Effects of *Davallia formosana* Hayata extracts on the proliferation and differentiation of osteoblastic MC3T3-E1 cells

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Abstract

In Taiwan, osteoporosis has become a serious health concern in the society as a consequence of the growing senior population over the years. Osteoporosis is a bone disease due to the imbalance of bone resorption and formation. Partial bone fractures are common clinical signs caused by osteoporosis that affects menopausal women and elderly people, thus finding new strategies that contribute to bone formation is imperative and indispensable. *Davallia formosana* Hayata is a native Taiwanese fern which is mainly used to stimulate osteogenesis and repair damaged osteocytes. To date there have been no results on MC3T3E1 studies regarding this plant, therefore the aim of this study was to evaluate the positive effect. *Davallia formosana* Hayata water extracts (DFWE) on proliferation and differentiation of pre-osteoblast cells. Pre-osteoblast MC3T3E1 cells were treated with different concentrations of DFWE (10ppm, 25ppm and 50ppm) for 24 hours and survival ability and protein expression of disease-associated membrane receptors were evaluated. Results showed that DFWE enhanced cell survival rate of MC3T3E1 cells to 100%, 110% and 112%, respectively. DFWE 25ppm and 50ppm in cell survival has increased significantly ($P<0.05$). Proteins expression of bone morphogenetic protein 2 (BMP2), collagen 1 (COl-1), alkaline phosphatase (ALP) and runt-related transcription factor 2 (RUNX-2) are 102%, 162%, and 216%, 134%, 210% and 228%, 111%, 148% and 148%, 106%, 165% and 204% compared to the control group, respectively. (-)Epicatechin-3-O-d-allopyranoside (CP) was isolated using column chromatography of DFWE. This compound, at the concentration of 0.01mg/mL, exhibits the higher survival ability and mineralization in MC3T3E1 cells. We postulate that CP is an active component in DFWE that may promote cell proliferation and differentiation in MC3T3E1 cells. Water extract of *Davallia formosana* Hayata may have potential to be used by patients with osteoporosis in the future.

Keywords: *Davallia formosana* Hayata, osteoporosis

Materials and methods

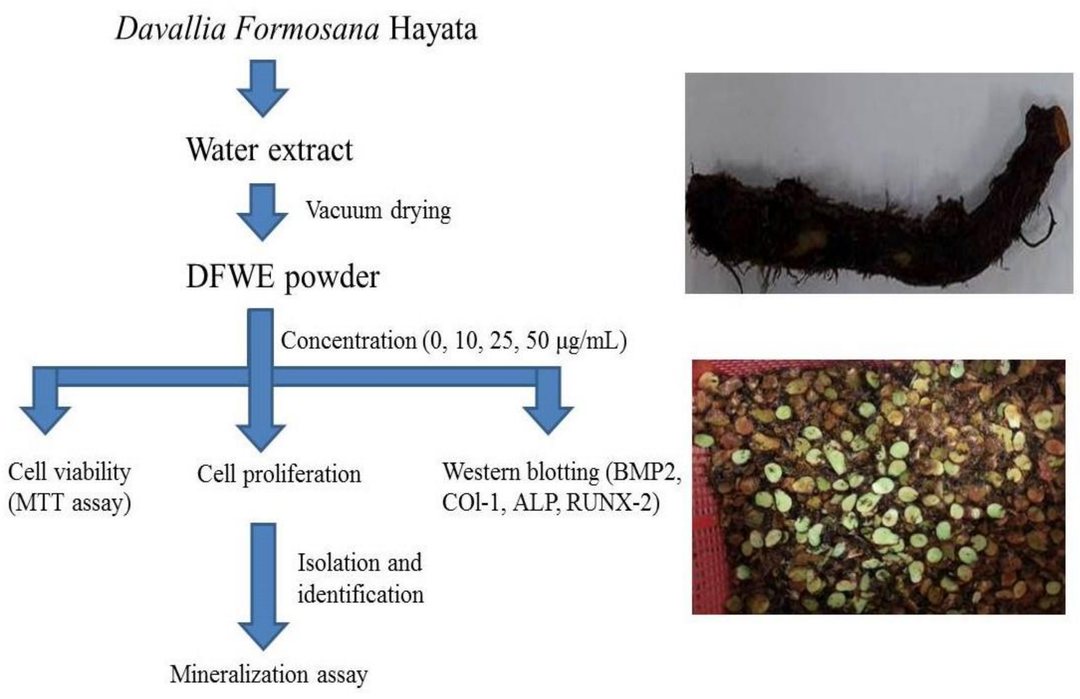

figure 1. Experimental structure.

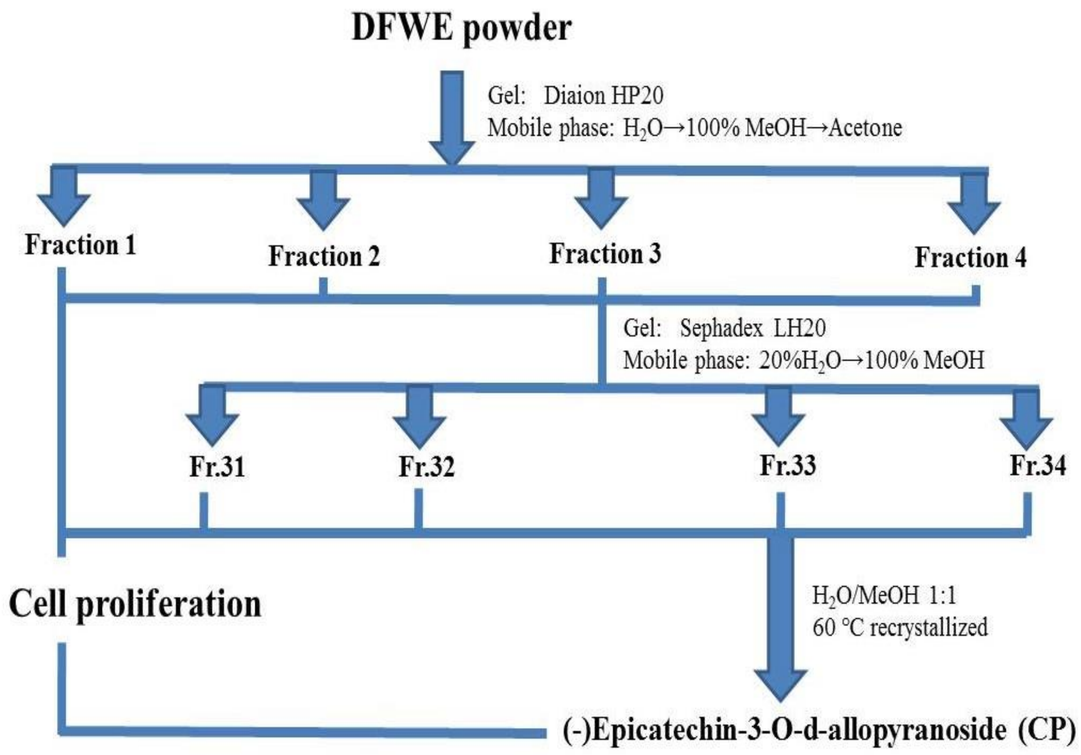

figure 2. The flow chat of isolation and identification from DFWE powder .

Results

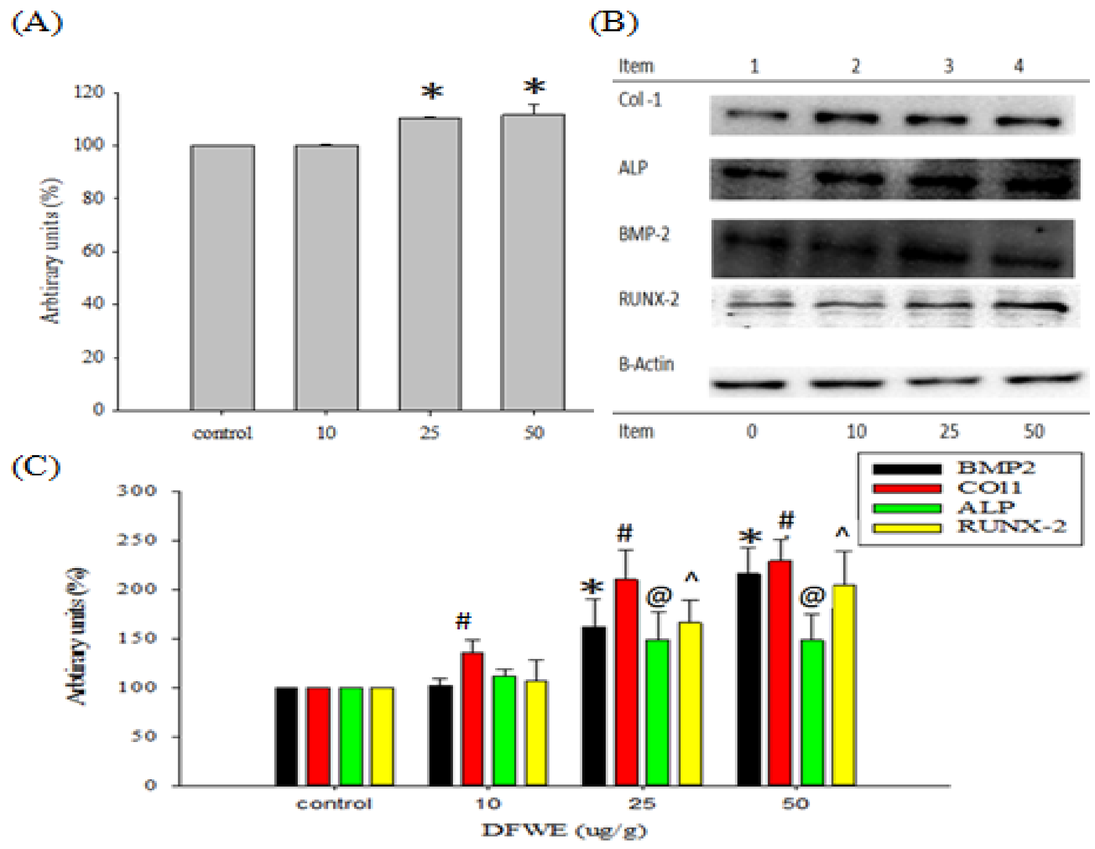

figure 3. Effect of DFWE on proliferation and differentiation in MC3T3-E1 cells. (A) The cell viability on MC3T3-E1 cells treated with DWFE. (B) Expressions of proliferation and differentiation proteins in MC3T3-E1 cells with DFWE treatment. (C) Western blotting data show the changes in BMP2, COl-1, ALP, RUNX-2 and quantitative western blotting data in MC3T3-E1 cells treated with DFWE at different concentrations (0–50 μg/mL) for 24 h. The different symbols indicates that the values differ significantly from the respective control, $p < 0.05$ ($n=3$) .

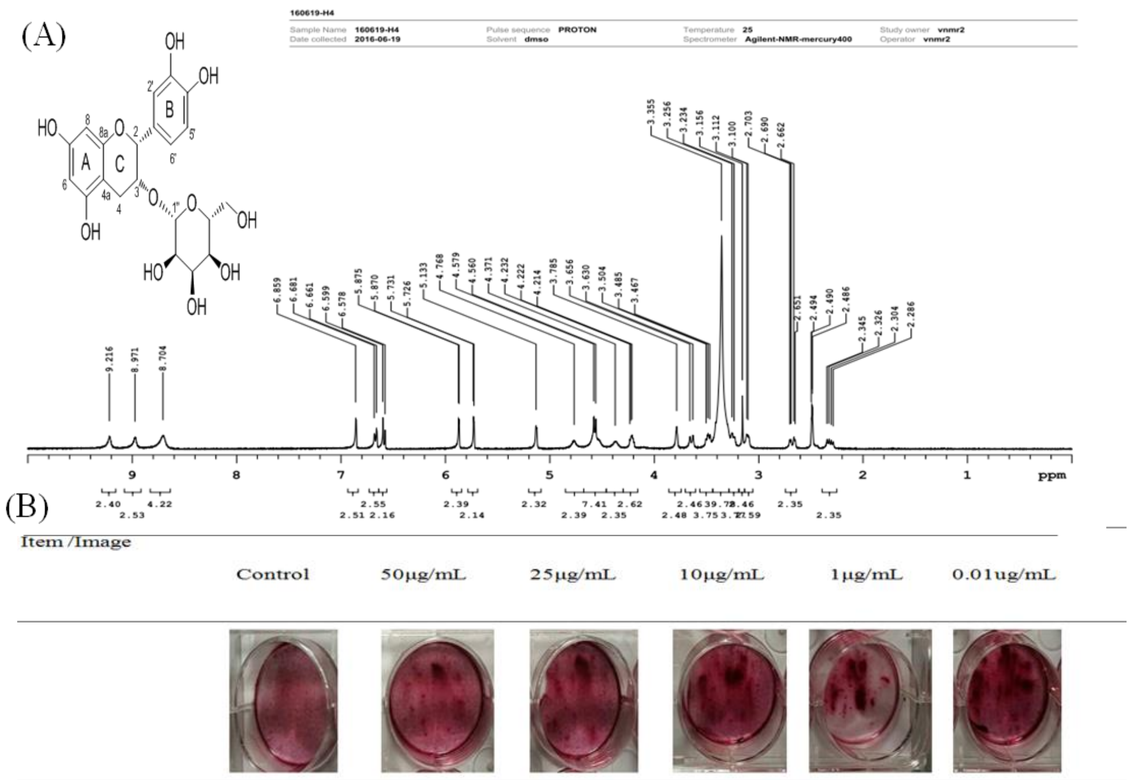

figure 4. (A) The 1H-NMR (DMSO) spectrum of (-)Epicatechin-3-O-d-allopyranoside from DFWE (B) and mineralization results on MC3T3-E1 cells.

Conclusions

BMP interacts with various downstream proteins including Runx-2, which induces bone differentiation factors. Additionally, ALP and COl-1 are produced as early osteogenic markers of matrix maturation while the late osteogenic mineralization markers are osteocalcin and osteopontin in our study. We isolated an active compound from DFWE identified as (-)Epicatechin-3-O-d-allopyranoside (CP), which significantly increased the mineralization in MC3T3E1 cells. Therefore, CP could be useful for preventing osteoporosis and as marker compound to figure out the quality of DFWE.