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Anti-inflammatory Activities of *Undaria pinnatifida* and *Laminaria japonica* (Phaeophyta)

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The anti-inflammatory activities of dichloromethane, ethanol, and boiling water extracts of the brown seaweeds *Undaria pinnatifida* (Harvey) Suringar and *Laminaria japonica* Areschoug were examined. Ethanol extracts (0.4 mg/ear) of *U. pinnatifida* inhibited inflammatory symptoms in mouse ear edema by 95.3%, and dichloromethane extract inhibited erythema by 65.5%. Dichloromethane and ethanol extracts (4 g/kg bw) of *L. japonica* demonstrated potent antipyretic activity. Activities of the seaweed extracts were similar to those of the commonly used drugs indomethacin and acetyl salicylic acid. No acute toxicity was observed after p.o. administration of each extract (5 g/kg bw). These results were in agreement with the claims of the health care industry and indigenous medicine that the above seaweeds can be used as an effective remedy for inflammation-related symptoms.

Key words: Anti-edema, Anti-inflammation, *Laminaria japonica*, Phaeophyta, *Undaria pinnatifida*

Introduction

Numerous studies have examined marine organisms, including seaweeds, in the search for new drugs from natural products. For drug development, the selection of samples for biological activity assays is often based on ecological observations of species with unique chemical mechanisms for coping with environmental pressures or assays of active ingredients from organisms used in folk or traditional medicine remedies (Smit, 2004). Several seaweed species are used as traditional medicines, foods, or health care products in various regions of the world. A brown seaweed *Undaria pinnatifida* (Harvey) Suringar, also known as Miyok or Heche, is used as food (Kang, 1968), and has many recorded uses in the treatment of fever, urination problems, lumps, and dropsy, without side effects, according to the Oriental medical textbook *Donguibogam* published in 1613 (Donguibogam Committee, 1999). The seaweed *U. pinnatifida* is well-known as a food among nursing Korean mothers, and almost all Korean women eat *U. pinnatifida* soup for a month or so after childbirth in the belief that it helps postpartum convalescence and cleanses the blood. Traditionally, the Japanese also eat *Undaria* during the postpartum period, and also use it to promote good hair and skin condition (Matsuzaki and Iwamura, 1980). In herbal medicine in China, *U. pinnatifida* is used to treat urinary diseases, dropsy, stomach ailments, hemorrhoids, anal fistulas, leucorrhea in women, and nocturnal emissions in men (Tseng and Chang, 1984). Another brown seaweed, *Laminaria japonica* Areschoug, also known as Dashima or Konpo is used as a food (Kang, 1968) and to treat dropsy, lump, urination problems, and facial swelling (Donguibogam Committee, 1999). The seaweed *L. japonica* is well-known as a rich source of glutamic acid (Ikeda, 2002) and is used extensively in Korean and Japanese cuisine to make a soup stock. In China, *L. japonica* is used to cure goiter, scrofula, urinary diseases, dropsy, stomach ailments, hemorrhoids, and anal fistulas (Tseng and Chang, 1984). Most of these medicinal effects of *U. pinnatifida* and *L. japonica* are directly or indirectly related to the anti-inflammatory action of the seaweeds. Annual productions of *U. pinnatifida* and *L. japonica* in 2006 were estimated at 287,169 tons (wet wt) and 106,408 tons (wet wt) by aquaculture in Korea (MOMAF, 2007). Thus, to evaluate the

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medicinal activity of *U. pinnatifida* and *L. japonica*, both abundant species with immense aquaculture potential, we examined the anti-inflammatory activeties of dichloromethane, ethanol, and boiling water extracts against hyperpyrexia, algesthesia, edema, erythema, and local blood flow in mice.

Materials and Methods

Seaweed materials

Thalli of the brown seaweeds *Undaria pinnatifida* (Harvey) Suringar and *Laminaria japonica* Areschoug were collected from a Kijang Aquaculture Farm (Busan, Korea) in March 2005 and March 2006. The scientific names of the seaweeds described in the Donguibogam were identified from their common or local names (Suh, 1997). A voucher specimen of each is deposited in our laboratory (Y.K. Hong). For 20 g of each seaweed powder, 1 L of dichloromethane or ethanol was used to extract solvent-soluble fraction by shaking at room temperature for 1 hr. For the water-soluble fraction, distilled water was boiled for 1 hr. To remove salt from the seaweed extracts, the extraction was repeated several times (from the previous solvent-soluble fraction) until the amount of salt was visibly negligible. In the case of *U. pinnatifida* powder, the dichloromethane extract was a brownish residue (1.3% yield), the ethanol extract was a greenish brown residue (5.3% yield), and the boiling water extract was brownish (14.6% yield). For *L. japonica* powder, the dichloromethane extract was a brownish residue (1.0% yield), the ethanol extract was greenish brown (1.9% yield), and the boiling water extract was brownish (3.8% yield).

Animals

BALB/c mice (8-10 weeks old; 20-25 g body weight) were used for assaying various activities. The animals were kept at room temperature (24±1°C) on a 12 hr light/dark cycle with free access to food and water. Animal experiments were performed in accordance with the U.S. NIH Guidelines for the Care and Use of Laboratory Animals.

Antipyretic activity

A Brewer's yeast-induced pyrexia model in mice was used to test the antipyretic activity of seaweed extracts (Teotino et al., 1963). When the rectal temperature peaked after 24 hr, either 4 g of extracts in 10 mL of 5% Tween-80 or 10 mL of 5% Tween-80 (control) per kg body weight were administered orally, and the rectal temperature (°C) was recorded after an additional 45 min using an electric thermometer connected to a probe, inserted 2 cm into the rectum. Relative temperature suppression (%) is expressed as [(value of the control - value of the extract) / value of the control] × 100. Acetyl salicylic acid (aspirin, 150 mg/kg, p.o.) was used as a standard.

Analgesic activity

In the tail-flick test (Gray et al., 1970), either extracts (1.5 g/10 mL of 5% Tween-80/kg body weight) or control was administered i.p. to mice, and tail-flick latency time (s) was measured 1 hr later using a tail-flick unit (Ugo Basile, Varese, Italy). Relative latency (%) was expressed as [(value of the extract - value of the control) / value of the control] × 100. Acetyl salicylic acid (150 mg/kg, p.o.) in the same volume of vehicle was used as the standard.

Anti-inflammatory activity

Stock solutions of extracts were prepared by adding ethanol (1 mL) to dried seaweed extracts (40 mg). Phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO, USA) in acetone (0.2 μg/10 μL/ear) was combined with the seaweed extracts in ethanol (0.4 mg/10 μL/ear) and topically applied to the whole inner side of the mouse's ear. Ear edema was measured after 10 hr using a spring-loaded micrometer (Mitutoyo Corp., Tokyo, Japan) (Griswold et al., 1998). Ear erythema was determined at 10 hr using digital photography, adjusted to balance white, and Photoshop 7.0 (Adobe, San Jose, CA, USA) to measure the magenta value. To confirm the anti-inflammatory activity of the seaweeds, local blood flow in the mouse ear was measured using laser speckle flowgraphy (Inflameter LFG-1; SoftCare, Fukuoka, Japan) (Lee et al., 2003). Edema (AU), erythema (AU), and blood flow (AU) values were calculated as $(I_{10} - I_0) / I_0$, where I_{10} is measurement 10 hr after PMA application and I_0 is the measurement at 0 hr. The relative inhibition rate (%) was expressed as [(value of the control - value of the extract) / value of the control] × 100. Indomethacin (0.3 mg/10 μL-ethanol / ear) was used as the standard.

Acute toxicity test

Mice were fasted for 6 hr, with water provided *ad libitum*. Extracts (5 g/10 mL of 5% Tween-80/kg bw) were administered orally to mice ($n = 5$, each). The animals were then observed for any abnormal behavior for 3 hr, and mortality was noted for up to 2 weeks. A group of animals treated with the Tween-80 served as the control.

Statistical analysis

All animal experiments were performed with at

least seven mice for each group, and the highest and lowest values were discarded. Data are reported as means ± S.E. The significance of the results was calculated using Student's *t*-test and was deemed statistically significant at $P < 0.01$.

Results

Effects of *U. pinnatifida* extracts

In preparing traditional medicines and health care foods, it is common to boil the materials in water or to soak them in beverage alcohol. To undertake more detailed investigations of the active substances, we prepared boiling water-, alcohol-, and dichloromethane-soluble extracts of the seaweeds, and determined their anti-inflammatory activities in mice. The seaweed extract's antipyretic activity was evaluated by measuring changes in rectal temperature. The mice were injected with brewer's yeast, and the rectal temperature peaked at 39.19 ± 0.07°C, which was above the normal 38.45 ± 0.06°C, at 24 hr. From the *U. pinnatifida* seaweed, oral administration of extracts marginally lowered rectal temperatures in hyperthermic mice (Table 1). Tail-flick behavior in mice was used to evaluate the analgesic activity of the seaweed extracts. As controls, mice injected with 5% Tween-80 responded by tail flicking in 3.08 ± 0.04 s on average. I.p. injection of ethanol extract showed activity against the algesia, with the latency increasing by 4.09 ± 0.15 s. The conditions for inducing mouse ear edema, erythema, and local blood flow by topical application of PMA were optimized by applying 0.2 μg PMA and measuring ear thickness after 10 hr (Fig. 1). PMA mixed with the ethanol extract (0.4 mg/ear) demonstrated an edema value of 0.04 ± 0.01 AU, about a 95.3% inhibition compared to the PMA control (Table 1). It revealed almost complete suppression of edema at this concentration. Indomethacin (0.3 mg/ear), the standard anti-inflammatory drug, showed 0.14 ± 0.01 AU, with 83.7% inhibition when applied with PMA to the mouse ear. The observed erythema value with 0.2 μg PMA was 0.58 ± 0.01 AU. The PMA plus dichloromethane extract (0.4 mg/ear) demonstrated an erythema value of 0.20 ± 0.02 AU, with 65.5% inhibition. Indomethacin showed 0.22 ± 0.08 AU, with 62.1% inhibition. To confirm the inhibition of edema and erythema, we measured local blood flow using laser speckle flowgraphy. The blood flow value for 0.2 μg PMA was 0.081 ± 0.005 AU, and the PMA plus dichloromethane extract demonstrated 0.036 ± 0.005 AU. From the *U. pinnatifida* seaweed, ethanol (Fig. 1) and dichloromethane extracts (i.e., likely non-polar compounds) showed potent ($P < 0.001$) anti-inflammatory activities, especially anti-edema and anti-erythema actions.

Effects of *L. japonica* extracts

The antipyretic activity of the seaweed extracts was evaluated by measuring changes in rectal temperature. From the *L. japonica* seaweed, oral administration of dichloromethane and ethanol extracts (4 g/kg bw) potently lowered rectal temperatures in hyperthermic mice to 34.58 ± 0.45°C and 35.10 ± 0.33°C, respectively (Table 2). Using acetyl salicylic acid (150 mg/kg bw) as the standard antipyretic drug, the temperature was 35.38 ± 0.58°C when administered p.o. in hyperthermic mice. Thus, the seaweed extract revealed stronger suppression of temperature than the standard drug. Tail-flick behavior in mice was used to evaluate the analgesic activity of the seaweed extracts. I.p. injection of the dichloro-

Table 1. Effect of *U. pinnatifida* extracts on anti-inflammatory activities against hyperpyrexia, algesthesia, edema, erythema, and local blood flow in mice. Each value represents the mean ± S.E.; $n \geq 5$. *$P < 0.01$ compared to control. **$P < 0.001$ compared to control

	Temperature (Antipyretic %)	Tail flick (Latency %)	Edema (Inhibition %)	Erythema (Inhibition %)	Blood flow (Inhibition %)
Control	36.92 ± 0.10 (-)	3.08 ± 0.04 (-)	0.86 ± 0.03 (-)	0.58 ± 0.01 (-)	0.081 ± 0.005 (-)
CH_2Cl_2 extract	36.53 ± 0.06 (1.1)	3.86 ± 0.23** (25.3)	0.22 ± 0.02** (74.4)	0.20 ± 0.02** (65.5)	0.036 ± 0.005* (55.6)
EtOH extract	36.54 ± 0.09 (1.0)	4.09 ± 0.15** (32.8)	0.04 ± 0.01** (95.3)	0.32 ± 0.03** (44.8)	0.069 ± 0.035 (14.8)
Boiling water extract	36.44 ± 0.33 (1.3)	3.39 ± 0.26 (10.1)	0.49 ± 0.06* (43.0)	0.39 ± 0.04** (32.8)	0.076 ± 0.025 (6.2)
Acetyl salicylic acid	35.38 ± 0.58* (4.2)	4.40 ± 0.30** (42.8)	- (-)	- (-)	- (-)
Indomethacin	- (-)	- (-)	0.14 ± 0.01** (83.7)	0.22 ± 0.08** (62.1)	0.024 ± 0.016** (70.4)

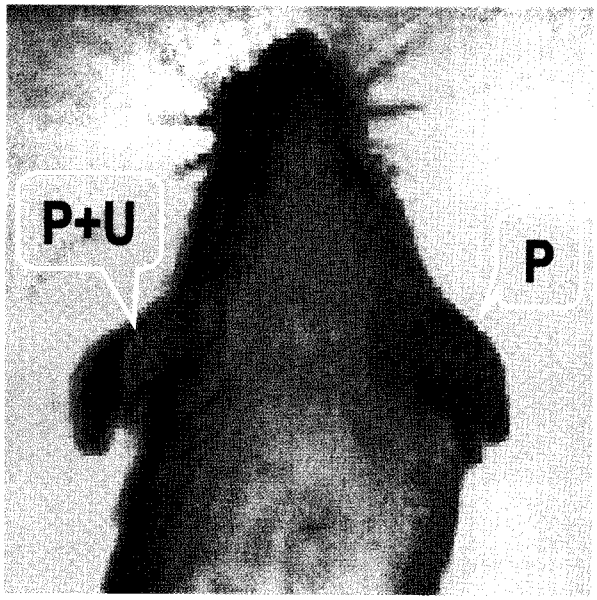

Fig. 1. Inflammation of mouse ear at 10 hr after PMA application. PMA (0.2 μg/10 μL-acetone/ear) was combined with the ethanol extract (0.4 mg/10 μL-ethanol/ear) of *U. pinnatifida* as a representative, and topically applied to the whole inner side of the left ear (P+U). Right ear (P) was applied with PMA as the control.

methane extract showed marginal analgesic activity, with a latency increase of 3.41 ± 0.17 s. To induce mouse ear edema, erythema, and local blood flow, we applied 0.2 μg PMA topically and took measurements 10 hr later. PMA mixed with the ethanol extract (0.4 mg/ear) demonstrated an edema value of 0.54 ± 0.05 AU (Table 2), and the PMA with the dichloromethane extract (0.4 mg/ear) produced an erythema value of 0.39 ± 0.04 AU. When we measured local blood flow, the PMA with the dichloromethane extract was 0.072 ± 0.015 AU. From the *L. japonica* seaweed, the dichloromethane and ethanol extracts (likely nonpolar compounds) showed potent ($P < 0.001$) antipyretic activity.

Acute toxicity

We evaluated any acute toxicity that the most active dichloromethane extracts might exhibit in mice, even though *U. pinnatifida* and *L. japonica* are staple foods in the daily diets of many Koreans and Japanese. Over the 2 weeks of observation, no death occurred in any group of five mice administered a dose of 5 g/kg bw. Most of the mice administered *U. pinnatifida* extract reacted immediately by jumping, sleeping, scaling, and writhing for 5 to 10 min. Mice administered *L. japonica* extract reacted by scaling and sleeping for a while and returned to normal behavior after 1.5 hr.

Discussion

The brown seaweeds *U. pinnatifida* and *L. japonica* are common along cold temperate coastal regions of the northeast Pacific, including Korea, Japan, and northern China. These brown seaweeds belong to the order Laminariales and grow on rocks and reefs in the subtidal zone to a depth of 1 to 15 m below tide-level. Both are also extensively farmed in Korea, Japan, and China.

In this study, we demonstrated that the ethanol extract of *U. pinnatifida* potently inhibited PMA-induced edema. Topical application of PMA induces a long-lasting inflammatory response, resulting from protein kinase C (PKC) activation associated with a

Table 2. Effect of *L. japonica* extracts on anti-inflammatory activities against hyperpyrexia, algesthesia, edema, erythema, and local blood flow in mice. Each value represents the mean ± S.E.; $n \geq 5$. *$P < 0.01$ compared to control. **$P < 0.001$ compared to control

	Temperature (Antipyretic %)	Tail flick (Latency %)	Edema (Inhibition %)	Erythema (Inhibition %)	Blood flow (Inhibition %)
Control	36.92 ± 0.10 (-)	3.08 ± 0.04 (-)	0.86 ± 0.03 (-)	0.58 ± 0.01 (-)	0.081 ± 0.005 (-)
CH_2Cl_2 extract	34.58 ± 0.45** (6.3)	3.41 ± 0.17 (10.7)	0.66 ± 0.09 (23.3)	0.39 ± 0.04** (32.8)	0.072 ± 0.015 (11.1)
EtOH extract	35.10 ± 0.33** (4.9)	3.29 ± 0.17 (6.8)	0.54 ± 0.05* (37.2)	0.40 ± 0.05** (31.0)	0.082 ± 0.025 (-1.2)
Boiling water extract	36.74 ± 0.08 (0.5)	3.08 ± 0.39 (0)	0.80 ± 0.05 (7.0)	0.57 ± 0.05 (1.7)	0.083 ± 0.017 (-2.5)
Acetyl salicylic acid	35.38 ± 0.58* (4.2)	4.40 ± 0.30** (42.8)	- (-)	- (-)	- (-)
Indomethacin	- (-)	- (-)	0.14 ± 0.01** (83.7)	0.22 ± 0.08** (62.1)	0.024 ± 0.016** (70.4)

transient increase in prostanoid production and marked cellular influx (Nishizuka, 1989). This high possesses powerful antioxidant activity (Heo et al., 2005), as it has been shown that the treatment of prostaglandin level is likely due to COX induction (Muller-Decker et al., 1995). The seaweed also mouse skin with a PKC activator, such as PMA, induces the formation of free radicals in vivo (Wei and Frenkel, 1992). The tail-flick response is believed to be a spinally mediated reflex (Chapman et al., 1985). Souza et al. (2002) reported that an intraplantar injection of PMA induces a painful response in mice. Several inflammatory mediators produce algesthesia by peripheral and spinal sensory fibers sensitization through protein kinase activation, including PKC (Scholz and Wolf, 2002). Algesthesia is associated with the formation of edema and erythema by PMA treatment (Tsuchiya et al., 2005), and thus the main active constituents in the seaweed extracts may contribute to inhibiting the pathway that mediates the pain, edema, and erythema associated with inflammation. Moreover, the dichloromethane and ethanol extracts of *L. japonica* were found to cause significant temperature decreases in yeast-induced fever. This result is consistent with the view that *L. japonica* influences prostaglandin biosynthesis, as prostaglandin is believed to be a regulator of body temperature (Dascombe, 1985). The production of prostaglandins in the central nervous system is the final common pathway responsible for fever induction (Howard, 1993). Inhibition of prostaglandin synthesis is thus a possible mechanism of the antipyretic action, as it is of acetyl salicylic acid (Akio et al., 1988).

Recently, the major active compounds from the seaweeds have been purified and their chemical identification is in progress. They appear to be similar to n-3 polyunsaturated fatty acids (n-3 PUFA), which are known for their anti-inflammatory effects related to their competition as substrates for cyclooxygenase and lipoxygenase, thus leading to decreased production of prostaglandins and leukotrienes (James et al. 2000). Dietary supplementation with n-3 PUFAs causes a reduction in the expression and activity of aggrecanases, inflammation-inducible cytokines (interleukin-1α and tumor necrosis factor-α), and cyclooxygenase-2, but not the constitutively expressed cyclooxygenase-1 (Curtis et al. 2000).

According to the WHO (1992), an herbal medicine is considered toxic if the LD_{50} is lower than 5 g/kg body weight. On this basis, the extracts of *U. pinnatifida* and *L. japonica* are not toxic because no mortality was observed at 5 g/kg. Our investigation suggests the extracts can be safely used by humans at moderate doses.

In conclusion, the present investigation demonstrated that extracts of the brown seaweeds *U. pinnatifida* and *L. japonica* have significant anti-edema and antipyretic activities, respectively, without any serious toxic effect at moderate doses. These findings reinforce the claims of the health care industry and indigenous medicine that those seaweeds can be used as remedies for inflammation-related symptoms.

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References

Akio, M., N. Tomoki, W. Tatsuo, O. Takuya and M. Naotoshi. 1988. Pattern differences in experimental fevers induced by endotoxin endogenous pyrogen and prostaglandins. Am. J. Physiol., 254, R633-R640.

Chapman, C.R., K.I. Casey, R. Dubner, K.M. Foley, R.H. Gracely and A.E. Reading. 1985. Pain measurement: an overview. Pain, 22, 1-31.

Curtis, C.L., C.E. Hughes, C.R. Flannery, C.B. Little, J.L. Harwood and B. Caterson. 2000. n-3 Fatty acids-specifically modulate catabolic factors involved in articular cartilage degradation. J. Biol. Chem., 275, 721-724.

Dascombe, M.J. 1985. The pharmacology of fever. Prog. Neurobiol., 25, 327-373.

Donguibogam Committee. 1999. Translated Donguibogam. Bubinmunwha Press, Seoul, 1-2198.

Gray, W.D., A.C. Osterberg, J.T. Scute. 1970. Measurement of the analgesic efficacy and potency of pentazocine by the D'Amour and Smith method. J. Pharmacol. Exp. Ther., 172, 154-162.

Griswold, D.E., L.D. Martin, A.M. Badger, J. Breton and M. Chabot-Fletcher. 1998. Evaluation of the cutaneous anti-inflammatory activity of azaspiranes. Inflamm. Res., 47, 56-61.

Heo, S.J., S.H. Cha, K.W. Lee, S.K. Cho and Y.J. Jeon. 2005. Antioxidant activities of Chlorophyta and Phaeophyta from Jeju Island. Algae, 20, 251-260.

Howard, M. 1993. Fever: causes and consequences. Neurosci. Biobehav. Rev., 17, 237-269.

Ikeda, K. 2002. New seasoning. Chem. Senses, 27, 847-849.

James, M.J., R.A. Gibson and L.G. Cleland. 2000. Dietary

polyunsaturated fatty acids and inflammatory mediator production. Am. J. Clin. Nutr., 71, 343S-348S.

Kang, J.W. 1968. Illustrated Encyclopedia of Fauna & Flora of Korea: Marine Algae, Korea. Samhwa Press, Seoul, 1-465.

Lee, M.C., N. Konishi and H. Fujii. 2003. Blood flow analysis of skin tissue under the sacrum using laser speckle flowgraphy. Optic. Rev., 10, 562–566.

Matsuzaki, S. and K. Iwamura. 1980. Application of seaweeds to human nutrition and medicine. In: Proceedings of the International Symposium for Food from the Sea. Noell, H., ed. Bremerhaven, Germany, 162-185.

MOMAF. 2007. Statistic Database for Fisheries Production. Retrieved from http://fs.fips.go.kr/main.jsp on July 14.

Muller-Decker, K., K. Scholz, E. Marks and G. Fustenberger. 1995. Differential expression of prostaglandin H synthase isozymes during multistage carcinogenesis in mouse epidermis. Mol. Carcinogen., 12, 31-41.

Nishizuka, Y. 1989. Studies and prospectives of the protein kinase C family for cellular regulation. Cancer, 63, 1892-1903.

Scholz, J. and C.J. Wolf. 2002. Can we conquer pain? Nature Neurosci., 5, 1062-1067.

Smit, A.J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: a review. J. Appl. Phycol., 16, 245-262.

Souza, A.I., F.A. Moreira, K.R. Almeida, C.M. Bertollo, K.A. Costa and M.M. Coelho. 2002. In vivo evidence for a role of protein kinase C in peripheral nociceptive processing. Br. J. Pharmacol., 88, 544-548.

Suh, K.T. 1997. Classification of herbs in decoction part of Donguibogam. Ph.D. Thesis, Kyungsung University, Busan, Korea. 1-138.

Teotino, U.M., L.P. Friz, A. Gandini and D.D. Bella. 1963. Thio derivatives of 2,3-dihydro-4*H*-1,3-benzoazin-4-one synthesis and pharmacological properties. J. Med. Chem., 6, 248-250.

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

Tsuchiya, M., A. Sakakibara and M. Yamamoto. 2005. A tachykinin NK1 receptor antagonist attenuates the 4beta-phorbol-12-myristate-13-acetate-induced ociceptive behaviour in the rat. Eur. J. Pharmacol., 507, 29-34.

Wei, H. and K. Frenkel. 1992. Suppression of tumor promoter-induced oxidative events and DNA damage in vivo by sarcophytol A: a possible mechanism of antipromotion. Cancer Res., 52, 2298-2303.

WHO. 1992. Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine. Regional Office for Western Pacific, Manila, Philippines, 1-38.

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