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Anti-Inflammatory and Analgesic Activity from Brown Algae *Sargassum polycystum*

Nanik Retno Buwono*[1], Yenny Risjani[1, 2], Sulastri Arsad[1, 2]

[1]*Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang 65145, Indonesia*

[2]*BioEcotox Research Center, Universitas Brawijaya, Malang 65145, Indonesia*

Abstract

The brown alga is widely distributed at many coastal areas in tropical zone such as in Indonesia. It has a potential as an antibacterial and analgesic agent, anti-inflammatory and antitumor properties. Therefore, an evaluation of analgesic and anti-inflammatory effect of Sargassum polycystum on mice model of fed-inflammatory is evaluated. The pain responses on normal and inflammatory mice model after it treated with extract of Sargassum polycystum at particular dose compared to untreated mice (control) is calculated. The analgesic effect was examined based on Writhing and Randall sellito method, and Paw Edema method for determining anti-inflammatory effect. The optimum inhibitory effect (86.67%) of analgesic and anti-inflammatory (48.15%) were sterol fraction (FSS) of Sargassum polycystum at dose 70 mg/kg, FSS fraction has a higher percentage of inhibition than aspirin (standard). The highest fucosterol concentration was obtained in hexane fraction compared to the fraction of chloroform fraction there were 3.89% and 3.64%, respectively.

Keywords: anti-inflammatory effects, analgesic, bioactivity, pain, Sargassum

INTRODUCTION

Macro algae have caused an emerging interest in the biochemical area due to their content on pharmacologically bioactive substances with great chances to be employed against bacteria, virus other pathogens and tumors (Smit, 2004; Torres et al., 2005). The algae can be used as antiviral, antibacterial and antifungal (Vitor et al., 2002). One of many problems in aquaculture is the attack of bacteria from genus of *Aeromonas* and *Vibrio* that cause fish disease. Alternative medical treatment with an organic substance considered safer for consumers. Several pharmaceutical researches show algae as a source of new anti-inflammatory, although the percentage is still low. One of a recent study from Bitencourt et al (2008), examined that marine red algae *Hypnea cervicornis* as an anti-inflammatory in vivo in mice and Wistar rats that showed activity of bioactive compounds produced inflammatory process control. Compounds potentially as anti-inflammatory can be found in nature such as alkaloids, terpenes, sterols glikoside (Vieira et al., 2004; Mayer and Hamann, 2004). Navarro et al (2001) reported that the sterol fraction isolated from acetone extract of *Sideritis foetens* has significant anti-inflammatory activity.

Pierong et al (2006) identified the presence of compounds 67Δ5-3β-sterols with sterenol, which is a carbon chain C19-C26-C23 to C30 of *Sargassum muticum* (Phaeophyta), brown algae. The brown algae contain plant sterols i.e. fucosterol, cholesterol, stigmasterol and clerosterol, which were not found in other algae (Kapetanovic et al., 2005). One of the abundant brown algae found in Indonesia is *Sargassum polycystum*. The present study was to investigate *analgesic and anti-inflammatory effect of Sargassum polycystum on mice model of fed-inflammatory*.

MATERIALS AND METHODS

Plant Material

The fresh Brown macro algae (*Sargassum polycystum*) were collected from Java Sea, Indonesia. The collected plant material were sorted, washed 3 times, and chopped. After air- dried during 2 weeks, it was powdered with mechanical grinder. The powdered sample was extracted with maceration method with 96% aqueous methanol for 2 days at room temperature. The macerated mixture was filtered and evaporated 35-40 °C (Aderogba et al.,2006). The crude extract of *Sargassum polycystum* was stored in the refrigerator until next experiment at Laboratory of Chemistry, Universitas Brawijaya in Malang.

Phytochemical Screening

Sterol from *Sargassum polycystum* was obtained by using two different solvents: hexane and chloroform after maceration as described in Indrayani *et al* (2006). It results fraction of *Sargassum polycystum*. The hexane and chloroform fractions were determined phytosterol concentration by using GCMS (Shimadzu GCMS-QP2010SRtx-5MS column, He, Temperature of column oven at 80 °C, injection at 310°C, final temperature of 29 °C, hold time of 34 min, p : 16.5 kPa). The sterol-contained fraction was used for analgesic and anti- inflammatory assays.

Animal

Female Wistar rats (*Rattus novergicus*) were acclimated and treated in the Laboratory of Pharmacology, Universitas Brawijaya, Malang. The animals were aged 2-4 months and weighing 150-250 g.

Analgesic Activity by Writhing Test

Rats were treated the fraction containing sterol orally 30 min before injected intraperitoneally with 0.6% acetic acid (10 ml/ kg body weight). After 10 min, the number of contraction was calculated per 20 min (Bitencourt et al., 2008). Analgesic effect can be determined as the decrease percentage of contraction number (percentage) by the following equation: decrease percentage of contraction number = contraction number of control – contraction number of treatment/ contraction number of control x 100 (Gupta et al., 2007).

Anti-inflammatory Activity

Rats were treated orally by fraction containing sterol 30 min before carragenan induction. Carragenan (1%; 0.1 ml) was injected intraperitoneally into right-back paw rat. Paw size was measured before (control, 0 min.) and after carragenan induction (30 and 90 min.). The paw size can be compared between control and treated one (Bitencourtet al., 2008). Anti-inflammatory effect can be measured by the decrease percentage of edema size (Ibironke and Ajiboye, 2007) as follow:

% decrease Randall sellito $(C_t - C_0)$control – $(C_t - C_0)$treated/$(C_t - C_0)$control x 100… (1)

Where C_t is edema size at t time and C_0 is edema size before carragenan induction.

Analgesic Activity by Randall Sellito

In this test, pain was measured in response to mechanical stimuli after treatment of inflammation. Analgesic respond of the withdrawal threshold of the paw was measured by analgesimeter (Pierre et al., 2006). Analgesic effect can be counted using Ibironke and Ajiboye (2007) formula:

% decrease Randall sellito $(C_t - C_0)$control – $(C_t - C_0)$treated/$(C_t - C_0)$control x 100 …....(2) Where C_t is edema size at t time and C_0 is edema size before carragenan induction.

RESULT AND DISCUSSION

Anti-inflammatory and Analgesic Effect

Result of GCMS analysis using hexane fraction from *Sargassum polycystum* (FHS) has 67 peaks, which have 6 higher peaks; there are *Hexadecanoic acid*, *Octadecanoic acid Neophytadiene*, *Stigmasta-5dien24-3-ol Fucosterol* and *Hexadecen-1-ol* (Figure 1 and Table 1). Hexadecanoic acids (peak no 25 and 27) has concentration of 31.15%. This compound is a group of palmitic acid, a derivate of unsaturated fatty acids, which having role in fatty acid biosynthesis (French et al., 2002). Several hexadecanoic and octadecanoic from pare fruit showed antitumor activity (Rita et al., 2007). Hexadecanoic acid from Sargassum plays role to inhibit bacterial growth (Junaedi et al., 2009).

Neophytadine (peak no 18) had 9.06% of concentration and *hexadecen-1-ol* (peak no 22) was 2.80%. According to Patton and Benson (1966) *hexadecen-1-ol* is a phytol group with unsaturated acid structure, which has important role in chlorophylls molecule placement in cellular plant chloroplast membrane. The concentration of *Stigmasta-5dien24-3-ol Fucosterol* (peak 67) was 3.89%, according Kapetanovic et al (2005) stigmasta-5-3-ol dien24 Fucosterol a phytosterol group.

Figure 2 shows the GCMS chromatogram and determination 6 higher peaks of chloroform fraction. Octadecenoic acids identified in peak no 34, 37, 36 with a total percentage of 22.11%; the compound is a group of oleic acid (Pacheco, 2006). Hexadecanoic acid concentration showed dominant with 21.80%, followed by stigmasta-5dien24-3-ol and stigmasta Fucosterol-4, 7.22 Trien-3beta-ol that is the phytosterol group were 3.64% (Table 2).

Between the two fractions (Hexane and chloroform), phytosterol concentration in hexane fraction (3.89%) was higher than in chloroform fraction (3.64%). Indrayani et al., (2006) used hexane and chloroform solvent for sterol determination in *Stachytarphetajamaicensis L. Vahl*. According to Bhakuni and Rawat (2005), fucosterol was dominant in brown algae; and Pierong et al (2006) has isolated sterol (24.98%) from *Sargassum muticum*, while other brown algae, *Stilophorarhizodes* (4.6%), *Punctarialatifolia* and *Punctariaplantaginea* (18.7%). This variation was caused by different environment condition of marine algae, such as salinity, temperature, and so on (Kamenarska et al., 2003).

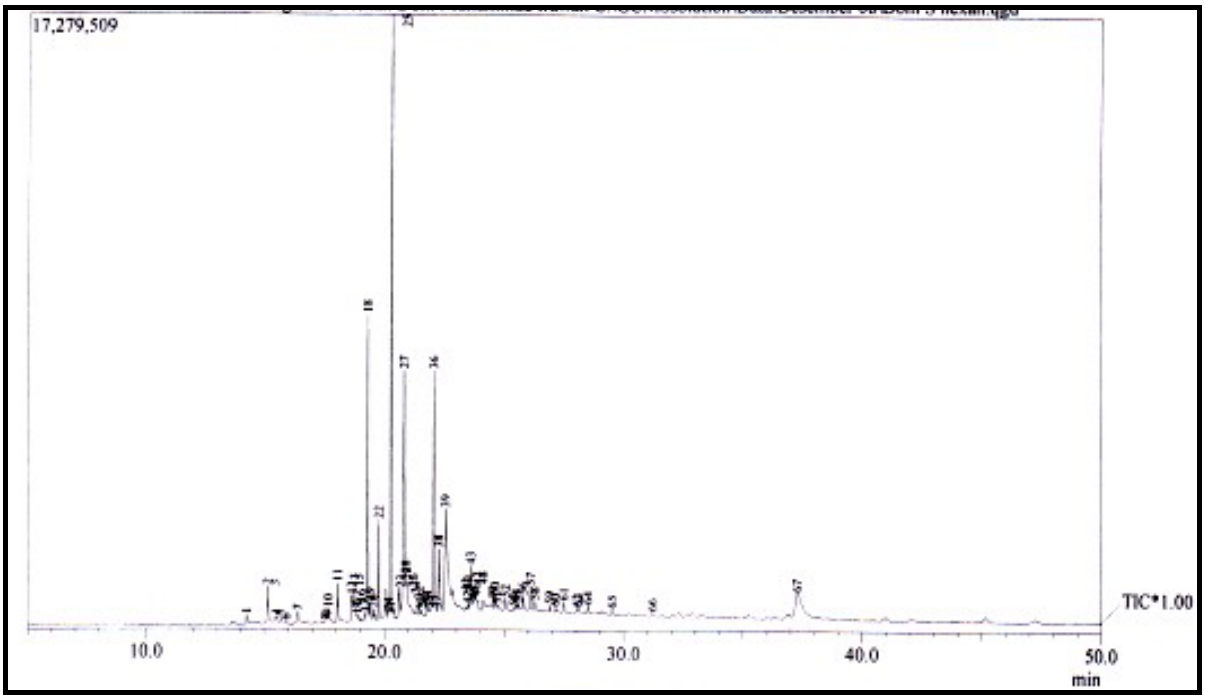

Fig 1. Determination of hexane fraction from *Sargassum polycystum* extract by GCMS

Table 1. Identification of the compounds contained in the hexane fraction of *Sargassum polycystum*

Peak no	Name	formula	Chemical group	MW	Concentration (%)
25	*Hexadecanoic acid*	$C_{17}H_{34}O_2$	Fatty acid	270	18,87
27	*Hexadecanoic acid*	$C_{16}H_{32}O_2$	Fatty acid	256	12,28
36	*Octadecenoic acid*	$C_{19}H_{36}O_2$	Fatty acid	298	12,27
18	*Neophytadiene*	$C_{20}H_{38}$	Fatty acid	278	9,06
67	*Stigmasta-5dien24-3-ol Fucosterol*	$C_{29}H_{48}O$	Phytosterol	412	3,89
22	*Hexadecen-1-ol*	$C_{20}H_{40}$	Fatty acid	296	2,80

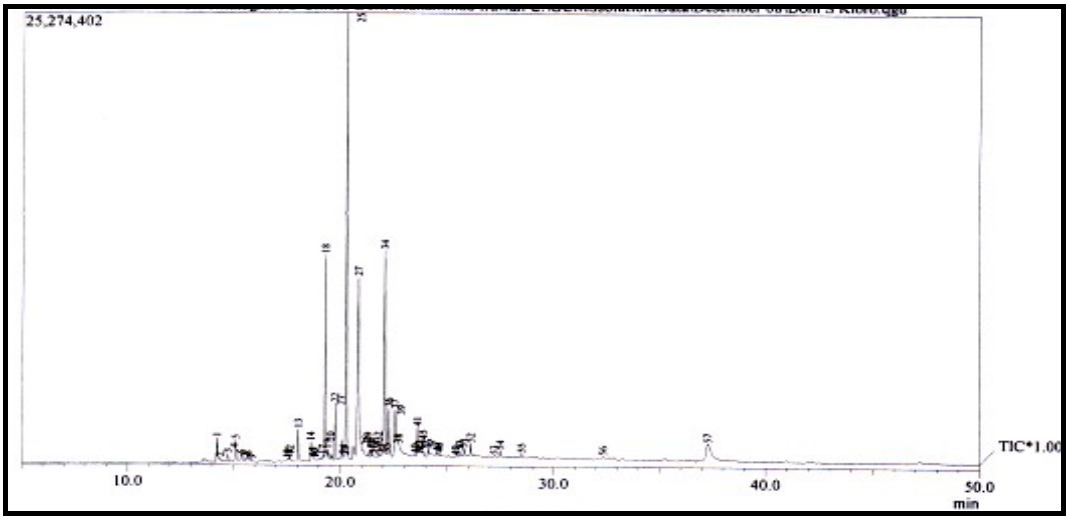

Fig. 2 Determination of chloroform fraction from *Sargassum polycystum* (FCS) extract by GCMS

Table 2. Identification of the compounds contained in the chloroform fraction of *Sargassum polycystum*

Peak no	Name	Formula	Chemical group	MW	Concentration (%)
25	*Hexadecanoic acid*	$C_{17}H_{34}O_2$	Fatty acid	270	21,80
34	*Octadecenoic acid*	$C_{19}H_{36}O_2$	Fatty acid	296	13,45
37	*Octadecenoic acid*	$C_{39}H_{72}O_5$	Fatty acid	621	5,70
57	*Stigmasta-5dien24-3-ol Fucosterol*	$C_{29}H_{48}O$	Phytosterol	412	3,64
36	*Octadecenoic acid*	$C_{19}H_{38}O_2$	Fatty acid	298	2,96
56	*Stigmasta-4,7,22 Trien-3beta-ol*	$C_{29}H_{46}O$	Phytosterol	410	0,21

Table 3. Analgesic value of FHS on Wistar mice

Treatment	Writhing test		Metode Randall Sellito				
	Number of contraction	% Decrease	Analgesic value				% Inhibition
			0 min	30 min	60 min	90 min	
C- (control)	41 ±1	0*	64±5,48	20±0	30±7,07	34±5,48	0*
SA (FHS 40mg/Kg BW)	23,6 ±2,30	42,44*	64±5,48	36±5,48	50±7,07	48±8,36	46,67*
SB (FHS 70mg/Kg BW)	13,2 ±4,55	67,8*	70±7,07	56±8,94	62±4,47	66±5,48	86,67*
SC (FHS 100mg/Kg BW)	16 ±4,69	60,9*	70±10	50±0	56±8,94	62±4,47	73,33*
C+ (Aspirin 100mg/Kg BW)	28 ±7,11	31,7*	66±5,48	48±4,47	50±0	56±5,48	66,67*

(*) significantly different (ANOVA)

% decrease of writhing = control – treatment/control*100

% decrease Randall sellito = $(C_t - C_0)$ control – $(C_t - C_0)$ treated/ $(C_t - C_0)$control*100

Where C_t is edema size at t time and C_0 is edema size before carragenan induction

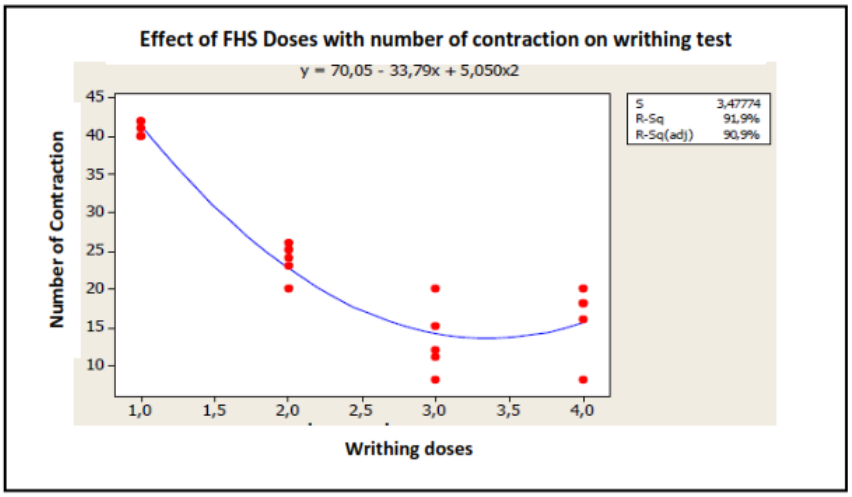

Fig 3. Relation between number of contraction and FHS doses resulted from Writhing test Doses: 40 mg/kg BW, 70 mg/kg BW, 100 mg/kg BW, five replications

Table 4. Paw edema test result in wistar mice

Treatment	edema (cm)			% inhibition
	0 min	30 min	90 min	
C- (control inflammation without treatment)	0,45±0,021	0,71±0,042	0,72±0,032	0*
SA (FHS 40mg/Kg BW)	0,39±0,034	0,49±0,025	0,55±0,046	40,74*
SB (FHS 70mg/Kg BW)	0,39±0,034	0,45±0,039	0,53±0,065	48,15*
SC (FHS 100mg/Kg BW)	0,44±0,018	0,53±0,035	0,59±0,017	41,48*
C+ (Aspirin 100mg/Kg BW)	0,45±0,019	0,52±0,022	0,62±0,033	35,56*

Edema size (cm) is average value with ± DS (*) significantly different (ANOVA)
% inhibition of paw edema = $(C_t - C_0)$ control – $(C_t - C_0)$treated/ $(C_t - C_0)$control*100
Where C_t is edema size at t time and C_0 is edema size before carragenan induction

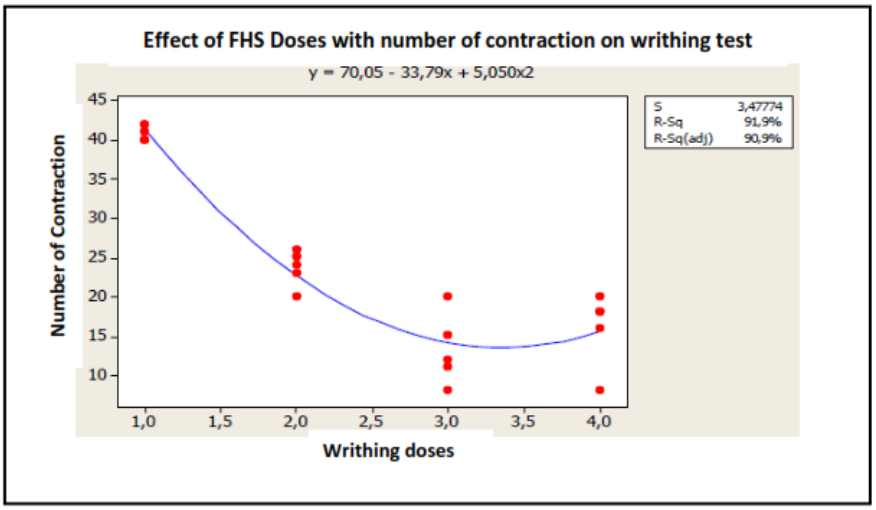

Fig 4. Relation between FHS doses on edema size. Doses: control (1.0) and the different FHS doses (40 mg/kg BW, 70 mg/kg BW, 100 mg/kg BW) with five replications

Analgesic test using Writhing and Randall sellito method was used to quantify analgesic effect of fraction containing sterol on Wistar mice. According to Bars et al (2001), it can be defined by contraction of abdominal muscle and body extension. This test is based on pain measured as the mechanical response after treatment with carrageenan inflammation (Guay et al., 2004). Analgesic value can be determined quantitatively using analgesimeter (Pierre et al., 2006). Writhing test used acetic acid for stimulus response, according to Deniz et al (2009), acetic acid can caused histological destruction that related to stimulus response. Randall sellito method is using carrageenan to inflict pain stimulus-response followed by physiological changes and edema in rats (Jlaakso, 2000). Writhing and Randall sellito methods on Table 3 show analgesic test result of FHS on Wistar mice. Figure 3 shows the relation of FHS concentration and contraction number of Wistar mice.

The data showed the highest analgesic effect found Hexane fraction at dose 70mg/Kg BW, with an average reduction of 67.8% (± SD = 13.2 ± 4.55%). The analgesic effect higher compared to Hexane fraction at dose40 mg per BW and BBW 100mg/ kg; and aspirin. However the fraction has lower analgesic activity compared to agglutinin isolated form *Hypnea cervicornis* (Bitencourt et al., 2008). The analgesic activity of the fraction was detected similar according two methods that we used either using Randall sellito or writhing test method. The Hexane fraction has the highest analgesic activity, with response value and inhibition percentage at 86.67% according to Randall sellito method. Noda (2000) has also reported that hexane, methanol and butanol extract of three Sargassum species such as *S. Tenerrium*, *S. Wightii* and *S. Cervicorne* using Writhing test showed a high value inhibition presentation (97%), which is higher than aspirin (53.5%).

We used Paw edema method to examine volume or thicknessof mice paw that has been induced by carragenan (Bitencourt et al., 2008; Rasool et al., 2006). The result of anti-inflammatory activity showed that the hexane fraction decrease edema size on mice model (Table 4.). Injection carragenan 1 ml at sub-cutanic paw mice increased edema at minute 30 that caused paw inflammation and then decreased at minute 90. The decreased of the edema was caused by hexane fraction treatment, especially at dose of 70 mg/kg BW. Furthermore paw edema size decrease consistent with correspond to increasing dose the hexane fraction (Fig.4). It indicates that the hexane fraction has anti-inflammatory activity with optimum dose 70 mg/kg BW.

According to Navarro (2001), sterol inhibit the pain by decreasing cellular membrane sensitivity, this can be function as stabilization. Stabilization of cellular membrane caused the inhibition of arachidonate metabolism and this will stop prostaglandin formation. This study shows that fucosterol may be having function as medical treatment for analgesic and anti-inflammatory effect. Pain inhibition and inflammation can be caused by cholesterol inhibition in cellular membrane by fucosterol (Ikeda et al., 1988; Umukoro and Asharobi, 2006). Effect of inflammation may caused inflammatory cells i.e. neutrophyle in the organic tissue (Bucci et al., 2004).

This study showed that Sargassum has a potential antibacterial and analgesic agent, anti-inflammatory. Sargassum has fucosterol may be have function as medical treatment for analgesic and anti-inflammatory effect. Our studies about bioactivity of the brown algae Sargassum could be recommended for the production of fucosterol, phlorotannin and composition for medical treatment.

CONCLUSION

Sargassum has fucosterol may be have function as medical treatment for analgesic and anti-inflammatory activity. The hexane fraction showed 3.89% of fucosterol content. Optimum test of the inhibitory effect of analgesic-Randall Sellito writhing method is a sterol fraction at a dose of 70 mg/ kg, while for the anti-inflammatory activity with Paw edema method also showed the same inhibitory effect on the optimum and the same fraction.

REFERENCES

[1] Aderogba M.A., E.K. Okoh, I.N. Okeke, A.O.Olajide, A.O. Ogundaini. Antimicrobial and Anti-inflammatory Effects of Piliostigma reticulatum Leaf Extract. *International Journal of Pharmacology*. 2006, *2*(1), 70-74. DOI:10.3923/ijp.2006.70.74.

[2] Bars, DL., M. Gozariu and SW Cadden. Animal Models of Nociception.*PharmacologicalReviews*. 2001, *53*(4), 598 643.

[3] Bhakuni D.S. and D.S. Rawat. Bioactive Marine Natural Products.Co-published by Springer. 2005. New York USA.

[4] Bitencourt, F.S, JG.Figueiredo, MR. L. Mota, CCR.Bezerra, PP. Silvestre, MR. Vale, KS.Nascimento, AH.Sampaio, CS. Nagano, SS Sampaio, WR. L. Farias, BS. Cavada, AM. S. Assreuy, NM. N. Alencar. Antinociceptive and anti-inflammatory effects of a mucin-binding agglutinin isolated from the red marine alga Hypneacervicornis. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2008, *377*, 139–148.

[5] Bucci, M, F Roviezzo, I Posadas, J Yu, L Parente, W C. Sessa, L J. Ignarro, G Cirino.Endothelial nitric oxide synthase activation is critical for vascular leakage during acute inflammation in vivo. 2004, www.pnas.org_cgi_doi_10.1073_pnas.0408906102.

[6] Deniz, M, Hasan H., SemaT., MelikeY., Buğra A., Şule Ç., and Berrak Ç. Nicotine withdrawal alleviates acetic acid-induced gastric injury in rats. *Environmental Toxicology and Pharmacology*. 2009, *27*(2), 200-205. doi:10.1016/j.etap.2008.10.006.

[7] French MA., Sundram K., Clandinin MT. Cholesterolaemic effect of palmitic acid in relation to other dietary fatty acids. *Asia Pacific J Clin Nutr*. 2002, *11*(Suppl), S401–S407.

[8] Guay J., Kevin B., Robert G., Joseph M. and Denis R. Carrageenan-induced Paw Edema in Rat Elicits a Predominant Prostaglandin E2 (PGE2) Response in the Central Nervous System Associated with the Induction of Microsomal PGE2 Synthase-1. *The Journal of Biological Chemistry*. 2004, *279*(23), 24866–24872. DOI 10.1074/jbc.M403106200.

[9] Gupta, M. Mazumdar, U.K, Gomathi, P. Anti-inflammatory and Antinociceptive Effects of *Galegapurpurea* Root. *International Journal of Pharmacology*. 2007, *3*(3), 210– 218. ISSN 1811-7775.

[10] Ibironke, G.F., Ajiboye, K.I. Studies on the Anti-inflammatory and Analgesic properties of *Chenopodium Ambrosioides* Leaf Extract in Rats. *International Journal of Pharmacology*. 2007, *3*(1): 111-115. ISSN 1811-7775.

[11] Ikeda, I., Kazunari T., Michihim S., George V. and Linda L. Inhibition of cholesterol absorption in rats by plant sterols. *Journal of Lipid Research*. 1988, *29*.

[12] Indrayani, L., Hartati S., Lydia S. Skrining Fitokimia dan Uji Toksisitas Ekstrak Daun Pecut Kuda (*Stachytarpheta jamaicensis* L. Vahl) terhadap Larva Udang *Artemia salina* Leach. *Berk.Penel.Hayati*. 2006, *12*, 57–61.

[13] Jlaakso. Rat Rat-forum Animal Models conference,1st Announcement.2000. jlaakso@healthtech.com.

[14] Junaedi, Risjani Y. and H Nursyam. Uji Aktifitas Antibakteri Ekstrak Rumput *Laut K alvarezii* dan *E denticulatum* terhadap bakteri *Aeromonas hydrophyla* dan *Vibrio alginolyticus*. 2008. Faculty of Fisheries and Marine Sciences. Universitas Brawijaya.

[15] Kamenarska, Z.G., Stefka D.D.K., Kamen L.S, Simeon S.P. A Comparative Study on The Sterol Composition on Some Brown Algae from the Black Sea. *J. Serb. Chem. Soc*. 2003, *68*(4-5), 269 – 275.

[16] Kapetanovic R., S Dusan, P. Simeon, Z Mario, K. Zoran, G. Miroslav J. Sterol composition of the adriatic sea algae *Ulva lactuca*, *Codiumdichotomum*, *Cystoseira adriatica* and *Fucusvirsoides*. *Journal of the Serbian Chemical Society*. 2005, *70* (12), 1395-1400. doi: 10.2298/JSC0512395K.

[17] Mayer, A.M. S. and MT. Hamann. Marine Pharmacology in 2000: Marine Compounds with Antibacterial, Anticoagulant, Antifungal, Anti-inflammatory, Antimalarial, Antiplatelet, Antituberculosis, and Antiviral Activities; Affecting the Cardiovascular, Immune, and Nervous Systems and Other Miscellaneous Mechanisms of Action. *Mar. Biotechnol*. 2004, *6*, 37–52. doi: 10.1007/s10126-003-0007-7.

[18] Navarro, A., Beatriz D.L.H., Angel V. Anti-Inflammatory and Immunomodulating Properties of a Sterol Fraction from *Sideritis foetens* CLEM. *Biol. Pharm. Bull*. 2001, *24*(5), 470 – 473.

[19] Noda. Effect of Sargassum Extract on Anti-Inflammatory and Analgesic Activity and Its Toxicity. 2000. prr.hec.gov.pk/Chapters/1765-4.pdf.

[20] Pacheco, Y., Beatriz B., Sergio L., José V., and Francisco JG. Ratio of oleic to palmitic acid is a dietary determinant of thrombogenic and fibrinolytic factors during the postprandial state in men1–4. *Am J Clin Nutr* . 2006, *84*, 342–9.

[21] Patton, S. and A.A Benson. Synthesis of phytanic acid. *Journal of Lipid Research*.1966, *7*.

[22] Pierong, W., XU Guanjun, Bian Lizeng, Zhang Shuichang and Song Fuqing. Study on sterols from brown algae (*Sargassum muticum*). *Chinese Science Bulletin*. 2006, *51* (20), 2520 - 2528. doi: 10.1007/s11434-006-2124-y.

[23] Pierre J. Estebe, F. Legay, M. Gentili, E. Wodey, C. Leduc, C. Ecoffey, and JP Moulinoux. An Evaluation of a Polyamine-Deficient Diet for the Treatment of Inflammatory Pain. *Anesth Analg*. 2006, *102*, 1781–8. Doi:10.1213/01.ane.0000205755.43562.2b

[24] Rasool, M., EP Sabina and B. Lavanya. Anti-inflammatory Effect of *Spirulinafusiformis* on Adjuvant-Induced Arthritis in Mice. *Biol. Pharm. Bull*. 2006 *29*(12), 2483—2487.

[25] Rita, W.S. Suirta, I. W. dan Sadikin, A. Isolasi dan Identifikasi Senyawa yang Berpotensi sebagai Antitumor pada Daging Buah Pare (*Momordicacharantia I*). Jurusan Kimia FMIPA Universitas Udayana, Bukit Jimbaran. *Jurnal Kimia*. 2007,*1*(2), 47-54.

[26] Smit, A.J. Medicinal and Pharmaceutical Uses of Seaweed Natural Products: A Review.*Journal of Applied Phycology*. 2004, *16*, 245-262. doi:10.1023/B:JAPH.0000047783.36600.ef.

[27] Torres MA, Barros, Marcelo P, Campos SCG, Pinto E, Rajamani S, Sayre RT, Colepicolo P. Biochemical biomarkers in algae and marine pollution: a review. *Ecotox Environ Safe*. 2008, *71*, 1-15.

[28] Umukoro, S., Ashorobi, R.B. Evaluation of the Anti-inflammatory and Membrane- Stabilizing Effects of *Eupatorium odoratum. International Jounal of Pharmacology*. 2006, *2*(5), 509-512. ISSN 1811-7775.

[29] Vieira, L.A.P., A.L.P. Freitas, J.P.A. Feitosa, D.C. Silva and G.S.B. Viana. The alga Bryothamnion seaforthii contains carbohydrates with antinociceptive activity. *Brazilian Journal of Medical and Biological Research*. 2004, *37*, 1071-1079. ISSN 0100-879X.

[30] Vitor J.M, Carvalho A.F.F.U, Freitas S.M, Melo V.M.M. Antibacterial Activity of Extracts of Six Macroalgae from the Northeastern Brazillian Coast. *Journal of Microbiology*. 2002, *33*, 311-313.