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Enzymes associated with anti-inflammatory potentialities of purified terpenoid extracts from the selected sea weeds

Research Article

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Abstract

Introduction: Macrophages are phagocytic WBCs involved in the immune defense and will be activated during inflammatory disorders. The synthesis of cytokines and mediators, particularly nitric oxide (NO) is triggered by the macrophage activator. NO induces many biological events. Therefore, NO regulation is proven to be potential for exploring anti-inflammatory drugs. Aim: The anti-inflammatory action of the purified terpenoid extracts from red algae such as *Hypnea musciformis, Gracilaria dura* and *Kappaphycus alvarezii* on LPS induced RAW 264.7 macrophages on lipoxygenase, cyclooxygenase, hyaluronidase, xanthine oxidase and myeloperoxidase inhibitory effects were evaluated. Methods: The methanolic extract of the sea weeds were subjected to silica gel column chromatography and the fraction was further subjected to GC-MS analysis. Then, the potentiality of the purified terpenoid extracts to inhibit various inflammation causing enzymes such as COX, LOX, hyaluronidase, xanthineoxidase and myeloperoxidase were carried out. Findings: The terpenoid extracts reduced the enzyme activities in a dose dependent manner as compared to control group. The extracts inhibited xanthine oxidase activity effectively at 250 µg/ml i.e., a maximum inhibitory activity of 62.1% as compared to the standard drug, allopurinol. The extract significantly inhibited lipoxygenase activity, with highest inhibitory activity at 100 µg/ml. The nitric acid synthesis was reduced to 8.5 µM by *Hypnea musciformis*. Conclusion: The present study revealed that the purified terpenoid extracts from *H. musciformis* exhibited potent anti-inflammatory activities followed by *G. dura* and *K. alvarezii* via regulating the anti-inflammatory enzymes. These findings provide justification for the traditional use of the red algae in inflammatory conditions.

Key Words: *Red algae, Inflammation, Nitric oxide inhibition, Terpenoids, Cytokine mediators, Enzymes.*

Introduction

Seaweeds are inbuilt with unique physiological and biochemical systems against adverse environmental conditions to combat life. From ancient time onwards Asian and European countries used the seaweeds as a source of polysaccharides for food and pharmaceutical uses. Currently, the seaweeds are proven to be a rich source of potent bioactive compounds that has immense importance in human life. Primary and secondary metabolites produced by seaweeds are of great interest. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivities varying from neurological issues in humans to algicidal, nematicidal, insecticidal etc (1). Seaweeds are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides. Currently, there is an increased interest in phyto products with valuable medicinal properties such as terpenoids. Epidemiological and experimental studies suggest that terpenoids from seaweeds may be helpful in the prevention and therapy of cancers and prostate inflammatory disorders. Seaweeds also have the valuable medicinal components such as antibiotics, laxatives, anticoagulants, antiulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins, enzymes and minerals essential for the human nutrition (2). The defensive strategy of many of the red algal species suggests that they possess many anti-oxidative and anti-genotoxic constituents in their cells. For this reason, interest in marine algae as a promising potential source of pharmaceutical agents has increased during the last few years. Similarly, seaweed possess wet, softening properties which according to traditional Chinese medicine enables them to dissolve hard nodules and tumors and to reduce swelling of the thyroid and lymph glands. Seaweed helps decongest swollen or

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inflamed lymph nodes; it can be consumed as the treatment for auto-immune illnesses, including chronic fatigue, HIV, arthritis and chronic allergies (3). In this juncture, the present study was designed to evaluate the anti-inflammatory action of the purified terpenoid extracts from red algae such as *Hypnea musciformis, Gracilaria dura* and *Kappaphycus alvarezii* on LPS induced RAW 264.7 macrophages on lipoxygenase, cyclooxygenase, hyaluronidase, xanthine oxidase and myeloperoxidase inhibitory effects.

Materials and Methods

Plant materials

The marine algae *Hypnea musciformis, Gracilaria dura* and *Kappaphycus alvarezii* were collected during March 2018, from the Mandapam coast (latitude 9˚ 17' N, longitude79˚ 22' E), Gulf of Mannar. Specimens for all the three sea weeds were collected, dried, properly identified and authenticated with the reference from CMFRI, Mandapam, Tamil nadu. Then 50 g washed samples were powdered and subjected to Soxhlet extraction with 250 ml of methanol. The extraction was repeated thrice. The crude extract was then filtered and kept at room temperature for evaporation. Fractionation of each sample was done by silica gel column chromatography (CC) using different ratios of petroleum ether and ethyl acetate as solvent combinations for each algal extract. The purified fractions obtained was collected and then quantified for the presence of terpenoids and further determined by GC-MS.

GC-MS analysis

For GC-MS analysis, the purified sample was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 ìM film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Helium was used as carrier gas with a flow rate of 1 mL/min; the injector was operated at 200°C and column oven temperature was programmed as 50 - 250°C at a rate of 10°C / min injection mode. A chromatogram was obtained and the mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library (4).

Enzyme inhibitory activity

5-lipoxygenase (LOX) inhibitory assay

5-LOX inhibitory activity of purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* was determined by the spectrometric method (5). OD was measured at λ = 234 nm for 10 min. Percentage inhibition of 5-LOX was determined by comparison of reaction rates of the extracts relative to control using the formula (E − S)/E × 100, where E and S were the activities of the enzyme with and without extracts, respectively. IC_{50} values were determined. Indomethacin was used as the reference standard.

Assay for cyclooxygenase (COX) inhibition

The COX inhibitor screening assay directly measures PGF2α by stannous chloride reduction of COX-derived PGH2 produced in the COX reaction (6). The reaction system consists of reaction buffer, haem, enzyme and plant extract pre-incubated at 37˚C for twenty minutes with background and enzyme controls. The absorbance was read at 420 nm. The data was plotted as % B/B0 (Standard Bound / Maximum Bound) versus log concentration using a 4-parameter logistic curve fit. The concentration of each sample was determined from a standard curve with appropriate dilutions and used to calculate the percent inhibition as per the formula given below:

Percent Inhibition (%) = (Activity of Control – Activity of Test) x 100 / Activity of Control

The percentage inhibition was plotted against the inhibitor concentration to determine the IC_{50} value (concentration at which there was 50% inhibition).

Hyaluronidase (HA) inhibitory assay

Hyaluronidase inhibitory activity of purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* was evaluated by a spectrometric method with slight modifications (7). Extracts were assayed at the concentrations of 100 to 500 µg/mL. Absorbance was measured at λ = 585 nm. Percentage enzyme inhibition was calculated as compared to the control. Tannic acid was used as the reference standard.

Xanthine oxidase (XO) inhibitory activity

Xanthine oxidase inhibitory activity of purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* was determined by a kinetic method (8). Extracts were tested at the assay concentration of 50 - 250 µg/ml. Absorbance was monitored with the change of absorbance at λ = 295 nm for 15 min. Percentage inhibition of xanthine oxidase was calculated using the formula (E − S)/E × 100, where E is the activity of enzyme without extracts and S is the activity of enzyme with extracts. Allopurinol was used as the reference standard.

Viability assay of LPS-activated RAW 264.7 macrophages

Murine macrophage (RAW 264.7) cells were cultured and maintained in DMEM supplemented with standard antibiotics and other standardized parameters. The plated cells were treated with different concentrations of the terpenoid extracts from the selected red algae (100-500 µg/mL) and incubated for 30 min (humidified atmosphere, 5% CO_2, 37°C), followed by the incubation with bacterial lipopolysaccharide (LPS, 1 µg/ml) for 24 h (9).

Nitric oxide (NO) assay

The inhibition of nitric oxide production was determined using the Griess assay (9) and absorbance was measured at λ = 540 nm. The nitrite concentration was determined using a standard curve of sodium nitrite (y = 0.012× + 0.036, R2 = 0.999). Percentage inhibition of nitric oxide formation by extracts was also calculated (10).

Myeloperoxidase (MPO) assay

Myeloperoxidase (MPO) activity was assayed as per the protocol (11). The change in absorbance at 460 nm was measured. MPO activity was presented as units per mL of cell lysate. One unit of MPO activity was defined as that degrading 1 µM of peroxide per minute at 25°C. Enzyme unit for MPO was determined using the formula: U= (OD x 4 x Vt x dilution factor)/ L x €460 x t x Vs. (OD = optical density, Vt = total volume in ml, L is light path in cm, €460 = extinction coefficient of tetraguaiacol, t = the time of measurement in minutes and Vs = sample volume in ml.)

Statistical Analysis

The values were three independent experiments and were expressed as mean ± standard deviation (S.D.) for n determinations where n=3 unless otherwise stated. Data analyses were performed using Sigma Plot version 12.5. The significance of differences from the respective controls was tested using one way ANOVA for each set of experiments.

Results and Discussion

The crude methanolic extracts of *H. musciformis, K. alvarezii* and *G. dura* were purified by silica gel column chromatography. Each fraction was eluted using petroleum ether and ethyl acetate solvent combinations. The fraction eluted using 95:5 solvent combinations of *H. musciformis*, 50:50 solvent combinations of *K. alvarezii* and 90:10 of *G. dura* showed significant amount of terpenoids. The fractions eluted by column chromatography were subjected to thin layer chromatography for confirming the presence of terpenoids. Parallelly, they were fractionated by GC-MS. Retention time and the relative abundance of each compound were recognized by GC-MS. The analysis of the 95:5 purified fraction of *H. musciformis* revealed the presence of 8 major peaks of terpenoids compatible with their fragmentation patterns (Fig:1). The 50:50 purified fraction of *K. alvarezii* showed 12 major peaks (Fig: 2) and 90:10 fraction of *G. dura* shows 4 major peaks of terpenoids (Fig: 3). The major terpenoids found in *H. musciformis* were Eicosane, Heneicosane, 2- Pentadecanone, Hexadecanoic acid, methyl ester, n-Hexadedcanoic acid, Hexadecanoic acid, ethyl ester, Heptadecanoic acid, methyl ester, 11- octadecanoic acid, methyl ester and in *K. alvarezii* were Eicosane, Heneicosane, Hexadecanoic acid, methyl ester, n-Hexadedcanoic acid and Beta amyrin whereas *G. dura* showed profound percentage of terpenoids such as Hexadecanoic acid methyl esters (15.58), n-Hexadedcanoic acid (80.78), 11- octadecanoic acid, methyl ester (80.78) and Phytol (3.65).

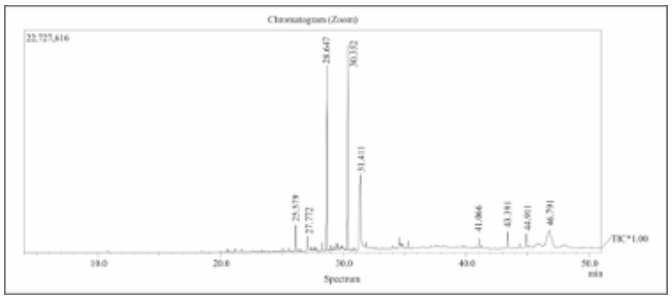

Fig. 1: GC- MS spectra showing terpenes composition of *Hypnea musciformis*

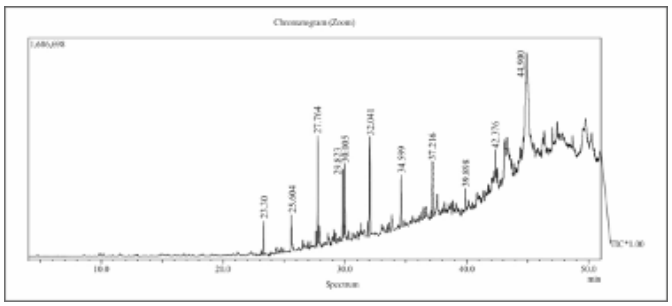

Fig. 2: GC-MS spectra showing terpenes composition of *Kappaphycus alvarezii*

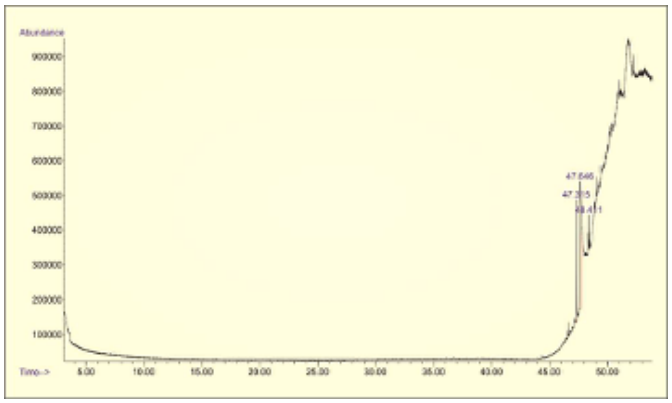

Fig.3: GC-MS spectra showing the terpenes composition of *Gracilaria dura*

5-lipoxygenase (5-LOX) inhibitory activity

The terpenoid extract from *G. dura* showed the highest 5-LOX inhibitory activity followed by *H. musciformis* and *K. alvarezii*. Based on the IC_{50} values, the extract of *G. dura* (IC_{50} = 40.8 µg/ml) was considered as remarkable in terms of 5-LOX inhibitory activity followed by *H. musciformis* (IC_{50} = 89.3 µg/ml) and *K. alvarezii* (IC_{50} = 100 µg/ml) (Fig. 4). The activities of the extracts were comparable with the positive control indomethacin, which also showed a dose dependent activity against 5-LOX enzyme inhibitory activity. The IC_{50} value was significant i.e., 19.3 µg/ml.

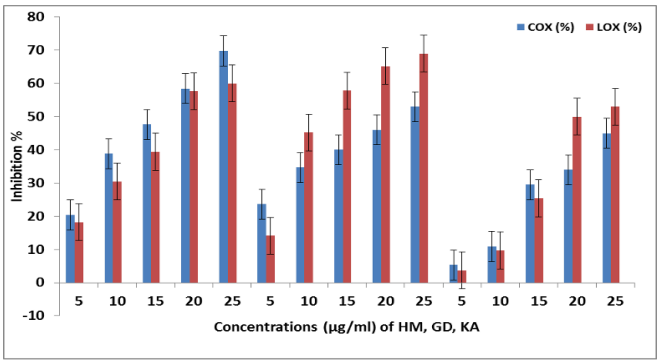

Fig.4: Terpenoid extracts from red algae on COX and LOX inhibitory activities

5- LOX is the first indexed enzyme involved in the arachidonic acid pathway leads to produce leukotrienes. Expression of 5-LOX has been connected with asthma, respiratory disorders, inflammatory bowel issues, cancer, cardiovascular disorders, atherosclerosis and stroke. Thus, LOX inhibitors are prompted as promising molecules for the treatment of inflammatory disorders on the basis of their roles in LOX pathways. Azad et al., proved that *Premna integrifolia* extracts as dual inhibitors of COX-2/5-LOX and thereby effective against inflammation and also as immune modulators (12). Sibata et al., reported inhibitory effects of brown algal phlorotannins on phospholipase A2s, lipoxygenases and cyclooxygenases. Inhibitors of these enzymes could become leading compounds in the development of new nonsteroidal anti-inflammatory drugs (13). Phycocyanin, a biliprotein isolated from *Spirulina platensis* significantly inhibited COX-2 with an IC_{50} value of 80 nM. The anti-inflammatory activity of phycocyanin might be due to its selective COX-2 inhibitory effect, coupled with its efficiency in scavenging free radicals and inhibit lipid peroxidation (14). Antony and Chakraborty were first isolated the antioxidant abeo-labdane type diterpenoid from intertidal red seaweed *Gracilaria salicornia* with 5- lipoxygenase inhibitory potential. They showed significantly greater activities against pro-inflammatory 5-lipoxygenase (IC_{50} 0.86 mg/mL) than non-steroidal anti-inflammatory agent ibuprofen (IC_{50} 0.92 mg/mL, $P < 0.05$) (15).

COX inhibitory activity

Subsequently, the purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* were screened for its effect on COX inhibitory activity. All purified terpenoid extracts of red algae were used as per the methodology to study the effect on the activity of COX and the results were showed in the fig 4. *H. musciformis* extracts showed a potent significant inhibition on the COX enzyme and was comparable with the positive control, indomethacin and results were expressed as percent inhibition of activity. *G. dura* revealed moderate activity but higher than *K. alvarezii.* The IC_{50} values were 74.2 (*H. musciformis*), 110.3 (*G. dura*) and 133.4 µg/ml (*K. alvarezii*).

Macrophages play leading roles in inflammatory disorders that are linked with up regulation of many inflammatory mediators. Further, PGE2 production is controlled by COX enzyme activity (16). Jiang et al., proved that γ-tocopherol is potential to inhibit COX activity and there by PGE2 production in macrophages and epithelial cells (17). The present results also showed that terpenoids inhibited COX enzyme activity effectively in a in a concentration dependent manner. Manju et al., reviewed the ideal and safer anti-inflammatory treatment via dual COX-2/5-LOX inhibition (18). Similarly, a new morpholine alkaloid was isolated from the thalli of red seaweed *Gracilaria opuntia* showed greater cyclooxygenase-2 (COX-2) inhibitory activity (IC_{50} 0.84 mg/mL) in conjunction with *in vitro* 5-lipoxidase inhibitory activity (IC_{50} 0.85 mg/mL) than non-steroidal anti-inflammatory drugs (19). A comparative study of *in vitro* anti-inflammatory activity of the sulphated polygalactan from two seaweeds *K. alvarezii* and *G. opuntia* was also reported (20). *G. opuntia* polygalactan exhibited greater COX-1 and COX-2 as well as 5-LOX inhibitory activity (0.24 mg/mL) than that of *K. alvarezii*. The sulphated polygalactan isolated showed greater anti-inflammatory activity in comparison with the positive control aspirin. Maneesh et al., reported that the ethylacetate : methanol fraction of *Sargassum wightii* exhibited significantly greater in vitro anti-inflammatory properties as determined by COX-1 and COX-2 inhibitory activities than those extracted by $CHCl_3$ and synthetic drug aspirin. It is significant to note that fraction of *S. wightii* also exhibited greater 5-LOX inhibitory activity than aspirin (21).

Hyaluronidase inhibitory activity

The hyaluronidase inhibitory activities of purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* were ranged from 9.8 to 58.6%. The *H. musciformis* extracts showed the highest activities followed by the extracts of *G. dura* and *K. alvarezii* (Fig.5). These two red algal extracts showed moderate to poor activities compared to the tannic acid (69.88%, 500 µg/mL) the synthetic drug used against inflammatory disorders.

Hyaluronidase (HA) hydrolyzes glycosaminoglycans including hyaluronan in the extracellular matrix during tissue remodeling process. Enhancement of HA activity was noticed in acute and chronic inflammatory diseases. Increased HA activity leads to degenerative changes in connective tissues. The cells, enzymes, and signaling modulators involved in these changes may be the targets for designinig compounds as dietary, pharmaceutical, and nutraceutical. The obtained results showed that purified terpenoid extracts have the potential to inhibit HA, which could regulate inflammation. Further, hyaluronidase triggers angiogenesis and facilitates tumor invasiveness. Sri Lankan medicinal plants were screened by Liyanaarachchim et al., to isolate novel cosmeceuticals i.e., the phytochemicals capable of inhibiting tyrosinase, elastase, hyaluronidase and promote antioxidant activities (22). Many studies have reported that HAase inhibition activity is a measure of anti-inflammatory potential of compounds. For example, caffeic acid oligomers from *Clinopodium gracile*, phlorotannins of

brown algae, pentacyclic triterpenoids from *Prismatomeris tetrandra* (23).The polyphenol extracts of five red algae including *Laminaria japonica, Porphyra* sp., *Spirulina platensis, Chlorella pyrenoidosa* and *Scytosiphon* sp. were analyzed for its antiallergic activity with the hyaluronidase inhibition assay (23). The anti-allergic activity of S*cytosiphon* sp. extract was even higher than the typical anti-allergic drug Disodium cromoglycate (DSCG). A number of studies have reported that polysaccharides have hyaluronidase-inhibitory effects. The hyaluronidase-inhibitory activity of a polysaccharide was proved from the unicellular marine alga *Porphyridium purpureum* (24). Six anti-allergic phlorotannins from the brown alga *Eisenia arborea* were examined for their inhibitory effects on hyaluronidase activities. Eckol inhibited the hyaluronidase activity similar to the typical inhibitors, EGCg and disodium cromoglycate. The other five phlorotannins also inhibited the enzyme activity stronger than the above inhibitors (25).

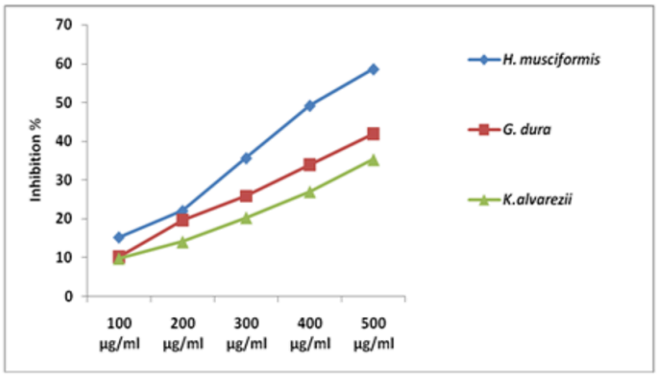

Fig. 5: Terpenoid extracts from red algae on Hyaluronidase inhibitory activity

Xanthine oxidase (XO) inhibitory activity

The purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* showed varied inhibitory activities against xanthine oxidase enzyme and activities ranged from 7.4 to 62.1%. The results revealed that, the terpenoid extracts from *H. musciformis* had the highest inhibitory activity followed by the extracts of *G. dura* and *K. alvarezii* (Fig.6) with respect to the reference standard allopurinol. The extract of *H. musciformis*, which showed the highest xanthine oxidase inhibitory activity in a dose dependent inhibitions within the concentration range of 50–250 µg/mL with a IC_{50} value of 208.5 µg/mL (Fig 6; Allopurinol: IC_{50}: 5.2 ± 0.01 µg/mL).

Inhibition of XO down regulates the uric acid synthesis which is related to primary or secondary gout issues. The application of phytochemicals useful for the management of gouty arthritic diseases are those which can inhibit xanthine oxidase and the generation of ROSs and the resulting inflammatory reactions linked with the accumulation of uric acid crystals in the joints and kidneys (26). Similarly, Djarmouni et al., established the role of xanthine oxidase inhibitory activities of *Santolina chamaecyparissus* in anti-inflammatory events (27). *In vitro* and *in vivo* xanthine oxidase (XO) inhibitory and antihyperuricemic activities of *Quercus acuta* by the active phytochemicals from the leaf extract (28). *In vivo* study using hyperuricemic mice induced with potassium oxonate demonstrated that the *Q. acuta* could inhibit hepatic XO activity at a relatively low oral dose (50 mg/kg) and significantly alleviate hyperuricemia to a similar extent as allopurinol. Trabsa et al., reported the kinetics of inhibition of xanthine oxidase by *Lycium arabicum* and its protective effect against oxonate induced hyper uricemia and renal dysfunction in mice (29). The extracts of *Paronychia argentea* were effective in both XO inhibiting and superoxide radical scavenging was evaluated by Moufida and Meriem (30). They established that these plant extracts can be used as a source of bioactive compounds useful as natural antioxidants and therapeutic agents for hyperuricemia, gout and other related diseases, where inhibition of XO and scavenging of superoxide radicals are necessary.

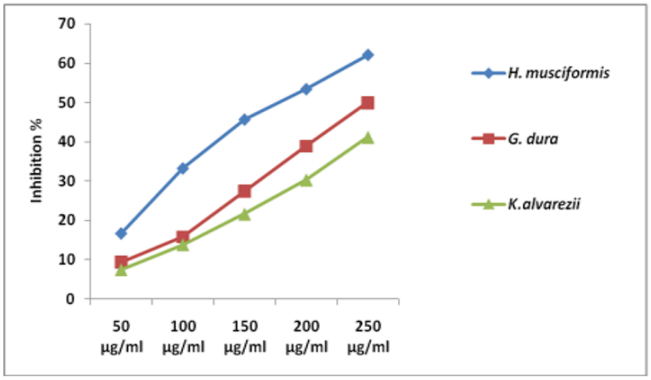

Fig.6: Terpenoid extracts from red algae on Xanthine oxidase inhibitory activity

Viability of LPS-activated RAW 264.7 macrophages

The purified terpenoid extracts from *H. musciformis*, *G. dura* and *K. alvarezii* showed high viability rate among RAW 264.7 macrophages (Cell viability > 90%) at the tested concentrations and there by suggesting non cytotoxicity of the terpenoid extracts from the red algae (Fig.7). This may be the reason for searching novel phytochemicals from plant based products.

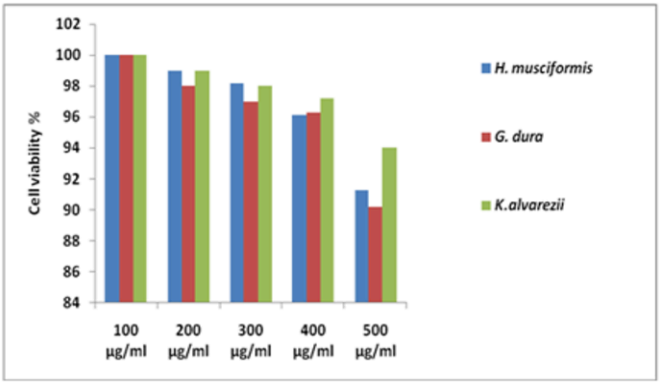

Fig.7: Terpenoid extracts from red algae on cell viability of RAW-264.7 macrophages

Kim et al., (31) evaluated the anti-inflammatory properties of *Stauntonia hexaphylla* fruit extract in LPS activated RAW-264.7 macrophages and revealed the safety of plant extracts in terms of viability in murine cell lines. Potential of *Anredrea cordifolia* and *Piper crocatum* extracts were observed through inflammatory markers such as TNF-α, IL-1, IL-6, and NO inhibitory activity assays in LPS induced macrophage cell line (RAW 264.7). Both *A. cordifolia* and *P. crocatum* extracts decreased TNF-α level in LPS-induced RAW 264.7, which was comparable to normal cell (32). Tseng et al., reported a major flavonoid component from *Morus australis* named morusin. *M. australis* extract together with morusin inhibited lipopolysaccharide-induced production of nitrite and prostaglandin E2 in RAW 264.7 cells (33). Inhibition of lipopolysaccharide induced inflammatory responses by *Sargassum hemiphyllum* sulfated polysaccharide extract in raw 264.7 macrophage cells was proven by Hwang et al., (34). The secretion profiles of proinflammatory cytokines were found to significantly reduce in a dose dependent manner.

Nitric oxide (NO) production inhibitory activity

The nitric oxide inhibitory activities of purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* were high to moderate in comparison to the reference standard L-NMMA and ranged from 4.9% to 64.7%. Of the tested terpenoid extracts, the extract of *H. musciformis* showed significant nitric oxide inhibitory activity, while that of *G. dura* and *K. alvarezii* showed moderate activities (Table 1).

Table 1: Production of NO (µM) and percentage of NO inhibition by terpenoid extracts from red algae

Concentration	*H. musciformis*		*G. dura*		*K. alvarezii*	
	NO (µM)	NO Inhibition (%)	NO (µM)	NO Inhibition (%)	NO (µM)	NO Inhibition (%)
25 µg/ml	39.6	10.2	42	7.5	46	4.9
50 µg/ml	28.3	18.6	30.6	14	35.5	10.2
75 µg/ml	21	24	25.3	29.4	30	24.7
100 µg/ml	14	50	19.5	40.3	24	29.87
200 µg/ml	8.5	64.7	11	50	13	40

NO: Nitric Oxide

Over production of nitric oxide is linked with many life style disorders such as arthritis, diabetes, and stroke, autoimmune and chronic inflammation. Similarly, pathogen infections also induce neurodegenerative disorders via the synthesis of nitric oxide. Inducible nitric oxide synthase (iNOS) triggers NO synthesis which in turn leads to vasodilation and hypotension noticed among septic shock and inflammation diseases. Therefore, NO inhibitors are lead molecules for the treatment of inflammatory issues.

For example, thyme oleoresin effectively inhibits NO production and iNOs gene expression in RAW 264.7 murine cells (35). Similarly, Gutierrez and Hoyo-Vadillo confirmed that the extracts of *Petiveria alliacea* in regulating NO synthesis and thereby anti-inflammatory disorders (36). Inhibitory activity of nitric oxide production by hirsutane-type sesquiterpenes was noticed from the red alga derived fungus *Chondrostereum* was also reported (37). Neorogioltriol, a tricyclic brominated diterpenoid isolated from the organic extract of the red algae *Laurencia glandulifera*, showed anti-inflammatory effects under *in vitro* condition in LPS-treated RAW 264.7 macrophages, and also *in vivo* using the carrageenan-induced paw edema model (38). In addition, anti-inflammatory effects of lipid extract of *Tetraselmis chuii*, *Chlorella sorokiniana* and *Chondrus crispus* in RAW 264.7 macrophages (39).

Myeloperoxidase (MPO) activity

The purified terpenoid extracts from *H. musciformis, G. dura* and *K. alvarezii* were effective in inhibiting myeloperoxidase (MPO). A concentration dependent increase in the inhibition of myeloperoxidase activity was exhibited by the terpenoid extracts. At higher concentrations inhibitory effect was significantly high. *H. musciformis* showed excellent MPO inhibition suggesting the ideal anti-inflammatory activity of the same. MPO inhibition at 100 µg/ml terpenoid extracts from *H. musciformis* (0.0083) was comparable with that of the standard drug indomethacin (0.0058) (Table 2).

Table 2. Effect of terpenoid extracts from red algae on Myeloperoxidase activity (µg/mL)

Conc.	*H. musciformis*	*G. dura*	*K. alvarezii*
12.5 µg/ml	0.0144	0.0166	0.0197
25 µg/ml	0.0140	0.0159	0.0192
50 µg/ml	0.0137	0.0147	0.0178
75 µg/ml	0.0131	0.0137	0.0161
100 µg/ml	0.0083	0.0098	0.0103

In the inflammatory reactions, oxidative stress is triggered by phagocytes containing myeloperoxidase, and these uplift ROS synthesis and down regulates oxidative defense. As an important indicator of neutrophil leaching, myeloperoxidase activity should be regulated during treatments. The protective effect of the terpenoid extracts in the present study was through balancing oxidative stress via inhibiting the MPO synthesis in the murine cells. Chniguir et al., revealed that *Syzygium aromaticum* aqueous extract inhibits myeloperoxidase and protects mice from LPS-induced lung inflammation (40). Inhibition of myeloperoxidase and neutrophil mediated hypochlorous acid formation in vitro and endothelial cell injury by epigallocatechin gallate was also reported (41). Further, the anti-inflammatory activity of a lectin extracted from the red seaweed *Bryothamnion triquetrum* by means of inhibiting myeloperoxidase activity in paw tissue (42). Similarly, inhibitive effect of quercetin on MPO mediated oxidant hypochlorous acid formation or scavenging of the oxidant hypochlorous acid was also evaluated (43).

Conclusion

Synthetic drugs such as steroidal and non-steroidal have been employed for the management of inflammatory diseases. Due to high costs and adverse effects, there is a profound interest in plant based research to identify novel agents which may be cheaper and have no side effect. The present study revealed that the purified terpenoid extracts from *H. musciformis* exhibited potent anti-inflammatory activities via regulating the enzymes such as COX, LOX, HA, XO, MPO and also by inhibiting NO synthesis. Therefore, the usage of many of these algae by locals as folk medicine is justifiable. This sort of research provides data to develop new drugs for future use.

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