

## A water-soluble high molecular weight substance isolated from Hyuganatsu orange (*Citrus tamurana*), suspected to be a polysaccharide, inhibits rat osteoclast cell formation

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Submission date: March 2, 2015; Acceptance date: May 31, 2015; Publication date: June 1, 2015

### ABSTRACT

**Background:** Osteoporosis is detrimental to aged women's health care. We previously reported that Hyuganatsu orange (*Citrus Tamurana*) contains active substances that inhibit osteoclast activities. Prior to conducting a human study, we sought to identify the biological active substance in the Hyuganatsu orange which suppresses osteoclast formation.

**Methods:** We isolated five fractions from a Hyuganatsu orange extract according to molecular weight. Each fraction was tested to determine its suppressive effect on the formation of osteoclasts in rats. We also used high-performance liquid chromatography (HPLC), infra-red (IR), and <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy to evaluate its chemical structure. Data was recorded as mean ± standard error of the mean. The Mann-Whitney test was used, and a p-value of <.05 was considered statistically significant.

**Results:** The highest and lowest molecular weight fractions showed significant suppression activity on rat osteoclast formation (p < .05). The lowest molecular weight fraction was identified as hesperidin using thin layer chromatography. Additionally, IR absorption revealed that the highest molecular weight fraction was not a flavonoid. With regard to chemical structure, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy suggested that the highest molecular weight fraction had signals compatible with a polysaccharide such as galacturonic acid.

**Conclusions:** Hyuganatsu orange contains a biological active substance other than hesperidin

that may be a polysaccharide and may suppress osteoclast formation.

**Key words:** *Citrus Tamurana*, TRAP positive cell, Polysaccharides, Rat osteoclast cell, in vitro study