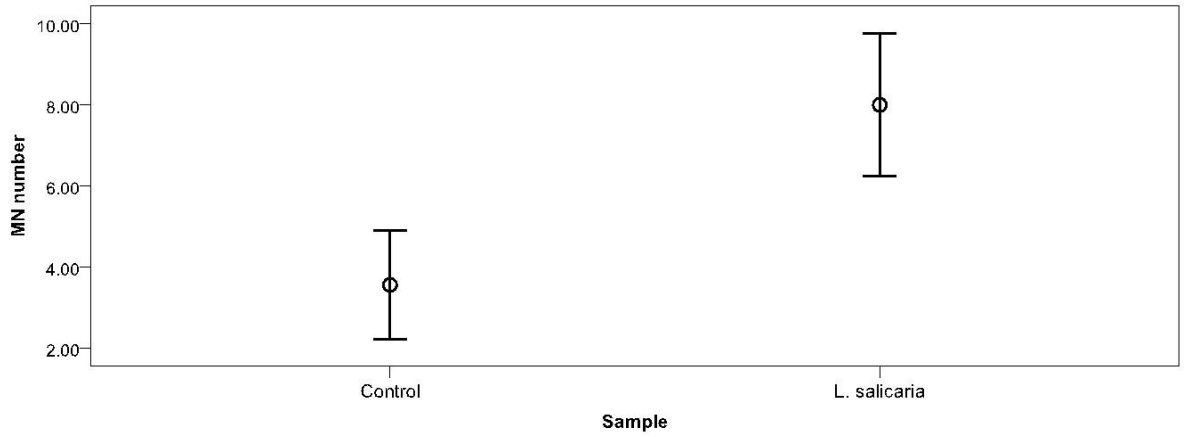
For the micronucleus test, *U. pictorum* specimens with length of 5-8 cm were used. Treatments were performed in 3 replicates. For the sample as well as for the control, aquaria of 3 l volume were used. Aquaria were aerated during the experiment, temperature was set at 22 ^{o}C. The assay was performed based on the protocol described by Wozniczki et al. [18], with some modifications. Exposure was 4 days, sample was renewed after 2 days, that is, fresh sample was used. Micronuclei were identified according to Fenech [8]. For each animal 250 cells were counted. The difference in mean MN numbers of control (Balaton Lake water) and *L. salicaria* extracts was determined using Students t-test. Total polyphenol was detected from dry aerial part samples [11]. Hydrolysed polyphenol analysis was made according to APHA [4].

Aqueous extract of *L. salicaria* had significant micronuclei induction, number of micronuclei/250 cells was 8.33 (t=4, 634, df=16, P<0.0001) (Fig.1.). Total tannin content of the dried aerial part was 0.2 mg/g, hydrolysable tannin content of the extract was 66.49 µg/ml.

Genotoxic potential of *L. salicaria* should be evaluated not only with regard to potential effects in the aquatic ecosystem, but also assessing its safe use as a medicinal herb. Assessment of the potential genotoxicity of traditional medicines is an important issue [6] and more and more plants are subjected to rigorous testing. In order to evaluate the genotoxic capacity of *L. salicaria*, the results of the MN test can be compared to medicinal herbs already tested. For example, Chukwujekwu and Van Staden [3] demonstrated the genotoxicity of the aqueous extract of the South African *Distephanus angulifolius* in the range of 1.3, 2.6, 5.3, and 10.6 g/l aqueous extract, using the *Allium cepa* bioassay. Genotoxicity was concentration-dependent, reaching its maximum at 5.3 g/l but in the highest concentration cytotoxic effect was observed. Fennell et al. [9] reviewed traditional African herbs for their safety and reported that of app. 50 species tested for genotoxicity, most had been found to exert mutagenic property in the range of 0.1, 0.5 and 2.5 g/l concentration, detected by micronuclei formation in human white blood cells. For *L. salicaria*, genotoxic effect elucidated by the 1 g/l concentration is in concordance with these results.

These authors stress the need for risk assessment of traditionally used medicinal herbs. In case of *L. salicaria*, as the aerial parts of the plant (as a decoction) and its preparations (fluid extract) are used, potential mutagenic effect cannot be neglected.

Figure 1. Micronucleus number in the control vs. *Lythrum salicaria* extract



References

1. Bolognesi, C., Fenech, M. (2012) Mussel micronucleus cytome assay. *Nat. Protoc. 7(6),* 1125-1137.
2. Chen, J., Zhang, H., Han, Z., Ye, J., Liu, Z. (2012) The influence of aquatic macrophytes on *Microcystis aeruginosa* growth. *Ecol. Eng. 42,* 130–133.
3. Chukwujekwu, J.C., J. Van Staden, J. (2014) Cytotoxic and genotoxic effects of water extract of *Distephanus angulifolius* on *Allium cepa* Linn. *S. Afr. J. Bot. 92,* 147-150.
4. Clesceri, L. S., Greenberg, A. E., Eaton, A. D. (Eds.) (1998) Standard Methods for the Examination of Water and Wastewater, 20th Edition. APHA American Public Health Association. pp.5-51.
5. Çoban, T., Çitoğlu, G.S., Sever, B., Işan, M. (2003) Antioxidant activities of plants used in traditional medicine in Turkey. *Pharm. Biol. 41,* 608–613.
6. Demma, J., Engidawork, E., Hellman B. (2009) Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *J. Ethnopharmacol. 122,* 136–142.

7. Economou, G., Travlos, I. S., Folinas, A., Karamanos, A. J. (2007) Greek oregano (*Origanum vulgare* ssp. *hirtum*) as allelopathic plant. *J. Food Agric. Environ. 5(1),* 348-351.
8. Fenech, M., Neville, S. (1992) Conversion of excision-repairable DNA lesions to micronuclei within one cell cycle in human lymphocytes. *Environ. Mol. Mutagen. 19(1),* 27-36.
9. Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E. et al. (2004) Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *J. Ethnopharmacol. 94,* 205–217.
10. Ferguson, L.R., van Zijl, P., Holloway, W.D., Jones, W.T. (1985) Condensed tannins induce micronuclei in cultured V79 Chinese hamster cells. *Mutat. Res. 158,* 89-95.
11. Graça, M. A.S., Bärlocher, F., Gessner, M.O. (Eds.) (2005) Methods to Study Litter Decomposition. Springer, pp. 97-100.
12. Gross, E. M., Erhard, D., Iványi, E. (2003) Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. *intermedia* (Wolfgang) Casper. *Hydrobiologia 506–509,* 583–590.
13. Labieniec, M., Gabryelak, T., Falcioni, G. (2003) Antioxidant and pro-oxidant effects of tannins in digestive cells of the freshwater mussel *Unio tumidus*. *Mutat. Res. 539,* 19–28.
14. Labieniec, M., Gabryelak, T. (2004) Response of DNA, proteins and membrane bilayer in the digestive gland cells of freshwater mussel *Unio tumidus* to tannins exposure. *Toxicol. in Vitro18,* 773–781.
15. Piwowarski, J. P., Kiss, A., K., Kozłowska-Wojciechowska, M. (2011) Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in

traditional Polish medicine for external treatment of diseases with inflammatory background. *J. Ethnopharmacol. 137*, 937– 941.

16. Rauha, J.P., Wolfender, J.L., Salminen, J.P., Pihlaja, K., Hostettmann, K., Vuorela, H. (2001) Characterization of the polyphenolic composition of purple loosestrife (*Lythrum salicaria*). *Z. Naturforsch. C 56*, 13–20.
17. Tunalier, Z., Koşar, M., Küpeli, E., Çaliş¸ I., Başer, K.H.C. (2007) Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. *J. Ethnopharmacol. 10*, 539–547.
18. Wozniczki, P, Lewandowska, R., Brzuzan, P., Ziomek, E., Bardega, R. (2004) The level of DNA damage and the frequency of micronuclei in haemolymph of freshwater mussels *Anodonta woodiana* exposed to benzo[a]pyrene. *Acta Toxicol. 12(1),* 41-45.