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Phycochemical analyses and pharmacological activities of seven macroalgae of Arabian Sea (Northern coast line)

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Abstract: Seaweeds have been used as nutraceuticals because of certain metabolites present in some species including polyphenols. Seven species of seaweeds collected from the Karachi coast were screened for the first time both for phycochemical analysis and pharmacological activities. The phycochemicals quantified included phenols, flavonoids, tannins and saponins. *Melanothamnus afaqhusainii* showed the highest content of phenols (2.16mg GAE/g) while highest flavonoids were observed in *Coelarthrum muelleri* (4.59mg RE/g). Tannins were found in low amounts while saponins were observed as major constituents among the seaweeds ranging from 1.34-3.47% in *Sargassum swartzii* and *Codium flabellatum*, respectively. Saponins were not analysed in Rhodophyta due to gel formation. Pharmacological screening revealed analgesic and anti-inflammatory effects. Both the methods of analgesic activities have shown significant increase in reaction time when methanol extracts were used. The reason for delayed anti-inflammatory activity of *Solieria robusta* and *C. muelleri* was found correlating with its gel forming ability. While *Cystoseira indica* and *C. flabellatum* exhibited highly significant effect from 1st to 5th h. Results suggested that selected seaweeds had potential in combating both acute and chronic inflammation and pain and hence can be used for therapeutic efficacy.

Keywords: Macroalgae, Arabian sea, phycochemistry, analgesic activity, anti-inflammatory activity.

INTRODUCTION

World's oceans are covering more than 70% of the earth's surface; and marine constitute about 90% of various life forms on earth. Marine is still a less explored frontier therefore the research community is paying more attention to marine natural products (NPs). The reason for this attraction is biological diversity of marine ecosystem representing an enormous resource for discovery of potential NPs. Generally complex structural chemicals are produced in the body of living creatures as a result of interaction with challenging surroundings. NPs are responsible for improving competitive survival of species (Gordon *et al*., 2012). As marine macro-algae are exposed to hostile and competitive environmental conditions, variety of secondary metabolites are produced. Often varying chemically, and hence physiologically from metabolites originating from terrestrial organisms, making these novel potentials as pharmaceutical leads. The well-known ability of marine algae to produce bioactive compounds has been reported widely for pharmaceutical purposes (Folmer *et al*., 2010). Diversity of the chemical constituents produced by seaweeds is outstanding and has shown variety of biological activities. Currently 9% of biomedical botanicals are reported from marine origin. Their potentials still need further exploration (Smith, 2004; De Almeida *et al.,* 2011; Hiorfumi *et al.,* 2011).

Analgesic and anti-inflammatory drugs relieve mild to moderate pain, decrease inflammation and fever. These drugs are used in joints pain, muscle pain and headache but not for discomfort in visceral organs like heart, liver and lungs. These analgesic and anti-inflammatory agents give curative effect by inhibiting various chemical substances which are released during inflammation and pain. These also play important role in maintaining body temperature. Use of pain relieving and anti-inflammatory drugs cause toxic and untoward effect particularly when treatment of pain, inflammation and fever recommend higher doses for long duration. The most commonly affected organs are liver, kidney and GIT (Hiorfumi *et al.,* 2011). Non-steroidal anti-inflammatory agents (NSAIDs) are mainly used to alleviate the symptoms of the diseases but are often linked with several side effects. Side effects produced by NSAIDs are contributing statistically in complications to deaths. It has been observed that one in five chronic clients of NSAIDs suffer gastric damage which is not immediately noticeable (Hiorfumi *et al.,* 2011). Consequently, there is a strong interest in search of novel analgesic and anti-inflammatory agents from natural sources. Such explorations lead to develop new drugs in order to combat most panic inflammatory diseases.

In the current study, seven species of seaweeds collected from Karachi coast were analysed for classes of

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secondary metabolites and their analgesic and anti-inflammatory activities.

MATERIALS AND METHODS

Materials, chemicals and reagents

Methanol, ethanol, *n*-butanol, and diethyl ether of analytical grade were purchased from DaeJung (Korea). Gallic acid (GAE), rutin (RE), tannic acid (TAE), Folin-Ciocalteu's phenol reagents, aluminium chloride ($AlCl_3$), sodium bicarbonate ($NaHCO_3$), ferric chloride ($FeCl_3$), hydrochloric acid (HCl), and potassium ferrocyanide ($K_3[Fe(CN)_6]$) were procured from Sigma Aldrich (USA). Similarly, chemicals used in phycochemical analyses were purchased either from Merck (Germany) or Sigma Aldrich.

Collection of samples

Fresh specimens of red algae *Coelarthrum muelleri* (Sonder) Borgesen, *Melanothamnus afaqhusainii* M. Sahmeel C. and *Solieria robusta* (Greville) Kylin; brown algae *Sargassum wightii* Greville*, S. swartzii* C. Agard and *Cystoseira indica* Thivy et Doshi Mairth S.; and green alga *Codium flabellatum* Nizamuddin INED are available as herbarium sheets in the CEMB, University of Karachi, with voucher specimen No. CYM-03, MYA-05, SYR-04, SYW-01, SYS-02, CYI-07 and CYF-06, respectively. All specimens were collected from Buleji, Karachi coast, during low tides.

Sample preparation for phycochemical analyses

Collected seaweeds were cleaned from associated debris and salt, and air dried in shade. Shade dried seaweeds were ground, sieved (1.0 mm mesh size), and sealed in amber sample bottles in a cold dark place for further analyses.

Determination of total phenols

Phenols contents were measured using method described by Singleton, *et al.*, 1999. Briefly, 2g of powder seaweeds was soaked in 20 ml of 80% methanol for 24h. Methanol extract was separated by decantation and residue was once again percolated with 80% methanol overnight. Both extracts were combined, filtered and transferred into pre-weighed vial. Filtrate were evaporated under reduce pressure. 2.5ml of 10% Folin-Ciocaltaeu's phenol reagent was added in 0.5 ml of methanol extracts (1 mg/ml), followed by time addition of 2.5 ml of 7.5% $NaHCO_3$ while mixing the samples in test tubes. Test tubes were incubated for 45 min at 45°C in water bath. A reagent blank was also prepared using only methanol. Absorbance was read at 765nm. Same procedure was repeated for standard solution. Gallic acid was used as standard. Results were expressed as mg GAE/g, using calibration curves prepared immediately before this analysis.

Determination of total flavonoids

Extraction protocol was similar as outlined for phenols. 2 ml of 2% $AlCl_3$ solution was added to 2 ml methanol extract (1 mg/ml) in a test tube. A reagent blank was also prepared with the same method. Sample was incubated at room temperature for an hour. Absorbance was read at 415nm. Same procedure was repeated for standard solution. Rutin was used as standard. Results were expressed as mg RE/g, using calibration curves prepared immediately before the analysis (Quettier *et al*., 2000).

Determination of tannins

2g of dried powder seaweeds sample was shaken with 5 ml of methanol in a test tube continuously for 1 min. Methanol extract was filtered. Quickly the tube was rinsed with an additional volume of methanol and the contents were poured at once into the filtering funnel. The filtrate volume was made up to 50 ml with distilled water. Extract was used for determination of tannins within an hour.

3ml of 0.1 M $FeCl_3$ in 0.1 N HCl was added to the 1ml of extract, followed immediately by time addition of 3ml of 0.008 M $K_3[Fe(CN)_6]$. Reagent blank was prepared containing only methanol. Absorbance was read at 720 nm after 10 min. Tannic acid was used as standard. Results were expressed as μg TAE/g, using standard calibration curves prepared immediately before these analyses (Price and Butler, 1977).

Determination of saponins

25g of powder seaweed sample was suspended in 250 ml of 20% aqueous ethanol and heated over water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and residue was re-extracted with another 200 ml of 20 % aqueous ethanol. Both extracts were combined and the combined extracts were concentrated to 40 ml over water bath at 90°C. The concentrated extract was transferred into a 250 ml separating funnel and extracted thrice with 20 ml of diethyl ether. The ether layers each time were discarded whereas the remaining aqueous layer was extracted thrice with 60ml of *n*-butanol. The combined *n*-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated over water bath, dried in the oven and weighed (Aliyu *et al*., 2008).

Total saponins were calculated by subtracting W1 from W2. Results were expressed as percentage.

Saponins % = [(W2 - W1)/W1] x 100

where, W1 = empty beaker

W2 = Beaker with saponins

Pharmacological activities

Seaweed powder was percolated at room temperature in 70% methanol thrice each for three days. Combined crude methanol extract was evaporated *envaccuo* and extracts were used after filtration for pharmacological activities.

Animals

Swiss albino mice (20-25 g) and Wistar rats (180-200g) were used. They were fed laboratory diet ad libitum. Mice

were given free access to food and water under standard conditions of lab trials in 12 h dark/light control. All the experiments were got approved from the local animal care committee. Animals were treated according to the ethical principles (approval No. IBC KU-138/2020).

Acute toxicity

Mice were administered with the doses (50-500 mg/kg bw) of algal methanol extracts or vehicle (10% DMSO) orally (10ml/kg). After administration of the extracts behavioural changes were noted continuously up to 2h. The mortality was observed for 48h. Animals were observed for 7 days (Singh and Singh, 1994).

Analgesic activity

Analgesic activity by tail immersion method

Mice were checked for sensitivity by immersing the tip of the tail in hot water at a temperature of 55±0.5°C. Mice which withdrew the tail within 5 sec were selected for activity. Each mouse served as its control at 0 and 10 min interval. The average of the two values was initial reaction time. The reaction time for the same group was taken at interval of 30, 60, 90, 120, 150 and 180 min, followed by the extract administration at the dose of 400 mg/kg bw orally. Control group received same volume of vehicle. Acetylsalicylic acid was used as standard in a dose of 100 mg/kg bw (Olabye *et al.,* 2000).

Analgesic activity by hotplate method

Hotplate method are used to evaluate the reaction latencies in mice (Shemmugasundaran and Venkataraman, 2005). The pre-screened mice were divided into nine groups (n=7). 10% DMSO with a dose of 10 ml/kg bw was given orally as vehicle to group I. Extracts were administered orally to group II to VIII with a dose 400 mg/kg bw while group IX received acetylsalicylic acid (standard) 100mg/kg bw orally. The mice were examined by setting on a hot plate at 55± 0.05°C. Thermal nociception was recorded as discomfort in type of hopping, withdrawal or licking of the paws. The response latencies were observed between 0 to 180 min.

Anti-inflammatory activity

The anti-inflammatory test was determined by using method of Winter *et al* (1962). The rats were arranged into nine groups with 7 rats per group. Prior to treatment, the size of the right hind paw of rat was measured three times by plethysmometer 7150, (Ugo Basile). Vehicle (10% DMSO), standard (Diclofenac at 50mg/kg bw) and extracts at 400mg/kg bw were introduced orally to the rats. 30min after the introduction of test samples, 1% of λ-carrageenan suspension (0.05 ml per rat) was inserted in each rat's right hind paw. The size of the right hind paw was measured again at 1st, 2nd, 3rd, 4th and 5th h after carrageenan induction.

Ethical approval

All experimental procedures were followed as per IBC approval IBC KU-138/2020, University of Karachi.

STATISTICAL ANALYSIS

Results are presented as mean ± standard deviation (SD). Based on hypotheses testing for variances among the different species, the p-values <0.001 for each of the phenols, flavonoids, tannins and saponins were calculated. Therefore, the non-parametric analyses of variance tests were applied. The corresponding p-values using Kruskal-Wallis test is <0.001 presenting statistically significant differences among different species for each of (secondary metabolites). Finally, for each class of secondary metabolite, the Dunnet's test (with unequal variances) was assumed. For pharmacological activity all experimental results are expressed as mean ± SEM followed by student's t tests. The value p<0.05 was considered significant whereas p<0.01 and p<0.001 as highly significant. All statistical analyses were performed using the SPSS software package, version 22.0.

RESULTS

Phycochemical analyses

Phycochemical screening showed that all selected seaweeds contained phenols, flavonoids, and saponins as shown in table 1. Alkaloids were also analysed but were not detectable.

Phycochemical analyses showed that total phenols range between 0.10 (*S. swartzii*) to 2.16 (*M. afaqhusainii*) mg GAE/g. Among Rhodophyta, phenol content in *M. afaqhusainii* was found higher followed by 0.24 in *C. muelleri.* In Ochrophyta phenol content ranges between 0.10 (*S. swartzii*) to 0.26 (*C. indica*) mg GAE/g. Total flavonoid content was found ranging from 1.12 (*S. robusta*) to (*C. muelleri*) 4.59 mg RE/g. Average results showed that flavonoid content in Rhodophyta and Ochrophyta were comparable. Total tannins were found in low amount ranging from 0.3 (*S. robusta*) to 140.8 (*M. afaqhusainii*) μg TAE/g. Saponins with a significant amount ranged from 1.34 (*S. swartzii*) (*C. flabellatum*) to 3.47%. Among Ochrophyta, *C. indica* possessed relatively high saponin content (2.61%).

Pharmacological activity

Acute toxicity

In acute toxicity test, all extracts up to 500mg/kg bw did not show any change in behavioural or neurological toxicity.

Analgesic activity by tail immersion method

Analgesic activity by tail immersion test is shown in table 2. Among all the species, *S. swartzii*, *M. afaqhusainii* and *S. robusta* exihibited significantly prolonged reaction time

that started at 30 min and remained effective for 180 min by resisting heat stimuli to the central nervous system.

Analgesic activity by hot plate method

In hot plate method, the significant analgesic effect was observed in *S. swartzii*, *C. flabellatum* and *S. robusta* for 180 min, while *C. indica* showed analgesic effect for 150 min. No analgesic effect was observed in *S. wightii* and *M. afaqhusanii*. Extract of *C. muelleri* showed analgesic effect only at 150 min (table 3).

Anti-inflammatory activity

The anti-inflammatory activity was assessed by carrageenan induced rat paw oedema method as shown in table 4. All extracts showed significant anti-inflammatory activity with $p<0.01$ and $p<0.001$ till 5^{th} h.

DISCUSSION

Phycochemical analyses

Phycochemical screening showed that all selected seaweeds contained phenols, flavonoids, and saponins (table 1). Total phenols in *C. flabellatum* (0.31 mg GAE/g) were found higher as compared to the previous study on sister species *C. bursa* and *C. fragil* (Frikha *et al*., 2011; Heffernan *et al*., 2014). Phenols contents were found statistically different ($p<0.05$) among all species. The observed total phenols in *Sargassum sp* were found lower when compared with the literature values (ranging from 3.20 to 216.65 mg CAE/g) in other species of the same genus (Thillaikkannu *et al.*, 2012; Seenivasan *et al*., 2012; Mhadhebia *et al*., 2014). In current study total phenols of *C. indica* were observed lowest in comparison to other species of genus *Cystoseira* (Chkhikvishvili and Ramazanov, 2000; Frikha *et al.,* 2011, Mhadhebia *et al*., 2014). Average results showed that Rhodophyta possess high phenol content followed by Chlorophyta and Ochrophyta. To the best of our knowledge phenol content of any species of *Melanothamnus*, *Coelarthrum* and *Solieria* are not documented in literature. Results showed that phenol content varies with species. The extraction yield is highly dependent on extraction method and solvent selected for extraction. Phenol content is highly affected by sunlight, climate, and geographical locations (Sullivan *et al*., 2011, Qasim *et al*., 2016). Variation in phenol content is evident in similar species collected from different locations. About 8000 phenolic compounds have been discovered from marine origin so far. Marine algae are a novel source of botanicals nevertheless their extractable polyphenols (less than 0.4%) are lower than that of other chemical constituents (Bocanegra, 2009). Marine origin phenols are usually more potent than terrestrial origin plant sources because of diversity in chemical structures. Sometime these phenols possess more than eight interconnected phenol rings (Sullivan *et al*., 2011). Phenols are accountable for numerous biological and physiological activities such as chemo-preventive properties e.g., antioxidant, anti-carcinogenic, anti-mutagenic and anti-inflammatory effects (Huang *et al*., 2010). These compounds act as anti-oxidant and can be utilized in food containing fats for preservation and to avoid the formation of various off flavours and undesirable compounds caused by the oxidation of lipids (Chi-Tang *et al*., 1992).

A direct relation was observed between the average phenol and average flavonoid contents both in Rhodophyta and Ochrophyta. In Rhodophyta, *C. muelleri* was found with highest flavonoid content (4.59 mg RE/g) while *S. robusta* and *M. afaqhusainii* were close to each other (1.12 and 1.28mg RE/g, respectively). Flavonoids were found statistically different ($p<0.05$) in all seaweeds. Literature survey revealed that with the exception of *S. wightii*, all other species in current study have never been analysed so far. *S. wightii* was recorded with relatively high content of flavonoids among studied Ochrophyta in current study and was found lower when compared with earlier reports (Seenivasan *et al*., 2012 and Sahayaraj, 2014). Flavonoids possess various biological activities, noteworthy among these are immunomodulatory, antiviral, anti-inflammatory, antimicrobial, anticancer and antithrombotic activities. Anti-inflammatory activity is a prime example of the therapeutic properties of flavonoids (Garcia-Lafuente *et al*., 2009). Cardiovascular diseases are very common and a major cause of death in developed countries. These deaths/diseases are generally related to hypercholesterolemia. Flavonoids possess the ability to reduce cholesterol level and hence play an effective role in minimizing the risk of diseases related to heart (Havsteen, 2002). The average amount of tannins and phenols were found directly co-related in Rhodophyta and Ochrophyta. Tannins content of all individual species of Rhodophyta and Ochrophyta have shown direct relation with flavonoids content.

Method used to analyse saponins in current study was not applicable in case of Rhodophyta due to gel formation in initial steps of extraction. Similar gel formation was observed in another set of separate experiment during pectin analyses (data not shown). Rhodophyta species are rich source of agar and carrageenan, responsible for the formation of gel and interference in analyses. Saponin content was found high in both *Sargassum spp* (*S. swartzii* (1.34%) and *S. wightii* (1.90)) when compared with the earlier findings (Sahayaraj, 2014). Saponins and phenols had a direct concentration relation in Ochraphyta. Significant difference was observed in saponins among all species. Saponins are accountable for various biological activities such as antibacterial, antifungal, antioxidant, etc., activities. Saponins due to their amphoteric polarity are natural surfactants and bear foaming properties. These saponins enhance surface properties of food and are used as preservative agents against microbial spoilage (Choon *et al.,* 2014). These are widely used in emulsifiers, detergents, and foaming agents. Saponins have also been

Table 1: Phycochemical composition of secondary metabolites

Seaweeds	Phenols (mg GAE/g)*	Flavonoids (mg RE/g)*	Tannins (μg TAE/g)*	Saponins (%)*
		Rhodophyta		
M. afaqhusainii	2.16 ± 0.07	1.28± 0.03	140.80 ± 0.00	Gel formation
C. muelleri	0.24 ± 0.03	4.59 ± 0.20	5.70 ± 0.00	Gel formation
S. robusta	0.15 ± 0.00	1.12 ± 0.02	0.30 ± 0.00	Gel formation
Average	0.85	2.3	0.05	-
		Ochrophyta		
C. indica	0.26 ± 0.04	1.83 ± 0.07	0.80 ± 0.000	2.61 ± 0.06
S. wightii	0.20 ± 0.02	2.38 ± 0.18	1.10 ± 0.00	1.90 ± 0.03
S. swartzii	0.10 ± 0.00	1.88 ± 0.00	0.70± 0.00	1.34 ± 0.03
Average	0.18	2.03	0.86	1.95
		Chlorophyta		
C. flabellatum	0.31 ± 0.01	1.45± 0.07	5.1 ± 0.00	3.47 ± 0.11

*mean and SD ($n = 9$)

Table 2: Analgesic activity by tail immersion test in mice

Group	Treatment	Dose (mg kg^{-1})	Reaction time in second at time (minutes) mean ± SEM						
			0	30	60	90	120	150	180
Control	10 % DMSO	10 ml	0.78±0.02	0.80±0.03	0.79±0.01	0.79±0.01	0.80±0.00	0.78±0.01	0.76±0.01
Standard	Acetylsalicylic acid	100	0.84±0.04	1.37±0.12*	3.27±0.31**	5.01±0.06***	4.93±0.26**	3.23±0.05***	2.02±0.06**
Test	*S.wightii*	400	0.89±0.11	1.75±0.17*	3.02±0.61	3.10±0.34*	2.16±0.08**	1.82±0.25*	1.23±0.11*
	S. swartzii	400	0.88±0.08	5.11±0.52**	5.16±0.99*	5.57±0.68**	4.39±0.33**	3.34±0.18**	3.22±0.05***
	C. muelleri	400	0.98±0.08	1.21±0.06**	2.65±0.27*	2.21±0.10**	2.01±0.07**	1.65±0.07**	1.40±0.06**
	C. indica	400	0.85±0.03	1.3±0.29	1.76±0.17*	2.19±0.48	2.63±0.19**	2.09±0.05***	1.67±0.09**
	M. afaqhusainii	400	0.89±0.07	3.24±0.17**	4.40±0.36**	4.71±0.45**	4.41±0.16***	3.82±0.11***	3.18±0.11***
	C. flabellatum	400	0.89±0.07	1.5±0.19*	1.81±0.18*	2.31±0.32**	2.82±0.19**	2.08±0.04***	1.79±0.08**
	S. robusta	400	0.89±0.04	2.58±0.39**	3.33±0.40**	2.66±0.43**	3.41±0.59**	3.23±0.74*	3.17±0.65*

Table 3: Analgesic activity by hotplate method in mice

Group	Treatment	Dose (mg kg^{-1})	Reaction time in second at time (minutes) mean ± SEM						
			0	30	60	90	120	150	180
Control	10 % DMSO	10 ml	7.33±0.33	7.67±0.33	7.33±0.33	6.67±0.67	7.33±0.33	6.67±0.33	6.67±0.33
Standard	Acetylsalicylic acid	100	7.67±0.33	8.67±0.67	12.00±0.58	13.33±0.88	14.33±1.20	11.00±0.58	9.33±0.33
Test	*S. wightii*	400	7.33±0.33	7.00±0.58	7.00±0.00	8.67±0.88	7.67±0.33	7.00±0.58	7.00±0.58
	S. swartzii	400	7.33±0.67	15.00±2.65	18.00±2.08*	15.67±0.88***	15.33±1.86*	13.00±1.00**	11.33±0.67**
	C. muelleri	400	7.00±0.58	7.33±0.88	8.67±1.20	9.33±1.20	10.67±1.86	9.00±0.58*	7.333±0.33
	C. indica	400	7.00±0.58	9.67±0.33**	11.33±0.33***	10.67±1.20*	10.00±0.58**	9.00±0.58*	8.33±1.20
	M. afaqhusainii	400	7.67±0.88	10.00±1.53	10.00±1.00	9.67±1.20	9.33±1.45	8.67±1.20	8.33±0.88
	C. flabellatum	400	6.33±0.33	9.67±1.20	12.33±1.33*	13.33±1.33*	12.33±0.33***	9.67±0.33**	9.00±0.58*
	S. robusta	400	8.09±0.28	12.80±1.65	17.76±3.22*	18.80±3.23*	19.60±3.52*	22.60±4.36*	21.20±3.54**

Table 4: Anti-inflammatory activity by carrageenan induced rat hind paw oedema

Group	Treatment	Dose mg kg^{-1}	Time in hours					
			0	1	2	3	4	5
Control	10% DMSO	10 ml	1.52±0.07	2.91±0.05	3.80±0.06	4.57±0.06	5.34±0.12	5.80±0.09
Standard	diclofenac	50	1.69±0.02	1.84±0.02***	1.83±0.05***	1.76±0.04***	1.74±0.03***	1.72±0.03***
Test	*S. wightii*	400	1.68±0.04	2.42±0.22	3.01±0.07***	2.92±0.06***	2.74±0.10***	2.71±0.11***
	S. swartzii	400	2.11±0.27	3.07±0.24	3.27±0.15*	3.20±0.06***	2.94±0.03***	2.82±0.02***
	C. muelleri	400	1.73±0.03	2.94±0.07	3.22±0.26	3.30±0.11***	3.08±0.10***	2.95±0.09**
	C. indica	400	1.69±0.02	1.77±0.01***	1.86±0.01***	1.92±0.02***	1.98±0.02***	1.99±0.04***
	M. afaqhusainii	400	1.67±0.03	2.60±0.36	2.84±0.24*	3.15±0.28*	2.79±0.24**	2.62±0.20***
	C. flabellatum	400	1.60±0.02	1.70±0.02***	1.80±0.01***	1.90±0.03***	2.01±0.05***	2.04±0.05***
	S. robusta	400	1.92±0.20	3.36±0.19	3.62±0.17	3.42±0.19**	3.50±0.17***	3.61±0.14***

Values are mean ± SEM. ($n = 7$), Where *p<0.05, **p<0.01, ***p<0.001 compared to control

used as raw material in production of hormones, immunological adjuvants and as medicine in pharmaceutical industries (Ozlem and Mazza, 2007). Literature survey revealed that marine derived saponins, specially from seaweeds, in comparison to terrestrial saponins are still untouched in terms of biological activities as well as in quantitative and qualitative chemical analyses. Therefore, chances to discover novel drug leads from marine derived saponins are brighter.

Pharmacological activity

Analgesic activity

Tail immersion and hot plate methods were used for assessing central analgesic effects. Both tests react against pain incentives directing through neuronal pathways. Tail immersion intervenes spinal reflexes to nociception incentives. Whereas hot plate is supraspinally organized response of pain. Opioid mediators reveal their analgesic effects both by spinal (μ2, k1, δ_2) and supraspinal (μ2, k3,

δ_1, σ_2) receptors (Chapman *et al*., 1985, Jinsmaa *et al.,* 2005).

All extracts showed significant and highly significant analgesic effect for 180 min. A significant increase in reaction time by selected algal extracts in both tail immersion and hot plate methods indicate the analgesic effect through central mechanism which involve endogenous opioid peptides and biogenic amines like 5HT (Bensemana and Gascan, 1978).

Anti-inflammatory activity

Carrageenan-induced inflammation is widely used in pharmacological field to develop non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors. The oedema induced by carrageenan is a biphasic process. The 1st phase starts with the secretion of a diverse set of mediators, such as histamine, serotonin and kinins up to 2.5 h. In the 2nd phase, prostaglandin-like substances are released during 2nd to 3rd h characterized by an intense neutrophil infiltrate (Marzouk *et al.,* 2010). In this study, crude methanol extracts of *S. robusta* and *C. muelleri* showed delayed action which started at 3rd h. On the bases of this observation and biphasic nature of carrageenan induced paw oedema, the indication may be due to the ability of extracts to inhibit the action of late mediators released in second phase during oedema induced by carrageenan. It is interesting to note that during the analyses of pectins (results not shown) and saponins gel formation in intermediate steps was observed. The amount of gel produced per unit mass was high in *C. muelleri* followed by *S. robusta* and *M. afaqhusainii*. Since Rhodophyta are known to have the carrageenan content, these must have evolved with some chemicals to protect themselves from the inflammatory effects of carrageenan. This might be interpreted as one of the reason of late anti-inflammatory response in case of Rhodophyta. Conversely high effectiveness of the extracts of *C. indica* and *C. flabellatum* from 1st to 5th h indicated that these extracts have the ability to inhibit all mediators released during inflammation and may be related to the absence of carragenan. Extracts of *S. swartzii* and *M. afaqhusainii* were observed to be weak inhibitors as first phase mediators while strongly inhibiting as the second phase mediators. Thus the results showed that the methanol extracts have potential in combating acute and chronic inflammation. Thus the analgesic and anti-inflammatory activity reflect that methanol extract may contain phenol, flavonoids, tannins and saponins. Phenols and flavonoids are reported to affect multiple targets as analgesic and anti-inflammatory agents. Literature survey revealed that the analgesic and anti-inflammatory activities were exerted by various flavonoids, sterols, terpenoids, saponins and other secondary metabolites (Thankarajan, 2014). The anti-inflammatory effect produced by selected algal extracts may be due to the inhibition of activities of cyclooxygenase and lipoxygenase and therefore reduced the formation of inflammatory metabolites (Nijveldt *et al*., 2001). Advance studies may be pursued in future to confirm the exact mechanism of action of selected extracts.

CONCLUSION

Current investigations have revealed high amount of saponins, significant amounts of flavonoids, followed by phenols, in studied seaweeds. The significant to highly significant analgesic and anti-inflammatory activities suggested that these seaweeds have a potential to use in combat with inflammatory diseases.

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