



Third International Congress on
Cocoa Coffee
and **Tea**

22–24th June 2015 Aveiro, Portugal

Program Booklet

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Structural features and macrophage immunostimulatory activity of the polysaccharides from *Fraxinus angustifolia* and *mentha suaveolens* hot water extracts

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The dried leaves of *Fraxinus angustifolia* and the dried shoots of *Mentha suaveolens* are used by the population of the Trás-os-Montes, which is a region located in the northeast of Portugal, to prepare hot water extracts due to their therapeutic properties. Recently, the contribution of polysaccharides for the therapeutic properties often attributed to various plants hot water extracts has been highlighted. This work will provide a structural characterization of the polysaccharides present in these hot water extracts and will also evaluate their possible macrophage immunostimulatory activity, which might contribute for the therapeutic properties reported for these hot water extracts.

The decoction during a total of 4 h, followed by dialysis of the extracted material, provided adequate amounts of high molecular weight material (HMWM) for the structural characterization of the polysaccharides from the hot water extracts of *Fraxinus angustifolia* dried leaves and *Mentha suaveolens* dried shoots. For both plants, sugar analysis of the HMWM revealed high proportions of uronic acid, which suggested the presence of pectic polysaccharides. Subsequently, the HMWM polysaccharides were sequentially fractionated by ethanol precipitation and anion exchange chromatography, according to their uronic acid content and methylesterification degree, respectively. Through linkage analysis it was possible to detect the presence of (1→4)-galacturonic acid residues together with (1→2)-rhamnose and (1→2,4)-rhamnose, which confirmed the presence of pectic polysaccharides. In addition, it was also possible to various linkages that suggested the presence of minor proportions of other polysaccharides, such as type II arabinogalactans.

Macrophage immunostimulatory activity assays evidenced that pectic polysaccharide rich fractions from *Fraxinus angustifolia* dried leaves and *Mentha suaveolens* dried shoots caused an increase in the macrophage nitric oxide production, without compromising its cellular viability. This suggests a possible contribution of polysaccharides for the therapeutic properties usually reported for these two hot water extracts.

Keywords: *Fraxinus angustifolia*, *Mentha suaveolens*, polysaccharides, immunostimulatory activity