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Purification and Characterization of anti-inflammatory constituents from the edible brown alga, *Undaria pinnatifida*

M.N.A. Khan, J.Y. Kang, N.H. Park, J.Y. Cho and Y.K. Hong

Department of Biotechnology, Pukyong National University, Namku, Busan 608-737, Korea

Introduction

Undaria pinnatifida, an edible brown alga, is well known as a health-care diet among east-asian people. It has also been used traditionally to treat fever, urination problems, lumps or swelling (Donguibogam Committee, 1999). In China, *U. pinnatifida*, is used as herbal medicine to treat urinary diseases, dropsy, stomach ailments, hemorrhoids, and fistulas, leukorrhea in women and nocturnal emission in men (Tseng and Chang, 1984). Most of these effects directly or indirectly related to the anti-inflammatory activity of this seaweed. Although scientific data on *U. pinnatifida* is very scanty, our group has recently reported the anti-inflammatory activity of methanol extracts of this seaweed on mouse model inflammation. In order to validate the use of *U. pinnatifida* as an anti-inflammatory drug for folk medicine, this study has been undertaken to isolate the anti-inflammatory active compounds as well as evaluate potential of isolated constituents of *U. pinnatifida*.

Materials and Methods

U. pinnatifida sample The brown seaweed, *U. pinnatifida* f. *distans* (Harvey), was collected between May 2001 and March 2006 from Kijang and Wando, Korea.

Extraction, purification and constituents The air dried brown alga, *U. pinnatifida* was extracted using acetonitrile (100%) and then subjected to silica gel open column chromatography separation. Following the in-vivo mouse ear inflammation assay, dichloromethane eluent was further purified using reverse-phase high performance liquid chromatography (HPLC). Eluted peaks and isolated compounds were determined through bio-assay. The structure of compounds was characterized by ^{1}H-NMR, ^{13}C-NMR spectroscopy.

Animals BALB/c mice were used in accordance with the U.S. NIH Guidelines for the Care and Use of Laboratory Animals.

Anti-inflammatory test Stock solutions of *U. pinnatifida* for anti-inflammatory assay were prepared using ethanol into crude extract and purified compounds. Phorbol myristate acetate (PMA; Sigma, St. Louis, MO, USA) was topically applied to the inner side of the mouse ear at 0.2μg in 10$\mu\ell$ acetone with an equal volume of the sample. Ear edema, erythema and blood flow were measured 10 h later using a spring-loaded micrometer (Mitutoyo Corp., Japan), analysis of digital photograph using Adobe Photoshop 7.0 and Laser Flowgraphy (LFG-1), respectively. The values were expressed as $(T_{10}-T_0)/T_0$, where T_{10} is 10 h value after PMA application and T_0 is at 0 h value.

Results and discussion

In the present study aiming at the identification of anti-inflammatory constituents from *U. pinnatifida* was investigated. The active peaks ATD-2 and ATD-4 were purified and characterized as stearidonic acid [18:4(n-3)] and eicosapentanoic acid [20:5(n-3)], respectively by comparison of their spectral data analysis.

To validate potential anti-inflammatory properties of purified compounds, we used mouse model ear inflammation by topical application of PMA. Ear swelling, erythema and blood flow values at 10h reached to 0.87± 0.01, 0.27±0.01 and 0.14±0.01, respectively. To measure inhibition rate of purified compounds against 0.2ug PMA in 10ul acetone, 10ul of different concentrations of both compounds were applied to the ear. The concentrations giving a 50% inhibition (IC_{50}) both for ATD-2 and ATD-4 were 55.5mM and 75.0mM for edema, 115.0mM and 150.5mM for erythema, and 80.0mM and 10.5mM for blood flow, respectively.

Recently we found an anti-inflammatory activity of *U. pinnatifida* methanol extract but there was no scientific information about the constituents responsible for anti-inflammatory activity of this seaweed species. The present study clearly revealed that *U. pinnatifida* contains potent anti-inflammatory constituents namely, stearidonic acid [18:4(n-3)] and eicosapentanoic acid [20:5(n-3)] which strongly supports our previous claims.

References

Donguibogam Committee, 1999. Translated Donguibogam. Bubinmunwha Press, Seoul, p.2198.

Tseng, C.K., Chang, C.F., 1984. Chinese seaweeds in herbal medicine. Hydrobiologia 116/117, 152-154