EMSG55: Evaluating Plant Leaf Extracts for Endocrine Activity & Reproductive Toxicity in Fish

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HIGHLIGHTS
- The goal of this research is to characterize the in vitro and in vivo activities of plant-derived endocrine active compounds (EACs) and to evaluate potential effects of relevance to fish reproductive health.
- Fallen leaves (beech, oak & reed) were collected from southwest England and processed for screening using a battery of (anti)estrogenic, (anti)androgenic & (anti)estripenic in vitro screens (the YAS, YES and YPS assays, respectively). This initial work addressed the impact of temperature and solvent on the extraction of dissolved organic carbon (DOC) and consequent responses in the YAS, YES and YPS assays (as both agonism and antagonism).
- Aqueous extracts (10 μL volumes) were directly screened in the YAS, YES and YPS assays. Aqueous extract subsamples (10 μL) were concentrated by solid phase extraction (SPE) onto a Sep-Pak C18 cartridge & the concentrated ethanol extracts were then also screened in the three yeast assays.
- Extraction temperature had a marked proportionate effect on aqueous DOC values. For the reed aqueous extracts, there was a DOC-related response in YES assay responses, while oak aqueous extracts gave DOC-related anti-YAS activity. Impacts of leaf extracts on fish reproduction will be addressed.

1. INTRODUCTION & OBJECTIVES

There is a growing interest in understanding the cumulative effects of both natural and synthetic endocrine disrupters on aquatic organisms. Recent publications have demonstrated the presence of endocrine active chemicals in leaf extracts from European ecosystems, with oak leaf extracts showing anti-androgenic effects in amphibians¹. Little is known, however, about the impacts of European plant leaf extracts on the reproductive systems of native fish species.

This CEFIC-LRfI funded project has been started in order to address two key questions:
1. The relative endocrine activity of plant leaves collected from wetland and woodland ecosystems in vitro;
2. To address the endocrine activity of the most potent leaf aqueous extracts in vivo for freshwater fish.

2. MATERIALS & METHODS

For each plant species sampled, beech (Fagus sylvatica), oak (Quercus robur) and reed (Phragmites australis), ca. 30L of dried leaves were collected from April to June 2011. Leaves were air dried (7d) before being resuspended in ethanol. The concentrated extracts were then eluted with methanol. The samples were dried under vacuum prior to solid phase extraction (SPE) onto a Sep-Pak C18 cartridge & the concentrated ethanol extracts were then also screened in the three yeast assays.

3. RESULTS & DISCUSSION

Extraction temperature had a marked proportionate effect on aqueous DOC values; ranging from 406 – 665 mg/L (BEECH-2011-1), 365 – 1030 mg/L (OAK-2011-1) and 650-984 mg/L (REED-2011-1).

Aqueous & ethanol extracts of the leaf extracts were run in the YAS, YES and YPS assays for agonist activity (Figures 3a-c).

Aqueous & ethanol extracts of the leaf extracts were run in the YAS, YES and YPS assays for antiestrogen activity (Figure 4).

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REFERENCES

¹ Hermanns B. et al., 2010. Aquatic leaf extracts display endocrine activity in vitro and disrupt sexual differentiation of male mesonephric fish larvae in vivo. General and Comparative Endocrinology 168, 245–253
5 Smith, A. et al., 2012. CEFIC report reference CEFIC-LRfI-EMSG55. We are grateful to the CEFIC and ECETOC project monitoring team for their valuable advice and to Professor John Sumpter & Kevin Gaido for generously providing the yeast cell lines used in the YAS, YES and YPS assays. Our thanks also to Andy Pratt for granting permission for reed sampling from Slapton Ley, UK and staff at the National Laboratory Service, Leeds for conducting the DOC analyses.

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