Phycochemical and pharmacological studies on *Ulva fasciata* Delile

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Abstract: Phycochemical and pharmacological studies were carried out on *Ulva fasciata* Delile. The ash content was found as 20.4812 % dry weight, moisture content 14.5514 %, total fat content as 0.1878% and 0.49341 %. Total carbohydrate was found as 54.5301-54.2246% dry weight, phenolic content as 0.022%, flavonoids found to be 0.0313% and tannins were 0.00003 %. *Ulva fasicata* showed central analgesic activity and significant anti-inflammatory activity at the dose of 400 mg/kg bw.

Keywords: *Ulva fasciata,* phycochemistry, pharmacology.

INTRODUCTION

A variety of seaweeds are found along the coastal belt of Sindh and Baluchistan as fresh (growing submerged) either as drifted material or entangled with rocks. In the coast line of Pakistan, there are about 70 species of 27 different genera of brown macroalgae. *Ulva fasciata, Chondria tennussima, Sargassum* spp., and *Valoniopsis pachyema* are abundantly found at Karachi coast (Aliya and Shameel, 1999; Samee *et al.*, 2009). The green macroalgal genus *Ulva* commonly called sea lettuce is widely distributed in marine and fresh water throughout the world (Canter and Lund, 1995; Guiry and Guiry, 2017). *Ulva fasciata* Delile belongs to phylum Chlorophycota, class Ulvophyceae, order Ulvales and family Ulvaceae is an abundant growing alga on the rocky shore of Buleji situated at the coastal belt of Karachi (Valeem and Shameel, 2006). In the Asian countries, *U. fasciata* is consumed raw in salad, soup and used for garnishing. In Tanzania, it is used for dressing wounds and cuts (Mshigeni, 1982).

Ulva fasciata is a dark green or bright grass green in color and may be colorless under stressed condition. The Thallus is thin and sheet like, consist of wide blades, basal portion 10-15 cm wide, tapering at the end, up to 15cm long, broad at the base and joint, but upper portion is divided deeply into many ribbon like segments, margin smooth, often undulate and holdfast is small. The cell wall polysaccharides in *Ulva* contain cellulose and water-soluble polysaccharides with sulphate groups. Sulphalted polysaccharide of marine algae have shown anticoagulant, antihyperlipidemic, antiviral, antioxidant and antitumor activities (Colliec *et al.*, 1994; Pereira *et al.*, 1999; Pengzhan *et al.*, 2003a; Pengzhan *et al.*, 2003b). *Ulva* is one of the important source of gelling polysaccharide (Lahaye and Axelos, 1993). Generally in seaweeds green and red contains higher protein (10-30% dry weight) where as in brown seaweed it is (5-15%) (Burtin, 2003; Matanjun *et al.*, 2009).

Ulva fasicata has shown antioxidant (Kokilam and Vasuki, 2014), antileishmanial (Sabina *et al.*, 2005), hypoglycemic (Subash-Babu *et al.*, 2008; Abirami and Kowsalya, 2013), antibacterial (Selvin *et al.*, 2004; Shahnaz and Shameel, 2006), antifungal (Febles *et al.*, 1995), insecticidal and nematicidal activities (Valeem *et al.*, 2011).

MATERIALS AND METHODS

Algal material

Ulva fasciata Delile was collected from its natural habitat at Buleji coast of Karachi in the month of September and October 2015. The alga was identified by a phycotaxonomist and voucher specimen and herbarium sheet was prepared with VS No. AUF-01-16.

Preparation of the powder and extract

For phycochemical analysis, algae was thoroughly washed with water to remove epiphytes, salt and debris then, dried under shade. Dried seaweed was grinded by electrical grinder that could pass through sieve No 20. The sample was store in bottles at room temperature for chemical analysis. For pharmacological studies, algal material 1kg was socked in 3L of methanol for seven days. The mixture was filtered and then concentrated by rotary evaporator at 40^{o}C. This methanol extract (MEUF) was used for pharmacological studies.

Phycochemical analysis

Ash content

Sample (1g) was taken in a preweighed crucible. The material was incinerated at 550^{o}C for 5h. The crucible was cooled and weighed. The procedure was repeated to get constant weight (AOAC, 1997).

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Moisture content
Sample (2 g) was taken in a crucible previously weighed and kept in oven at 105°C until constant weight. The moisture content in the algal sample was determined by subtracting the dried sample weight from the air dried sample (AOAC, 1997).

Determination of total fat
Total fat were extracted by Soxhlet apparatus. 10 g sample was taken into a thimble. Empty extraction flask was weighed. 250 ml of n-hexane was used as extraction solvent. The refluxing was continued for 8 h at the boiling point of n-hexane. After extraction, the solvent was evaporated from the flask and weighed the flask with extracted crude oil. The percentage of extracted oil was calculated by using the equation.

% of oil = extracted oil/dry sample x100 (AOAC, 1997)

Determination of total fat by Folch method
Sample (1 g) was homogenized with 20 ml of chloroform and methanol 2:1 (v/v) mixture and left over night for extraction. Sample was filtered with fat free filter paper. Crude extract mixed thoroughly with 2 ml of salt solution and was kept to separate into two phases, without interfacial fluff. Organic layer was collected in preweighed vial and evaporated to dry and weighed. Then calculate the % of fat (Folch *et al.*, 1957).

Determination of total protein
Dried sample 100 mg, was soaked in distilled water for 24 h at 4°C with continuous stirring. Sample was centrifuged at 13400 rpm for 20 min (10 ml) at 4°C. Supernatant was separated carefully. 10 ml beta-marcaptoethanol was added in residue and stirrer for 1h at room temperature then the suspension was centrifuged at 13400 rpm for 20 min. The supernatant was separated and mixed with previous supernatant and was used for ammonium sulphate precipitation of protein (Lowry *et al.*, 1951)**.**

Ammonium sulphate precipitation of protein
Ammonium sulphate (7 g) was added gradually in 10 ml supernatant already chilled in ice bath and stirrer for 1h and kept the beaker chilled. It was centrifuged at 4°C for 15 min. The supernatant was removed carefully and pallet was dissolved in 10 ml buffer pH 6-7 and stored in refrigerator for protein analysis (Lowry *et al.*, 1951).

Estimation of protein
Estimation of protein from seaweed extract was carried out by Lowry method. 1ml of extract was taken in a test tube then Lowry solution (2 ml) was added. The test tubes were incubated for 10 min at room temperature then 0.2 ml of diluted Folin-phenol reagent was added. Shaken vigorously and incubated for 30 min in dark, at ambient temperature. Same procedure was repeated with standard Bovine Serum Albumin (BSA) solution. In blank, distilled water was used instead of extract and absorbance was noted at 600, 660 and 750 nm.

Total protein was calculated as percentage by using following equation.
Protein (% w/w) in sample = [(C x V x D) / m] x 100
C = mg / L of protein in sample from calibration curve
V = Liter of solvents used for extraction
D = dilution factor,
m = mg of biomass taken (Lowry *et al.*, 1951)

Determination of carbohydrates by difference method
Total carbohydrate was calculated by difference method. By using equation
100-[moisture + ash + protein + fat + crude fiber] = %. (Pádua *et al.*, 2004)

Determination of crude fiber
Sample (2 g) was boiled with 200 ml of 0.255N sulfuric acid for 30 mins using anti-bumping chips. After cooling and filtration through muslin cloth the residue was washed using boiling water till it was no longer acidic then residue was boiled with 200 ml of 0.313 N of NaOH solution for 30 min and again filtered with muslin cloth. Then residue was washed successively with 25 ml of boiling 1.25% sulphuric acid, three 50 ml portions of water and 25 ml alcohol respectively. Then residue was transferred to a weighed crucible and dried in oven for 2 h at 130 ± 2°C then weighed. It was then ignited for 30 mins at 600 ± 15°C. Then it was cooled by keeping in a desiccator and reweighed. Loss on ignition was estimated as crude fiber (Patil and Gaikwad, 2011).

Extraction of phenol and flavonoids
Powdered seaweed sample (2 g) was soaked in 15 ml of 80 % methanol for 24 h. Methanol extract was filtered and transferred into preweighed vial and dried. The obtained extract was kept in sterile sample tube till analysis at 4°C (Singleton *et al.*, 1999; Quettier *et al.*, 2000)**.**

Determination of total phenols
Folin-Ciocaltaeu's reagent 2.5 ml was added in 0.5 ml of diluted methanol extract (concentration 1mg/ml), followed by time addition 2.5 ml of 7.5% $NaHCO_3$. While mixing, the sample was kept for 45 min at 45°C on water bath. A reagent blank was prepared using methanol instead of sample. Absorbance was noted at 415 nm (Shimadzu UV-visible spectrophotometer UV-240). The results were expressed as %, using calibration curves prepared immediately before this analysis and gallic acid was used as standard (Singleton *et al.*, 1999)**.**

Determination of total flavonoids
Dried methanol extract was diluted to 1mg/ml then 2 ml of 2 % $AlCl_3$ solution (in methanol) was added into 2 ml diluted methanol extract (concentration 1mg/ml). A

reagent blank was also prepared with the same method. Sample was incubated for an hour at room temperature. Absorbance was read at 415nm. The experiment was conducted in replicates for each analysis of test and standard (rutin) and the results were expressed as %, using calibration curves prepared immediately before this analysis (Quettier *et al.*, 2000).

Determination of total tannins

Seaweed sample (2 g) was shaken for 1 min with 5 ml of methanol in a test tube and filtered. The tube was immediately rinsed with 5 ml of methanol and the material was transferred immediately into the filtering funnel. The volume of the filtrate was made up to 50 ml with distilled water and analyzed within an hour. 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCl was added to the extract, then immediately by timed addition of 3 ml of 0.008 M $K_3[Fe(CN)_6]$ after 10 mins. The absorbance was measured in cuvettes at 720 nm. A reagent blank sample was prepared. The results were presented as tannic acid equivalents, by using standard calibration curves prepared immediately before the analysis (Price and Butler, 1977).

Determination of alkaloids

A sample of 3 g was taken in a beaker, and 200 ml of 20 % ethanolic acetic acid was added. The beaker was covered and left for 4 h. The extract was filtered and volume was reduced to one quarter of the initial, using water bath. Then concentrated NH_4OH was added slowly drop wise to the extract until the precipitation get completed. The precipitates were left for settling then filtered, dried and weighed (Poornima and Ravishankar, 2009).

Pharmacological studies

Animals

Male Wistar albino rats (180-200g) and male Swiss albino mice of 20-22 gm were used. The mice and rats were grouped then housed in polyacrylic cages in standard laboratory conditions (temperature 25± 2ºC). The animals were kept under observation for seven days before starting of the experiment.

Acute toxicity test in mice

Mice were administered with different doses (50-500 mg/kg) of *Ulva fasciata* methanol extract. The changes were observed for 2 h continuously and the mortality, if any was noted for a period of 24 - 48 h. However, animals were observed for one week (Singh and Singh, 1994).

Analgesic activity by tail immersion method

Three groups of 7 mice in each group were made. Group I (control) received normal saline 5 ml/kg b.w. Whereas group II and III received 400 mg/kg b.w. of MEUF and standard acetyl salicylic acid (100 mg/kg b.w, orally). The tail (about 5 cm) was dipped into a water bath maintained at 55±0.5ºC and the time in seconds to withdraw the tail from hot water was noted as the reaction time. The observations were noted at 30, 60, 90, 120, 150 and 180 min after administration (Janssen *et al.*, 1963).

Analgesic activity by hot plate method

Three groups (7 mice in each) were made. The group I animals were served as control and received the vehicle (normal saline, 5 ml/kg b.w.). The extract MEUF at the dose of 400 mg/kg b.w. was given orally to group II while group III received acetyl salicylic acid (100 mg/kg b.w, orally). The mice were placed individually on an aluminum hot plate at temperature 55±0.5ºC for maximum 30 sec. The reaction time was noted at which animals showed the licking response of their paws. The response was recorded at 0, 30, 60, 90, 120, 150 and 180 min (Eddy and Leimbach, 1953).

Anti-inflammatory activity by rat paw edema

The anti-inflammatory activity was carried out in rats (Winter *et al.*, 1962). Before administration, the volume of the right hind paw of each rat was measured three times by plethysmometer 7150, (Ugo Basile). Vehicle (normal saline) to group I, standard diclofenac sodium 50 mg/kg to group II and methanol extract of *Ulva fasciata* at 400 mg/kg were given orally to the animals. 30 minutes after the treatment of extract, an injection of 1% λ-carrageenan, suspension 0.05 ml was administered in hind paw of rats. Observations were taken at 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th}hour, after carrageenan administration.

STATISTICAL ANALYSIS

The values of results are given as Mean ±SEM. The statiscal analysis was performed by student t-test. Values were significant at $p<0.05$ while highly significant at $p<0.01$.

RESULTS

Phycochemical studies

Proximate composition of Ulva fasciata

Ulva fasciata was analyzed for its chemical composition in which ash, moisture content, total fat, protein, carbohydrate, crude fiber, phenols, flavonoids, tannins and alkaloids were determined (table. 1 and 2; fig. 1).

Pharmacological studies

Acute toxicity test in mice

Acute toxicity test was evaluated on 7 mice in each group fasted for 12 h before experiment. The extract was administrated in 50-500mg/kg orally to respective groups. The mice were observed for 2 h after administration for any immediate signs of toxicity then at 24 and 48 h. The mortality was noted for up to 7 days. Concentration above 500 mg/kg was not used and therefore LD_{50} could not be calculated.

Table 1: Proximate composition (%) of nutrients in *Ulva fasciata*

Ash	Moisture	Fat	Protein	Carbohydrate	Fiber
20.4842	14.5514	0.1878	3.8897	54.5301	6.3567

Table 2: Composition (%) of secondary metabolites in *Ulva fasciata*

Phenols	Flavonoids	Tannins	Alkaloids
0.0224	0.0313	0.00003	Not detected

Table 3: Analgesic activity of *Ulva fasciata* by tail immersion method

Treatment	Dose mg/kg	Reaction time in second at time (min)						
		0	30	60	90	120	150	180
NS	5ml / kg	0.78±0.020	0.79±0.026	0.79±0.008	0.79±0.012	0.80±0.004	0.78±0.006	0.76±0.008
ASA	100	0.83±0.039	1.37±0.116	3.27±0.310	5.01±0.063^{c}	4.93±0.257^{b}	3.22±0.052^{c}	2.01±0.061^{b}
MEUF	400	0.87±0.047	4.25±0.403^{b}	4.25±0.148^{c}	3.86±0.495^{b}	3.15±0.265^{b}	3.10±0.206^{b}	2.79±0.165^{b}

NS: Normal saline; ASA: Acetyl salicylic acid; N = 7; MEUF: Methanol extract of *Ulva fasciata*; Each value is presented as the Mean ± SEM; $p < 0.05^{a}$, $p < 0.01^{b}$ and $p < 0.001^{c}$.

Table 4: Analgesic activity of *Ulva fasciata* by hot plate method

Treatment	Dose mg/kg	Reaction time in second at time (min)						
		0	30	60	90	120	150	180
NS	5ml / kg	7.33±0.333	7.66±0.330	7.33±0.333	6.66±0.666	7.33±0.333	6.66±0.333	6.66±0.333
ASA	100	7.66±0.333	8.66±0.666	12.00±0.577^{b}	13.33±0.881^{b}	14.33±1.201^{a}	11.00±0.577^{a}	9.33±0.333^{a}
MEUF	400	6.66±0.667	12.33±1.203^{a}	9.00±1.156^{a}	11.33±2.336^{a}	13.66±1.454^{a}	8.33±0.667	8.00±0.578

NS: Normal saline; ASA: Acetyl salicylic acid; N = 7; MEUF: Methanol extract of *Ulva fasciata*; Each value is presented as the Mean ± SEM; $p < 0.05^{a}$, $p < 0.01^{b}$ and $p < 0.001^{c}$.

Table 5: Anti-inflammatory activity of *Ulva fasciata*

Treatment	Dose mg/kg	0	1 hr	2 hr	3 hr	4 hr	5 hr
NS	-	1.52±0.066	2.91±0.053	3.80±0.063	4.56±0.056	5.34±0.124	5.79±0.124
DS	50	1.68±0.020	1.83±0.023^{a}	1.83±0.051^{a}	1.75±0.044^{a}	1.73±0.032^{a}	1.73±0.032^{a}
MEUF	400	2.42±0.237	3.08±0.352	3.27±0.426	3.52±0.290	3.31±0.290^{a}	3.18±0.305^{b}

NS: Normal saline; DS: Diclofenac sodium; N = 7; Each value is presented as the Mean ± SEM; $p < 0.05^{a}$, $p < 0.01^{b}$ and $p < 0.001^{c}$.

Analgesic activity

The analgesic activity was evaluated by tail immersion and hot plate methods. In tail immersion test *Ulva fasciata* methanol extract showed significant analgesic effect from 30 to 180 min in 400mg/kg b.w. dose. The standard acetyl salicylic acid was significantly effective at 90 to 180 min (table. 3, fig. 2). In hot plate method, *Ulva fasciata* started significant effect at 30 min till 120 min, where as acetyl salicylic acid showed significant analgesic effect from 60 to 180 min in the dose of 100 mg/kg (table. 4, fig. 3).

Anti-inflammatory activity

Methanolic extract of *Ulva fasciata* was given to rats 1 h before carregeenan injection in 400 mg/kg b.w. dose. A significant anti-inflammatory effect at 4th and 5th hour was observed where as standard diclofenac sodium started significant effect in the 1st hour which remains till 5h (table. 5).

DISCUSSION

Phycochemical studies

Ash content

The ash content of *Ulva fasciata* was found as (20.4842 % dry weight). The amount of ash obtained was similar with the previous work of the same genus *Ulva fasciata*, 20.61% DW (Padua *et al.,* 2004) and 25.40% DW (Mcdermid and Stuercke, 2003), *Ulva rigida,* 25.74% DW (Frikha *et al.*, 2011) and *Ulva lactuca,* 21.30% DW (Wong and Cheung, 2000). The ash content in the current study was also in agreement with those in other previous findings (Rupérez, 2002; Sánchez-Machado *et al.*, 2004). Seaweeds possess large amount of ash or mineral contents (8-40%) and therefore are appropriate to take in diet for good health. The high amount of ash or mineral contents of this species is supposed to be the high content of minerals such as Na, Fe, S, I, Ca, Mg, P and K, which are important for our body (Nisizawa *et al.*, 1987; Rupérez, 2002).

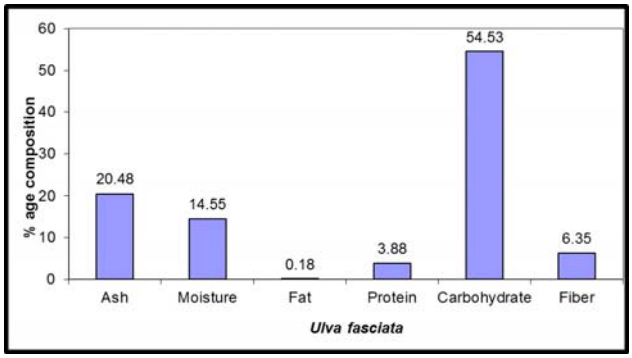

Fig. 1: Proximate composition (%) of nutrients in *Ulva fasciata*.

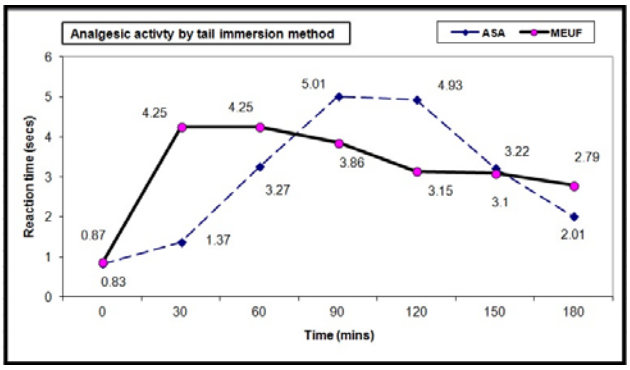

Fig. 2: Analgesic activity of *Ulva fasciata* by tail immersion method.

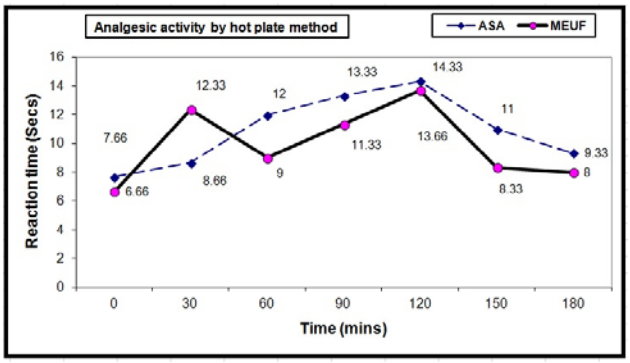

Fig. 3: Analgesic activity of *Ulva fasciata* by hot plate method.

Water and moisture content

Water content of *U. fasciata* was recorded as 77.847% and the moisture content was analyzed by the subtraction of oven dry sample weight from air dried sample weight. The moisture content was found as 14.5514% dry weight which is lowest in comparison to reported value (Pádua *et al.*, 2004).

Fat content

The total fat in the present study was investigated by two different extraction methods, Folch method and standard AOAC Soxhlet extraction method. Most algae contained fat less than 4% dry weight but several marine algae have high values such as *Dictyota acutiloba,* 16.1% DW and *Dictyota sandvicenis* 20.0% DW (Mcdermid and Stuercke, 2003).The total fat contents of *Ulva fasciata* was determined by AOAC standard method is (0.1878%) of DW. The result is consistent with the previous findings for *Ulva fasciata,* 0.2% DW (Carvalho *et al.*, 2009) by soxhlet extractor. The fat content of *Ulva fasciata* is comparable with some brown and red algae (Ruperez and Saura-Calixto, 2001). While the fat content was analyzed by Folch method is 0.4934% DW which is comparable to the previous findings 0.51% and 0.5% (Rameshkumar *et al.*, 2012; Kokilam and Vasuki, 2014). The value is also in consistent with the previous study 1-3% DW (Fleurence, 1999). However it is lower when compared to the same species 3.6-5.1% DW by (Mcdermid and Stuercke, 2003). This difference in quantity might be due to the geographical region and climate factors (Benjama and Masniyom, 2011).

Current results showed different fat content in the same species which may be due to the effect of different solvent extraction method. In AOAC method, n-hexane and in Folch method chloroform: methanol 2:1 is used as extraction solvents. Algal fat consists of both polar and nonpolar components, therefore the fat extraction depends on the polarity of the solvent or a mixture of solvents. As n- hexane is a non-polar solvent and extracts only non-polar fat while methanol is polar and can extract the polar fat and can penetrate in the cell wall, and making non-polar fat more accessible. Thus the difference in extraction yield was found and the extracted fat content by Folch method was three times higher than AOAC method. It has also been reported that seaweeds contain low fat constituent eicosapentaenoic acid, C20:5ω3, which may be used for cardiovascular diseases, thrombosis and atherosclerosis. These also possess antiviral, antibacterial, anti tumor, anti-oxidant and anti-inflammatory activities. Despite the fact that poly unsaturated fatty acids are very important for human digestive system, they also act as hormone like signaling molecules, such as prostaglandins, leukotrienes and thromboxanes. These properties showed the potential of fat as nutraceutical and pharmaceutical agent (Pereira *et al.*, 2012).

Protein

Seaweeds contain high content of protein (10-47%) but the concentration of protein varies with species, seasonal and geographical location (Bocanegra *et al.*, 2009). Proteins are the vital constituent of living cell and are essential for human and animals. Proteins content of *Ulva fasciata* in this study was found very low 3.8897% DW when compared to the previous study 8.8% DW (Mcdermid and Stuercke, 2003) and 17.08% DW (Kokilam and Vasuki, 2014) for the same species. Extraction of protein from marine flora is difficult due to phenolic compounds and enormous amount of cell wall

polyanionic mucilage. Phenolic compounds can destroy protein structure in oxidizing condition while cell wall mucilage produce gel like solution during extraction which may cause hindrance in protein extraction and purification (Wong and Cheung, 2001).

Carbohydrates

Carbohydrates are essential part of metabolism and provide energy not only for respiration but also for several metabolic processes. High content of carbohydrates is a noticeable characteristic in seaweeds. The total carbohydrates found in the current study were 54.5301% D.W. of *Ulva fasciata*. Usually algal carbohydrates are not completely digested by human and are referred as dietary fiber. Human gastrointestinal enzyme do not produce required enzyme which are responsible for degradation of these carbohydrates hence energy bioavailability is low (Gressler *et al.*, 2011).

Crude fiber

The content of crude fiber of *Ulva fasciata* was 6.3567% DW in the present study. The result is lower when compared with the previous findings, 9.32-11.91% for the *U. lactuca* (Pádua *et al.*, 2004). The economic, cultural and scientific developments of society have great impact in nutritional habitat and life style. Diet with high calories, rich in saturated fats and sugar, low fiber and complex carbohydrates is common in daily diet which may cause obesity problem, heart disease, hypertension, and diabetes. Seaweeds possess high fiber contents which are different from the fiber of terrestrial plants. Human consumption of algal fiber give positive influence on health and reduce the risk of constipation, colon cancer, diabetes, hypercholesterolemia and obesity (Ortiz *et al.*, 2009).

Total phenolics

Seaweeds are also a major source of bioactive compounds. Currently more than 8000 phenolic compounds are known and most of them are of marine origin isolated from marine algae. Total phenolic contents of *U. fasciata* was recorded as 0.0224% and found lower than reported values (Kokilam and Vasuki, 2014; Sahayaraj *et al.*, 2014). The variation may be due to environmental conditions, geographical location and extraction method. These phenolic compounds showed wide range of biological effects like anti carcinogenic effect, also influence on cardiovascular health, antioxidant potential and age related neuronal degeneration. Human cell may suffer oxidative stress during disease condition or optimum nutrition is deficient. Under these conditions "reactive oxygen species" initiate fat peroxidation and damage to other biomolecules. The high concentrations of phenolic compounds in seaweeds act as antioxidant which may helpful in reducing oxidative reactions lethal to health (Zubek *et al.*, 2012).

Flavonoids

The flavonoids from *U. fasciata* were found 0.0313 % which was lower when compared to the previous study (Kokilam and Vasuki, 2014; Sahayaraj *et al.*, 2014). Flavonoids are responsible for many biological activities including anti-cancer, anti-microbial, antiviral, anti-inflammatory and immunomodulatory. The anti-inflammatory activity of flavonoids is commonly used in Chinese traditional medicine. Furthermore, inflammation can gradually causes various chronic diseases like arteriosclerosis, neurodegenerative cancer and obesity. Rutin has been reported effective in adjuvant arthritis (Guardía *et al.*, 2001).

Tannins

Tannins were found in trace quantity 0.00003%. Tannins have also been reported as HIV-1 inhibitor. They also have ability to absorbed UV radiation and act as photo protective for cell against photo destruction. The structure of algal tannins consist of 8 phenol rings while the tannins produce from terrestrial plants comprises of 3 to 4 rings (Zubek *et al.*, 2012).

Alkaloids

Alkaloids were not found in the methanol extract of *Ulva fasciata*.

Pharmacological studies

Analgesic activity

Pain is an unpleasant, emotional and sensory experience which associated with actual or potential tissue damage. It is primarily a protective process and produce discomfort and several side effects. Analgesics are the medicines used to reduce or treat pain. Many synthesized analgesic compounds have shown side effects such as GIT bleeding, ulceration, drowsiness, respiratory distress, nausea etc. (Ezeja *et al.*, 2011). Therefore, there is a need to search and develop new compounds from medicinal plants and marine natural products with more potent analgesic effect and fewer side effects.

Different animal models like acetic acid induced writhing, tail immersion and hot plate test are commonly employed to investigate the analgesic effect of natural products. These tests for analgesic agents not only determine nociception but also the reaction of animals to different painful stimuli which may be chemical as formalin test and acetic acid-induced writhing test, thermal as tail immersion or hot plate tests, or mechanical method as tail or paw pressure tests (George *et al.*, 2009).

The tail immersion test is used to observe morphine-like agents which selectively prolong the reaction time of tail withdrawal reflex in mice where as in hot plate test, the paws of mice are sensitive to heat at 55±1^{o}C. In both models the reaction time shows the degree of analgesia (Ramabadran and Bansinath, 1986; Toma *et al.*, 2003).

Both tail immersion and hot plate test are employed to investigate analgesics which are centrally acting. In both tests, sensory nerves sensitize the nociceptors and therefore the roll of endogenous mediators like prostaglandins are reduced (Bachhav *et al.*, 2009). In the present study *Ulva fasciata* showed significant analgesic effect in both tail immersion and hot plate tests. Therefore, it may be said that the analgesic effect of the methanol extract of *Ulva fasciata* may be due to inhibition of prostaglandin pathway.

Anti-inflammatory activity

Inflammation has a complex pathophysiology. It involves different signaling molecules which are produced by mast cells, macrophages and leukocytes due to cellular responses including phagocytic uptake and also the production of inflammatory mediators such as prostaglandin (PGE_2), nitric oxide and tumor necrosis factor (TNF-α) (Kinne *et al.*, 2007). These factors causing edema formation due to proteins, fluids and leukocytes at the site of inflammation (White, 1999). The cytokines, which are formed by either immune or cranial neural crest (CNC) cells, may sensitize the peripheral nociceptors while Prostaglanidins (PGs) induce hyperalgia as they affect nerve endings (Khan *et al.*, 2007). Therefore, pain, inflammation and fever are produced due to formation of prostaglandins. The inflammation occurs as swelling to redness, heat and pain to remove injurious stimuli and to start healing, but if it is not control it can cause of certain diseases like vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis (Punchard *et al.*, 2004; O'hanlon *et al.*, 2010). Carrageenan is commonly used to produce inflammation for the evaluation of natural products. It produces severe inflammatory reaction in 30 min when injected on the rat paw (Marzouk *et al.*, 2010). In the present study, *Ulva fasciata* showed significant anti-inflammatory activity in the 4^{th} and 5^{th} hr.

Therefore, it is suggested that the methanol extract of *Ulva fasciata* may possibly acts by inhibiting the release and/or action of prostaglandin as the extract showed a significant anti-inflammatory activity at the third phase after 180 min of the edema formation.

The phycochemical study of the methanol extract of *Ulva fasciata* revealed the presence of phenols, flavanoids and tannins. The analgesic and anti-inflammatory activity of phenols, flavonoids and tannins have been reported (Harborne and Williams, 2000; De Sousa Araújo *et al.*, 2008).Therefore it may be said that the analgesic and anti-inflammatory effects shown by methanolic extract of *Ulva fasciata* are due to the presence of these secondary metabolites.

CONCLUSION

The phycochemical analysis of *Ulva fasciata* showed the presence of fat, protein, carbohydrate, phenols, flavonoids and tannins. In pharmacological studies the methanol extract of *Ulva fasciata* Delile did not show any toxicity upto the dose of 500 mg/kg b.w, orally and also showed central analgesic and significant anti-inflammatory effects. Both effects are due to several classes of active secondary metabolites present in *Ulva fasciata*. Further studies are needed to determine the mechanism of action and testing of potential isolated compounds for pharmacological effects.

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