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## Potential bioactive effects of softwood knot extracts

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Softwood knots, i.e. the branch base encased in the tree stem, belonging to the genera *Pinus* and *Picea*, contain exceptionally large amounts of bioactive phenolic compounds<sup>(1)</sup>, which can be 50–100 times greater than in adjacent stemwood. They constitute a valuable resource with a potential for use as technical antioxidants, biological antioxidants in foodstuffs, functional food ingredients and natural biocides<sup>(1)</sup>. Previously it has been reported that in human Jurkat T cells knot extracts from *Pinus banksiana* Lamb. (Jack pine, Saskatchewan, Canada) are more cytotoxic than *Picea sitchensis* (Bong.) Carr. (Sitka spruce, Co. Mayo, Republic of Ireland), with half maximal inhibitory concentration values of 153.0 µg/ml and 376.1 µg/ml respectively<sup>(2)</sup>. Thus, the aim of the present study was to further assess the potential bioactivity of these wood knot samples by investigating their effect on cell membrane integrity, antioxidant enzyme activity and cytokine production in human Jurkat T cells. In addition, their potential protective effects against oxidant-induced cell injury and DNA damage were determined.

Cell membrane integrity was determined using the lactate dehydrogenase-release assay kit (BioGenesis, Poole, Dorset, UK). Catalase activity was determined using a Calbiochem colorimetric kit (Merck Chemicals Ltd, Nottingham, UK). Supplementation with non-toxic doses of the Jack pine and Sitka spruce knot extracts (10 and 30 µg/ml) for 24 h had no effect on Jurkat cell membrane integrity or catalase activity.

To assess potential immunomodulatory effects Jurkat cells were treated with concanavalin A (Con A; 25 µg/ml) in the presence or absence of softwood knot extracts (10 and 30 µg/ml) for 24 h. IL-2 production was determined by an eBioscience ELISA kit (Insight Biotechnology Ltd, Wembley, Middlesex, UK). Jack pine knot extract at a concentration of 30 µg/ml was the only sample to significantly suppress ( $P < 0.05$ ) ConA-induced IL-2 production.

Cytoprotection was assessed by measuring the viability of Jurkat cells supplemented with Sitka spruce and Jack pine knot extracts (10 and 30 µg/ml) for 24 h followed by treatment with H<sub>2</sub>O<sub>2</sub> (125 and 250 µM) for 1 h. Cell viability was determined by the MTT assay II kit (Roche Diagnostics, Burgess Hill, West Sussex, UK). Pre-treatment with Sitka spruce (10 and 30 µg/ml) and Jack pine (10 µg/ml) protected ( $P < 0.01$ ) against cell injury induced by the presence of 125 µM-H<sub>2</sub>O<sub>2</sub>. Both softwood extracts significantly enhanced ( $P < 0.01$ ) cell viability following exposure to 250 µM-H<sub>2</sub>O<sub>2</sub> compared with cells treated with the oxidant alone. Caco-2 cells were pre-incubated with the wood knot extracts for 24 h followed by exposure to H<sub>2</sub>O<sub>2</sub> (50 µM for 30 min). DNA damage was assessed using the comet assay by the method of Tice *et al.*<sup>(3)</sup> None of the extracts at the concentrations tested protected the cells against H<sub>2</sub>O<sub>2</sub>-induced DNA damage.

From the findings it is evident that there are interesting opportunities for phytochemicals found in tree species that need to be further evaluated and researched. In particular, further investigation into the immunomodulatory and cytoprotective effects of softwood knot extracts is warranted.

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