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Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS ON THE MACRO MARINE ALGAE SARGASSUM TENERRIMUM J. AGARDH

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ABSTRACT

Sargassum tenerrimum J. Agardh, a macro marine alga collected from the Mandapam, Gulf of Mannar region and was extracted with non-polar chloroform solvent. The crude extract of the same was subjected to study the preliminary phytochemical investigation. It indicates the presence of carbohydrates and alkaloids. GC-MS analysis of the extract showed 12 peaks among them N-hexadecanoic acid (36.4%) and Oleic acid (12%) represented high volume. Carrageenan induced paw edema model in Wistar rat showed the significant reduction of edema. The percentage of inhibition in carrageenan induced paw edema with 100 mg/kg S.tenerrimum extract and Indomethacin was 51% and 52% respectively and it showed significant anti-inflammatory action in the model. Chloroform extract of S.tenerrimum showed statistically significant dose dependent anti-inflammatory activity.

KEYWORDS: *Seaweed, Phytochemicals, Inflammation, Paw Edema, Sargassum Tenerrimum.*

INTRODUCTION

Seaweeds are evaluated for the exploration of bioactive compounds with a wide range of biological activities, such as antibiotics, antifungal, antibacterial, antiviral, antioxidant and anti-inflammatory [1]. Seaweed is closely associate with human life and been utilize in numerous ways as a source of food, feed, fertilizer, medicine since time immemorial. In earlier revealed the traditional Chinese medicine, *Sargassum* used to treat goitre from the 25–220 AD [2]. It has high concentration of trace elements along with protein, carbohydrate, lipid, iodine, bromine, vitamins and substances like antibiotic nature. Several marine macro algae are renewable living resources, which supply raw materials for the preparation of various bioactive molecules. The biochemical composition varies to species to species due to geographical location, temperature, wind, rainfall also influence growth and life cycle of algae. Seaweeds have been extensively explored for its antibacterial activities and in quest of bioactive compounds; the extracts are being subjected to purification techniques [3, 4] and are considered as effective anti-inflammatory products and antioxidants etc [5].

The fresh seaweed production from worldwide coastal regions is about 28.5 million tons [6]. *Sargassum wightii, S.myriocystum* and *S.ilicifolium* are cultivated throughout the year for algin production. Approximately 27,000 tons of *Sargassum* spp. harvested in Mandapam region and also standing stock of seaweed in Indian coastal 6,82,758.87 tons [7].

Three fractions of *S.plastiquinones* were isolated from marine alga *S.micracanthum* for the inhibition of measles and cytomegalovirus[8]. Commonly in seaweed presence of polysaccharide, lipid, flavonoids, terpenoids, coumarins, phlorotanins and phytosterols instead of the presence of fucoidan and alginate. Alginates can absorb water and become viscous gum, it has been used as thickeners, stabilisers and gelling agents in the food and pharmaceutical industries and well validate product. However, modern pharmacological research found alginates isolated from various species of this genus had anticancer (in vivo), antiviral (in vitro) and hypolipidemic (in vivo) activities. Isolated a compound apo-9′-fucoxanthinone from the S.muticum which has potential to inhibit the production of nitric oxide and Prostaglandin E2 and induce nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 in RAW 264.7 cell line [1].

Comparatively obtain better antimicrobial activity in fresh seaweeds than dry seaweeds [9]. Several investigations have demonstrated that a high dietary intake of natural phenols with the presence of several types of antioxidants such as flavonoids [10, 11] in seaweed prolong the life of packed material and reduce the chronic disease and cancer. The new drugs established from seaweed also reduce toxic effects.

Utilizing chemically synthesized drug has adverse side effect in living system at the same time; studies from natural

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products are safe, easily accessible and available, and reduced the harmfulness. Many beneficial biological activity from this family such as anticancer [12], antimicrobial [13], antiviral [8, 14], antioxidant [15] anti-obesity [16], anti-diabetic [17], antipyretic, analgesic and wound healing activity [18] were reported. Between 1981 and 2014 approved drugs 42% are from natural and their derivative products [19]. *S.tenarrimum* has been studied for exploiting its potential as antimicrobial agents [20]. Medicinal aspect studies on pharmacokinetics, bioavailability, efficacy, safety measure and chemical modify should be clear elevation needed. In the present study, we make an attempt to evaluate the anti-inflammatory activity of *S.tenerrimum.*

MATERIALS AND METHODS

Sample collection and extract preparation:

Fresh specimens of *Sargassum tenerrimum* was collected from the intertidal region of the Mandapam coastal area and immediately brought to the laboratory in plastic bags containing water to prevent evaporation. Then the plants were washed thoroughly with tap water to remove extraneous materials and epiphytic. Seaweed material as a whole was shade dried for 7-10 days to prevent photolysis and powdered with a mixer grinder. The algal sample stirred with chloroform ambient temperature for 20-30 days. The extract was then concentrated and dried under reduced pressure and the semi solid used for experiments.

Preliminary Phytochemical screening:

Chloroform extracts of *S.tenerrimum* are subjected to qualitative tests for identification of phytochemical constituents according to standard procedures [21].

Fehling's test:

Equal volume of Fehling A and Fehling B reagents is mixed together reagent 2ml of it was added to 2ml of crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test:

2ml of crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Test for Terpenoids:

2ml of crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated sulphuric acid was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids.

Test for Alkaloids:

2ml of crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's reagent added. A white creamy precipitation indicated the presence of alkaloids.

Test for Phenols and Tannins:

Crude extract 2ml was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Saponins:

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Alkaline reagent test:

2ml of crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

GC-MS analysis:

GC-MS studies carried out by using Clarus 680 GC and employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument and the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Animal studies:

Male albino Wistar rats weighing between 150-200g animal were used for the experiments (NCP/IAEC/688-2016-17/14). They were kept in polypropylene cages under standard laboratory conditions (12: 12 hr light/dark cycle at 24˚C. Rats were provided with commercial rat diet and water ad libitum. Animals were quarantined and acclimatized to laboratory conditions for 7 days prior to study initiation. Animals were observed for general health and suitability for testing during this period.

Carrageenan induced rat Paw Edema:

The anti-inflammatory activity of the test compounds was evaluated in albino Wistar rats employing the method by Winter et al. [22]. Animals were fasted overnight and were divided into control, standard and different test groups. Animals in the standard group received indomethacin at the dose of 10 mg/kg by oral route. Before this all the test compounds. Extracts were administered by oral route at the dose of 25, 50 and 100mg/kg. Required quantity of the test compound was weighed and made suspension with 0.5%w/v Carboxymethyl cellulose. This suspension was administered orally. The rats in the control group received the vehicle solution without test compounds. One hour after test drugs administration, rats in all the groups were challenged with 0.1 ml of 1% carrageenan in the subplantar region of right hind paw. Paw thickness was measured before and every one hr up to 4hrs after the challenge of carrageenan using Vernier caliber. The percent inhibition of paw thickness for treated groups was calculated by comparing with mean paw thickness of control group.

$$\% \text{ Inhibition} = 100\ (1 - Vt/Vc),$$

Where, Vc- Control mean paw thickness

Vt – Test mean paw thickness

Statistical analysis:

All the values were expressed as mean ± standard error mean (SEM). Data were analyzed by using One-way ANOVA followed by Dunnett's test. One-way ANOVA followed by Dunnett's test. P value <0.001 considered as significant and the minimum level of significance was fixed at $p<0.05$.

RESULTS

The qualitative tests for preliminary phytochemicals from the crude chloroform extracts of *S.tenerrimum* showed the presence of carbohydrates and alkaloids in the extracts (Table 1). Other tests are not responded positively.

Table No. 1: Preliminary phytochemicals observed from qualitative tests

Fehling's test	+
Benedict's test	+
Terpenoids test	-
Alkaloids test	+
phenols and tannins test	-
Saponins test	-
Alkaline reagent test	-

Table No. 2: GC-MS analysis of *Sargassum tenerrimum* chloroform extract

Peak	RT	Compound	Mol. Wt.	Structure	Area (%)
1	13.478	Phenol, 2,4-bis(1,1-dimethylethyl)-	206	$C_{14}H_{22}O$	3.58
2	13.663	Phenol, 3,5-bis(1,1-dimethylethyl)-	206	$C_{14}H_{22}O$	9.30
3	17.924	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	12.88
4	18.145	N- hexadecanoic acid	256	$C_{16}H_{32}O_2$	36.42
5	19.460	Z,E-2-Methyl-3,13octadecadien-1-ol	280	$C_{19}H_{36}O$	3.14
6	20.050	Oleic acid	282	$C_{18}H_{34}O_2$	12.01
7	20.436	Hexatriacontane	506	$C_{36}H_{74}$	4.27
8	21.236	Tetratetracontane	618	$C_{44}H_{90}$	3.76
9	22.006	Hexatriacontane	506	$C_{36}H_{74}$	4.49
10	22.751	Hexatriacontane	506	$C_{36}H_{74}$	4.27
11	23.462	Tetratriacontane	478	$C_{34}H_{70}$	3.22
12	30.990	Propionitrile, 3-(3,5-di-tert-butyl-4-hydroxyphenyl)thio-	291	$C_{17}H_{25}ONS$	2.78

GC–MS analysis revealed the presence of 12 peaks they may be 12 different biocomponds (Fig. 1). The GC-MS results revealed that, compounds such as N- hexadecanoic acid presence high peak (36.4%), Oleic acid(12%), Phenol, 2,4-bis(1,1-dimethylethyl (3%), Phenol,3,5-bis(1,1-dimethylethyl) (9%), Z,E-2-Methyl-3,13octadecadien-1-ol,Tetratetracontane, Hexatriacontane, Tetratetricontane, Propionitrile, 3-(3,5-di-tert-butyl-4-hydroxyphenyl)thio (Table 2). Other principles constituted minor portions of the extract and appeared trace amount in the spectrum.

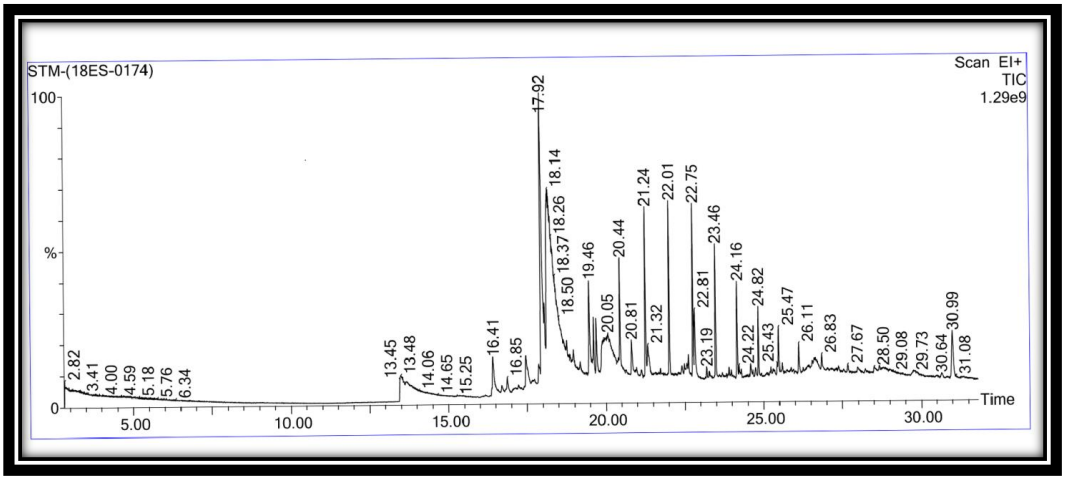

Fig. 1: GC-MS peak analysis of *S. tenerrimum*

Carrageenan induced rat paw edema:

The sample on carrageenan induced rat paw edema at one hour interval study was compared to that control for the evaluation of anti-inflammatory activity on the basis of mean ± standard deviation (M±SD) inhibition of paw edema volume (Table 3). The experiment showed the extracts exhibited statistically significant compare with standard drug (Indomethacin) in the group IV whereas 51% of inhibition of 100mg/kg. In 25 mg/kg of 3rd group have 24% inhibition range 1.7±0.02, 3.2±0.04, 4.3±0.02, 5.1±0.03 and 4.5±0.02. Then 50mg/kg of 4th group has 43% of inhibition 1.3±0.03, 3.0±0.02, 3.6±0.01, 3.8±0.01 and 3.4±0.04. The non-polar extract shown anti-inflammatory activity.

Table No. 3: *S. tenerrimum* inhibition percentage of paw edema test

GROUP	Paw thickness in mm					% Inhibition at 3hr
	0 hr	1hr	2hr	3hr	4hr	
Group I: Carrageenan (control)	1.3±0.02	3.3±0.04	5.4±0.06	6.7±0.04	4.8±0.02	------
Group II: Indomethacin 10mg/kg	1.5±0.02	2.1±0.02**	2.8±0.04**	3.2±0.02**	2.5±0.06**	52
Group III: 25mg/kg	1.7±0.02	3.2±0.04	4.3±0.02**	5.1±0.03**	4.5±0.02	24
Group IV: 50mg/kg	1.3±0.03	3.0±0.02*	3.6±0.01**	3.8±0.01**	3.4±0.04**	43
Group VI: 100mg/kg	1.5±0.02	2.7±0.04**	3.1±0.04**	3.3±0.06**	2.8±0.04**	51

Values were mean ± SEM, n=6, *P<0.05, **P<0.01 Vs control. All the values were expressed as mean ± standard error mean (SEM). Data were analyzed by using One-way ANOVA followed by Dunnett's test.

DISCUSSION

The present study showed the positive presence of carbohydrates and alkaloids in preliminary phytochemical screening but in contrast *S.wightii* reported verities of metabolites such as steroids, flavonoids, phenol [23]. The active polysaccharide has been reported from *S.tenerrimum* [24] and the study is coinciding with the present study. *S.polycystum* and *S.duplicatum* have reported varieties of metabolites such as steroids, alkaloids, phenolics, glycosides, flavonoids and saponins. It may be due to different polarity of extractives [25]. Preliminary phytochemical screening of *S.tenerrimum* reported to have 13 different compounds such as alkaloids, phenols, flavonoids, protein, saponins, steroids, tannins, xanthoprotein, carbohydrates, terpenoids, xylose, amino acids and glycosides [26].

Aqueous-alcoholic extract of *Juniperus phoenicea* has hexadecanoic acid is saturated fatty acid in GC-MS analysis and anti-inflammation activity is reported [27] and oleic acid (C18:1 n-9) monounsaturated fatty acid which increase in vascular endothelial growth factor α and interleukin1β [28]. The chloroform extract of *S.wightii* showed an inhibition of 56.32 ± 6.3 at a dose of 100mg/kg, 200mg, 300mg showed 48.32±1.3, 40.5 ± 2.2 [29]. Methanolic extract of *S.ilicifolium* effect was observed at a dose of 50mg/kg at 3hrs of 87.5% inhibitory effect [30]. In contrast to the present study, GC-MS analysis of methanolic extract of *S tenerrimum* reported to have 12 different compounds and the major compounds are 1,2-Benzodicarbonsaeure and Cyclopropane pentanoic acid [31]. These compounds have possessed antibacterial activities.

In seaweeds richest bioactive compound present better than the terrestrial plant [32]. Extracted from natural products play a significant role in drug discovery searching this area well developed in last two decades. Inflammation is a normal physiological and immune response to tissue injury and occurs when the human body attempts to counteract potentially injurious agents, such as invading bacteria, viruses, and other pathogens. Anti-inflammation is due to the synergistic action of many components.

In vivo studies carrageenan induced inflammation during the initial hour of injection is attributed to the release of histamine, 5-Hydroxytryptamine, then the second phase release of prostaglandins which can be measured around 3-4hr time [33, 34]. There is an increase in control group during initial stage against standard drug/positive control ($p<0.01$). The groups treated with 25 mg/kg, 50 mg/kg and 100mg/kg of *S.tenerrimum* chloroform extract showed the significant decrease ($p<0.05$ and $p<0.01$) respectively then the 100mg/kg of extract showed well significant ($P<0.01$) level. And previous study viewed hexadecanoic acid, and oleic acid as anti-inflammatory activity which are intervening with the prostaglandin pathways [18]. The activity may be due to the presence of phytochemical constituents present in the extract. The further studies are needed for isolation and characterization active principles responsible for the activity.

CONCLUSION

Searching of novel phytochemical leads for pharmacological properties is a continuing process of researcher due to arising modern diseases parallel in time. Terrestrial sources are already exploited much for screening and identification of promising drugs now turned to marine sources from Algae and other marine biota. *Sargassum* and it further purification of compound as a good source for potential exploration of promising safe future drugs.

REFERENCES:

1. Yang EJ, Ham YM, Lee WJ, Lee NH, Hyun CG. Anti-inflammatory effects of apo-9 fucoxanthinone from the brown alga *Sargassum muticum*. DARU J Pharm Sci **2013**; 21:62.
2. Liu L, Heinrich M., Myers S, Dworjanyn SA. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in traditional Chinese medicine: A phytochemical and pharmacological review. J Ethnopharmacol **2012**;142:591–619.
3. Leary D, Vierros M, Hamon G, Arico S, Monagle C. Marine genetic resources: A review of scientific and commercial interest. Marie Policy **2009**;33:183-194.
4. Rao PSP, Rao PS, Karmarkar SM. Antibacterial activity from Indian species of *Sargassum.* Botanica Marina **1988**; 31(4):295-298.
5. Patra JK, Patra AP, Mahapatra NK, Thatoi HN, Das S, Sahu RK, Swain G.C. Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika lake, Orissa. India. Malaysian J Microbiol **2009**;5(2):128-131.
6. Food and agriculture organization of the United Nations. FAO. Rome **2014**; p. 61.
7. Kaliaperumal N, Kalimuthu S. Seaweed potential and its exploitation in India. Seaweed Res Util **1997**;19:33–40.
8. Iwashima M, Mori J, Ting X, Matsunaga T, Hayashi K, Shinoda D. Antioxidant and antiviral activities of plastoquinones from the brown alga *Sargassum micracanthum* and a new chromene derivative converted from the plastoquinones. Biol Pharm Bull **2005**;28:374-377.
9. Padmini Sreenivasa Rao P, Sreenivasa Rao P, Karmarkar SM. Antibacterial substances from brown algae II. Efficiency of solvents in the evaluation of antibacterial substances from *Sargassum johnstonii* Setchell et Gardner. Botanica Marina **1986**;29:503–507.

10. Bartolomeu WSS, Miguel AC, Joana TM, Mafalda ACQ, Antonio CSF, Jose AT,Antonio AV. Antioxidant potential of two red seaweeds from the Brazilian coasts. J Agri and Food Chem **2011**;59:5589–5594.
11. Manivannan K, Thirumaran G, Karthikaidevi G, Hemalatha A, Anantharaman P. Biochemical composition of seaweeds from Mandapam coastal regions along Southeast coast of India. Amer-Eura J Bot **2008**;1(2):32-37.
12. Thinh PD, Menshova RV, Ermakova SP, Anastyuk SD, Ly BM, Zvyagintseva, TN. Structural characteristics and anticancer activity of fucoidan from the brown alga Sargassum mcclurei. Marine Drugs. **2013**;6;11(5):1456-1476.
13. Vinayagamoorthi P, Balasubramanian C. Antimicrobial properties of marine seaweed *Sargassum muticum* against human pathogens. J Coastal Life Med **2015**;3(2): 122-125.
14. Zhu W, Ooi VE, Chan PK, Ang-Po JR. Isolation and characterization of a sulfated polysaccharide from the brown alga *Sargassum patens* and determination of its anti-herpes activity. *Biochem and Cell Biol* **2003**;81:25-33.
15. Siti AB, Sri R, Djagal WM, Iwan YBL. Antioxidant activity of brown algae *Sargassum* species extracts from the coastline of Java island. Am J Agri and Bio Sci **2012**;7(3):337-346.
16. Awang AN, Ng JL, Matanjun P, Sulaiman MR, Tan TS, Ooi YB. Anti-obesity property of the brown seaweed, *Sargassum polycystum* using an in vivo animal model. J Appl Phyco **2014**;26:1043–1048.
17. Stalin, S. and Sampathkumar, P. Investigations on the anti-diabetic potential of novel marine diabetic potential of novel marine seaweed *Sargassum longiotom* against alloxan induced diabetes induced diabetes induced diabetes mellitus: A pilot study. Bang J Pharma **2014**;9: 194-197.
18. Kang JY, Khan MNA, Parka NH, Cho JY, Lee MC, Fujii H, Hong YK. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice. J Ethnopharmacol **2008**; 116(1):187-90.
19. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Pro **2016**;16;79: 629-66.
20. Sherwani SK, Nazim K, Khan M, Ahmed M, Malik MW, Noor AA, Khan MU, Ali QM, Alam SI. Phytochemical and antibacterial screening of crude extract of *Sargassum tenerrimum* J. Agardh against potential human pathogens. FUUAST J Bio **2012**;2(2):65-68.
21. Harborne JB. Phytochemical methods - a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall;**1984**;4-16.
22. Winter CA, Rissey EA, Nuss, GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drug. Proc Soc Exp Biol Med **1962**;11:544–547.
23. Jeeva S, Antonisamy, Cosman Domettila, Babu Anantham, Mony Mahesh. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. Asian Pac J Trop Med **2012**;2(1):S30-S33.
24. Sinha S, Astani A, Ghose T, Schitzler P, Ray B. Polysaccharides from *Sargassum tenerrimum*: structural features, chemical modification and anti-viral activity. Phytochem **2010**;71:235-242.
25. Kanimozhi AS, Johnson M, Malar TRJ. Phytochemical composition of *Sargassum polycystum* C.Agardh and *Sargassum duplicatum* J. Agardh. Int J Pharm Pharm Sci **2015**;**7**(8):893-897.
26. Sasikala M., Indhumathi E, Radhika S, Sasireka R. Effect of seaweed extract (*Sargassum tenerrimum*) on seed germination and growth of tomato plant (*Solanum lycopersicum*). Int J ChemTech Res **2016**;9:285-293.
27. Bouassida KZ, Makni S, Tounsi A, Jlaiel L, Trigui M, Tounsi S. Effects of *Juniperus phoenicea* hydro alcoholic extract on inflammatory mediators and oxidative stress markers in carrageenan induced paw oedema in mice. BioMed Res Int **2018**;3785487.
28. Leonardo MP, Elaine H, Edgair, FM, Flavia O, Edson AL, Sandra HF, Rui, Tania, CP. Effect of oleic and linoleic acids on the inflammatory phase of wound healing in rats. Cell Biochem and Func **2007**;26(2):197-204.
29. Raman R, Parthiban SSK, Muthuraman M, Sivasubramanian A. Antimicrobial and anti inflammatory studies on Sargassum wightii extracts. Int J Pharm Pharm Sci **2014**;6(7):611-614.
30. Simpi CC, Nagathan CV, Karajgi SR, Kalyane NV. Evaluation of marine brown algae *Sargassum ilicifolium* extract for analgesic and anti-inflammatory activity. Phcog Res **2013**;5:146-9.
31. Kumar P, Senthamilselvi S, Govindaraju M. GC-MS profiling and antibacterial activity of *Sargassum tenerrimum.* J Pharm Res **2013**;6:88-92.
32. Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. Marine natural products. Nat Prod Rep **2012**;29:144-122.
33. Vinegar R, Schreiber W, Hugo, R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther **1969**;166:96-103.
34. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--differences and similarities. New Eng J Med **1991**; 13;324(24):1716-25.

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