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## Digested and fermented seaweed phlorotannins reduce DNA damage and inhibit growth of HT-29 colon cancer cells

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Brown seaweeds are a rich source of phlorotannins, a characteristic class of phenolic compounds which are unique to seaweeds of this type and can comprise 5 to 15% of the dried weight<sup>(1)</sup>. Unlike other classes of polyphenols, there is a lack of knowledge regarding phlorotannins and their bioactivity, particularly after their metabolism by the gut microbiota.

We investigated the effects of a phlorotannin-rich Food Grade Extract (FGE) from the brown seaweed *Ascophyllum nodosum* and its High Molecular Weight (HMW) and Low Molecular Weight (LMW) fraction on colon cancer cells after being subject to *in vitro* gastrointestinal digestion (GID)<sup>(2)</sup> and colonic fermentation (CF)<sup>(3)</sup>.

HT-29 cells were exposed to the GID and CF extracts and we tested the ability of the three extracts to reduce H<sub>2</sub>O<sub>2</sub> induced cellular DNA damage (24 h, COMET assay) and inhibit cancer cell growth (48 h, SRB assay). The statistical analysis was conducted by one-way ANOVA followed by a Bonferroni post-hoc *t*-test.

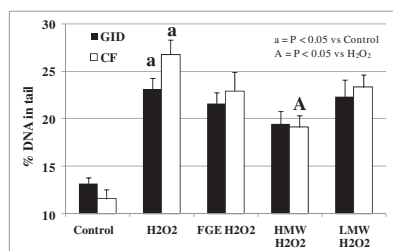


Fig. 1. COMET assay.

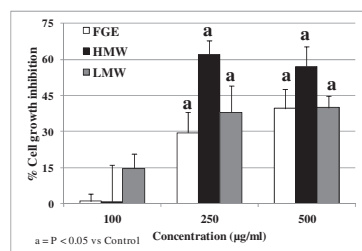


Fig. 2. SRB assay.

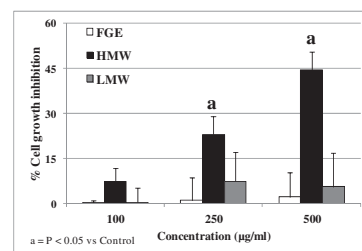


Fig. 3. SRB assay CF.

As shown in Fig. 1, a low level of DNA damage (% of DNA in tail) was measured in the controls whereas in cells challenged with H<sub>2</sub>O<sub>2</sub>, the DNA damage was significantly increased ( $P < 0.05$ ). The pre-treatment of cells with GID extracts did not significantly reduce DNA damage compared to H<sub>2</sub>O<sub>2</sub> alone, however the CF extract HMW was significantly effective ( $P < 0.05$ ), whereas the CF extracts LMW and FGE did not induce any significant protective effect. Fig. 2 shows that all GID extracts significantly inhibited ( $P < 0.05$ ) HT-29 cell growth (at 250 and 500 µg/ml), with HMW being the most effective. When the cells were treated with the CF extracts (Fig. 3), only HMW was able to significantly inhibit ( $P < 0.05$ ) the growth of the cells (at 250 and 500 µg/ml).

In conclusion, the gut microbiota metabolism of the seaweed extracts increased their ability to counteract the H<sub>2</sub>O<sub>2</sub> induced DNA damage, whereas it reduced the ability of the extracts to inhibit the cell growth. In both cases, HMW was the most effective, indicating that the high molecular weight phlorotannins potentially exert a stronger beneficial effect in the colon.

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