Supporting Information

Anti-adipogenic activities of *Alnus incana* and *Populus balsamifera* bark extracts, Part I: Sites and mechanisms of action

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Effects of *Alnus incana* and *Populus balsamifera* on lipolysis in 3T3-L1 adipocytes

**Lipolysis**

The effect of extract treatment on lipolysis of intracellular triglyceride stores was assessed in fully mature and triglyceride-laden adipocytes on their 8th day of differentiation. Cell medium was changed immediately prior to the start of the experiment and medium was collected at the end of a 24 h treatment period. The experiment was conducted in serum- and insulin-supplemented differentiation medium. Glycerol content in the medium was quantified with Free Glycerol Reagent (Sigma-Aldrich), according the manufacturer’s instructions. Epinephrine (purity ≥ 98%; Sigma-Aldrich) was used as a positive control.

These experiments served to test whether the induction of lipolysis could contribute to the observed inhibition of triglyceride accumulation. The effect of a 24 h extract treatment on glycerol release was thus measured in fully mature adipocytes undergoing their 8th day of culture in adipogenic conditions. Epinephrine (1 μM), used as a positive control, more than doubled the amount of glycerol released into the culture medium over the 24 h culture period. *Alnus* did not significantly increase glycerol release. However, *Populus* increased lipolysis by slightly more than 50%. Assessment of intracellular triglyceride content by AdipoRed dye showed no difference between groups, indicating that triglyceride content reduction through lipolysis was below the threshold of detection (not shown).
**Supplemental figure 1S**

![Bar chart showing Glycerol Release (ug/well/24 h) for different treatments: Vehicle, Alnus, Populus, Epinephrine.](image)

**Fig. 1S:** *Populus balsamifera* extract stimulates lipolysis in fully mature 3T3-L1 adipocytes. Glycerol released into the culture medium as a result of lipolysis over a 24 h treatment period with vehicle, extract, or 1 μM epinephrine in 3T3-L1 adipocytes undergoing their 8th day of differentiation was assessed using a standard kit. Treatment was conducted in complete medium without insulin supplementation. Data are presented as mean ± SEM for n=6 combined from 2 separate experiments. * indicates a significant (p≤0.05) difference from the vehicle-treated control group.