Purification and characterization of water soluble natural compounds from the marine algae Padina that interact with the membrane progesterone receptor (mPR)

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Abstract of Dissertation

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Abstract : Marine algal flora significantly contributes in the productivity, flow of energy in the ecosystem, remediation of contaminants and nutrient cycling between terrestrial and aquatic ecosystems. Moreover, marine algae also release some chemical, which has some hormonal like activity. As we know that progestins are the synthetic forms of the hormone progesterone, which occurs naturally in the body and acts a very significant role in controlling reproduction. The physiological effects of progesterone are mediated through modulating the expression of genes linked with nucleus progesterone receptors (nPRs), but the discovery of membrane progestin receptors (mPRs) brought new insights into progesterone action. Scientists are now focusing their efforts on identifying the new mPR ligand. Previously, our laboratory group conducted one of the research projects on detecting the membrane progestin receptor (mPR) interacting compound of coral sea water, for which they collected samples from Mauritius. Other mPR ligands, such as DES, DHP, and Org OD 02-0, were discovered in early research after the discovery of membrane progestin receptors (mPRs) as a nongenomic signaling pathway.

Current study attempted to cultivate the marine algae known as "*Padina*" in *in-vitro* condition to collect the natural active compound. The samples were collected from the at marine field of Shimoda Marine Research Center, University of Tsukuba or Mochimune marine filed of Faculty of Agriculture Regional Field Science Education and Research Center, Shizuoka University. All the samples were transported into aquarium in the day of sampling. Algae were planted in gravel stone in 120 cm long aquariums and cultivated for several months in culture room of Mochimune marine filed, Shizuoka University. Filtering systems and LED lights were set on the aquariums. Firstly, the species of the *Padina* was identified by DNA sequencing. The

Molecular analyses using mitochondrial *cox3* genes as molecular markers confirmed the species as *Padina arborescens*. The secreted compounds were accumulated by filtration system and the compounds were collected from the filter by using the absolute ethanol. Then the samples were concentrated in the ODS column by peristaltic pump. High performance liquid chromatography (HPLC) was implemented to separate the compound from column. The samples were fractionated with four different colors. Several steps of HPLC analysis were accomplished to obtain the final refined samples. The binding affinity of the samples were significantly potent

towards the mPRα, which was governed by the steroid binding assay with the crushed cells membrane of transfected cell comprising the mPRα gene. Finally, fraction with mPRa-interacting activity was purified by TSKgel Phenyl-5PW RP Glass with two peaks. The existence of specific chemical compound in the samples were unveiled by ESI mass spectrometry. According to the major signal, the molecular mass was recorded for peak 1 sample as 554 Dalton while the compound of peak 2 showed the molecular mass 554 and 437 Dalton. The fluorogenic attributes of the purified compounds were also determined through spectrometric analysis, the absorbance and fluorescent scanning pattern at maximum excitation indicated that the peak 1 was excited at 418nm and emitted at 668nm while the peak 2 was excited at 412nm and emitted at 672nm. Meanwhile, the purified chemical exhibited the antagonistic attributes with 17alpha, 20beta-dihydroxy-4-pregnen-3-one (17,20beta-DHP) in order to induce the zebrafish oocyte maturation and ovulation *in-vivo*. The purified compounds were unable to induce the fish oocyte maturation and ovulation. On account of this, we attempted to evaluate their antagonistic activity on inducing activity by natural hormone, 17,20ß-DHP. In case of *in vivo* assay, oocyte maturation and ovulation was inhibited significantly by both compound though the oocyte maturation was not prevented completely by the compound from peak 2. Meanwhile, the oocyte maturation was also inhibited by in vitro assay for both compounds.

Based on the current findings, it can be concluded that two secreted compounds from Padina were purified and characterized in this study. Purified compounds interact with the membrane progesterone receptor, or mPR. *In vitro* and *in vivo* assay revealed that the compounds inhibit fish oocyte maturation and ovulation outstandingly. Our purified compounds were highly fluorogenic. However, determination of the chemical structure of the compound which has not yet successful would be our future prospect as new pharmaceutical candidate as well as to make the compound commercially available.