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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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 ¹H and ¹³C 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,
¹H and ¹³C 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