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Ulva Lactuca and Its Polysaccharides: Food and Biomedical Aspects

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Abstract
Seaweeds are getting importance in various fields ranging from food to medical. In this respect *Ulva lactuca* (L), a green macro algae and sometimes known as sea lettuce has been well studied and documented. Current review cites some published literature for *U. lactuca* chemical and structural composition, and its food and biomedical applications. With significant amount of micro and macro-nutrients, plentiful functionally bioactive compounds have also been determined in *U. lactuca*. It has been reported that this green algae has sufficient antioxidants, antimicrobial, antiviral, antihyperlipidemic, antitumor and anti-inflammatory properties which suggest it a potent source against various ailments. Additionally, reports regarding its polysaccharides' use in drug delivery, wound dressing and tissue engineering authenticate its candidacy in biomedical applications. However, advancements should be made by blending *U. lactuca* polysaccharides with other untested natural or synthetic polymers of non-cytotoxic nature.
Keywords: *Ulva* sp., polysaccharides, medicinal plant, bioactive agents

Introduction

Of the total 221worldly known seaweed species, about two-third are reported for food applications (Zemke-White and Ohno, 1999). These seaweeds, since prehistoric time, had been remained a staple and vital part in Chinese, Japanese and Korean diet. Interestingly, China and Japan are the main contributors in world production-consumption scenario. Twenty percent of Asian diet is comprised of seaweeds that are relished not for their nutritional viewpoint but of unique and enchanting flavour. But in Western diet, the seaweeds are just used as food additives or extracts (Carvalho *et al*., 2009). In line with several beneficial flora, seaweeds have been widely analyzed by scientific community for their health promoting benefits which were believed historically. With the growing interest in nutraceuticals and functional foods, seaweeds are being intensively considered, where they deliver a multitude of functionalities ranging from a simple nutritional improvement to physiologically complex mechanisms. Nutraceuticals can be stated as a food that induces favourable physiological functions, upsurges the welfare and declines the chances for suffering of a particular ailment. It is widely believed that these functionalities are preventative than curative. Functional food or nutraceutical is often obtained from traditional sources that are rich in bioactive agents, which are known for health promoting action for human. Furthermore, new types of functional products derived from seaweeds, have recently been developed and studied extensively. Sometimes, these type of products are taken as whole food or as a food supplement, and mostly these are sold as pills or tablets.

It was suggested that the seaweeds contain many biologically active components, which could be used as therapeutic agents in dietary supplements (Madhusudan *et al*., 2011). Similarly, *Ulva* sp. (green algae) has been known for various functional bioactive compounds that have studied for their activities. In genus *Ulva,* approximately 50 species have been identified and reported (Nic Dhonncha and Guiry, 2002). Our focus is *Ulva lactuca* (L.), commonly known as "sea lettuce" or "green laver". Morphologically, it has color variations from green to dark green depending on its occurrence i.e., underwater or at beach. It forms irregular, but round ruffled edge shaped translucent soft sheet fronds (leaf blades) from slight yellowish to blackish green. Fronds are attached to rocks with the help of holdfast. It gains 20-30 cm diameter that is quite smaller (Braune and Guiry, 2011; Pereira *et al*., 2010). *U. lactuca* is harvested from beaches in the world every year in huge quantity (Braune and Guiry, 2011; Tian *et al*., 2015a). It has prehistoric record for treatment of hyperlipidemia, sunstroke and many urinary diseases in the form of food supplement in Chinese medicine (Tseng and Chang, 1984), *U. lactuca* is also a source of methane, hydrogen sulfide and some other gasses after being decomposed (Tian *et al*., 2015b). *Ulva* polysaccharide possessed huge amount of SDF and IDF but, mainly SDF (Lahaye *et al*., 1994; Lahaye and Robic, 2007). It provides 20.53 and 31.55% of soluble (SDF), and 34.37 and 21.54% of insoluble IDF from polysaccharide complexes determined by Prosky and Englyst methods (Yaich *et al*., 2015). Table 1 presents some studies of crude composition of *U. lactuca*. Kylin firstly introduced the term "ulvan" originally used for referring the different water soluble sulfated fractions (fibre) of *U. lactuca*. This now has been generalized to refer the sulfated polysaccharides from members of Ulvales, mainly *Ulva* species. Ulvans are widely distributed in the cell wall structures of thallus (Bobin‐Dubigeon *et al*., 1997).

Table 1. Crude Chemical Composition of *U. lactuca*

Components	Values (%)			References
	a	b	c	
Ash	19.59	21.3-22.8	12.4– 29.9	[a]Yaich et al. (2015); [b]Wong and Cheung, (2000); [c]Rohani-Ghadikolaei et al. (2012)
Protein	8.46	17	27.2	[a] Ortiz et al. (2006); [b] Rohani-Ghadikolaei et al. (2012); [c] Yaich et al. (2015)
Lipid	7.87	1.5-3.6	0.3	
Total dietary fibre	54.90	50.3-55.4	60.50	[a] Ortiz et al. (2006); [b] Wong and Cheung (2000); [c] Yaich et al. (2015)
Insoluble fibre	34.37	24.2-32.6	33.30	
Soluble fibre	20.53	15.8-8.0	27.20	[a] Lahaye and Jegou (1993); [b] Ortiz et al. (2006); [c]Yaich et al. (2015)
Dry weight	50.3 -55.4	31.8-59.1	-	[a] Lahaye and Jegou (1993); [b] Rohani-Ghadikolaei et al. (2012); [c] Yaich et al. (2015)
Carbohydrate	59.10	61.50	-	[a] Ortiz et al. (2006); [b] Rohani-Ghadikolaei et al. (2012)
Moisture	6.80	12.60	-	

SDF have health benefits for host by improving gastrointestinal immunity, dropping blood glucose and lipids, reducing colorectal cancer and cardiovascular risks (Gunness and Gidley, 2010). While IDF is associated with emission of bile acids, reduction in passage time through gastrointestinal tract and enhancement of fecal bulk. The binding ability (of SDF and IDF) with organic and inorganic complexes, and the degradability and/or fermentability by the intestinal microflora is considered of additional significance (Croog *et al*., 1986).

Complexcity in dietary fibre (DF) extraction from seaweeds is due to the complicated polysaccharides' chemical composition, and other contributing factors are maturity, season, origin, species and growth conditions (Costa *et al*., 2012; Nagaoka *et al*., 2000). *U. lactuca* has been established as a better source of DF as compared to fruits and vegetables (Webster and Gadd, 1996). DF extraction from marine sources is an important intervention for improvement of healthy foods industry. Extracted DF addition into diet is a health promoting supplement, and a renewable source of raw material too. *U. lactuca* polysaccharides were analysed (Percival and Wold, 1963) and found that these contain glucose, glucuronic acid, xylose, rhamnose and sulfate groups. Brading *et al*. (1954) extracted a water-soluble polysaccharide from *U. lactuca* by boiling and subsequent ethanol precipitation with Na_2CO_3 solution. The resulted composition was 31, 9.4, 19.2 and 7.7% of rhamnose, xylose, glucuronic acid and glucose, respectively. Recently, ultrasonic-assisted extraction has been adopted for isolation of *U. lactuca* polysaccharides (Tian *et al*., 2015a).

Physicochemical and Structural composition

The nutritional properties of most seaweeds are well known. Similarly, a number of reports have been suggested chemical composition of *U. lactuca*. Total crude protein, although vary with season, source and physiological maturity, but reported between 4.3-8.5% (Lahaye and Axelos, 1993; Yaich *et al*., 2011). A multitude of essential and nonessential amino acids have been reported in *U. lactuca* proteins (Table 2). A handsome amount of lipids 7.9-10.69% has also been reported (Tabarsa *et al*., 2012; Yaich *et al*., 2011). Lipids are constituted by variety of mono and polyunsaturated fatty acids (Table 3). Many mineral elements are required by the human body due to its complex structures, and about all the essential minerals have been reported to be present in seaweeds. Mineral profile of *U. lactuca* (Table 4) is also variable depending upon the method and the experimentation conditions. But the variability of these nutritional composition is basically a resulted from numerous factors including the geographical origin, physiological maturity, season and environmental variations. Functionally, fibres effect differently with the degree of solubility. Total dietary fibre reported are 54.90%, with insoluble and soluble fractions of 34.37 and 20.53%, respectively (Yaich *et al*., 2011). Physico-functional properties of *U. lactuca* polysaccharides have been investigated and it was observed that 1.0 g of fibre concentrate could hold about 1.10 g and 11.0 g of oil and water, respectively (Yaich *et al*., 2015).

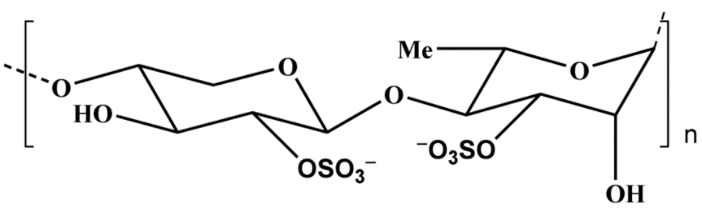
Table 2. Amino acids profile in Flour (F) and Total Fiber Concentrate (TFC) of *U. lactuca*

Amino acids	Values (mg/100 g)	
	F [(a)]	TFC[(b)]
Aspartic	1487.0	13300
Glutamic	1508.4	12640
Serine	833.2	7220
Histidine	133.9	1460
Glycine	815.6	6800
Threonine	797.8	6580
Arginine	486.6	6330
Alanine	1096.4	9400
Proline	0.7	4250
Tyrosine	435.2	6190
Valine	339.2	9320
Methionine	671.7	2620
Cystine	55.0	1850
Isoleucine	550.0	5010
Leucine	1034.5	8630
Phenylalanine	1245.4	2520
Lysine	723.3	6720

[a]Ortiz et al., (2006), [b]Yaich et al., (2015)

Various reports are available in determining the structural composition of *U. lactuca* polysaccharides and the composition is variable. In a chemico-enzymatic degradation study, rhamnose, glucose, galactose, xylose and glucuronic acid were the constitutive sugars detected upon complete hydrolysis. Chemical hydrolysis of ulvan (polysaccharides) showed about 2% (dry weight) of ulvanobiouronic acid (β-D-GlcA-(1, 4)-L-Rha). However, the additional hydrolysis by purified β-D-glucuronidase resulted in the degradation of ulvanobiouronic acid into glucuronic acid and rhamnose. So by this method of chemico-enzymatic analysis helped in estimating the acidic and neutral sugars, especially the iduronic acid in this sulfated polysaccharides (Quemener *et al.*, 1997).

Ulvan from *U. lactuca* was chemically composed of 47% total carbohydrates, 23.2% uronic acids, 29.9% ash, 17.1% sulfate groups and 1.0% N_2 (Pengzhan *et al.*, 2003). Gas chromaotographic (GC) analysis indicated the presence of main neutral sugars namely: glucose, rhamnose and xylose, with the smaller fractions of arabinose, galactose and mannose. Further analysis using ^{13}C-NMR (Nuclear Magnetic Resonance) and FTIR (Fourier Transform Infrared Spectroscopy) indicted that the repeating units are (α-L-IdopA-(1→ 4)-α-L-Rhap 3S) and (β-D-GlcpA-(1→ 4)-α-L-Rhap 3S) **(Figure 1).** By seaweed histology method (Alcian blue staining) sulfated polysaccharides presence was authenticated, while the chemical composition was found in the order of total sugar (65.4%), uronic acid (17.2%) and sulfate contents (17.4%) (Sathivel *et al.*, 2008). A rapid determination method of *U. lactuca* cell wall composition was reported using FTIR (Robic *et al.*, 2009). GC analysis showed that rhamnose was the main component of structure, while variable contents of glucose and fucose with traces of galactose, mannose and xylose were also observed (Mao *et al.*, 2006).



[→ 4)-β-D-Xyl 2S(1→ 4)-α-L-Rha3S(1→]

Figure 1: Structural repeating units of main disaccharide in *Ulva* sp. (Lahaye and Robic, 2007)

Antimicrobial activity

Pathogenic microbes are ubiquitous and are the main cause of human health problem issues. In medicine various preventive and curative interventions have been adopted. Development of synthetic and natural drugs is one of the critical way to treat the human health problems. With the passage of time there is an increasing interest in natural drugs which are thought to have the least side effects. Similarly, *U. lactuca* has been evaluated as a potent source of controlling the human pathogenic microorganisms.

U. lactuca extract was tested against some human pathogenic bacteria namely, Salmonella paratyphi, Pseudomonas aeruginosa, Vibrio cholera, Staphylococcus aureus, Shigella dysentriae and Klebsiella pneumonia. Extract activity against bacteria was tested using inhibition zone method. It was observed that 11.2 mm of inhibition zone for extract against Pseudomonas aeruginosa (Vallinayagam et al., 2009).

Table 3. Fatty acid composition of *U. lactuca*)

Minerals	Total Fatty Acids (%)		References
	a	b	
C14:0	7.6	1.14	[a]Rohani-Ghadikolaei et al. (2012); [b]Ortiz et al. (2006)
C16:0	50.7	14	
C18:0	2.8	8.39	
C20:0	0.8	0.19	
C22:0	3.8	0.27	
C24:0	0.6	9.45	
C16:1n-7	10.6	1.87	
C20:5n-3 (EPA)*	1.3	1.01	
C22:6n-3 (DHA)**	1.1	0.8	
C14:1n-5	0.9	-	[a]Rohani-Ghadikolaei et al. (2012)
C18:1n-9	11.5	-	
C20:1n-9	0.4	-	
C24:1n-9	0.5	-	
C18:3n-3	2.5	-	
C18:2n-6	3.5	-	
C20:2n-6	0.5	-	
C20:4n-6 (AA)***	1.1	-	
C20:1	-	4.21	[b]Ortiz et al. (2006)
C22:1n-9	-	0.79	
C20:4x6	-	0.34	
C18:4x3	-	0.41	

* eicosapentaenoic, **docosahexaenoic, ***arachidonic, - no results were found

Another *in vitro* analysis of *U. lactuca* extract was made against some Gram positive and negative bacteria, and a fungus (Kim *et al*., 2005). Bioactive components were extracted using Ethyl-ether; broad spectral activity was observed. Especially, a stronger activity was observed against *Staphylococcus aureus* that was resistant to methicillin.

Table 4. Mineral profile of *U. lactuca*

Minerals	Values (mg/100 g)		References
	a	b	
Iron	46.4	2.35	[a]Khairy and El-Sheikh (2015); [b]Rohani-Ghadikolaei et al. (2012)
Potassium	515.6	7.5	
Magnesium	79.1	14.6	
Zinc	1.6	0.17	
Copper	0.34	0.56	
Manganese	1.5	-	[a]Rohani-Ghadikolaei et al. (2012)
Cobalt	0.07	-	
Calcium	71.2	-	
Sodium	8.9	-	
Chromium	0.1	-	
Lead	0.04	-	

(-) no results were observed

Similarly, Tuney *et al*. (2006) also reported antimicrobial activity of fresh and dried *Ulva* sp. against some bacteria, yeast and fungus of pathogenic nature. Various solvents were nominated for extraction, and the disc diffusion method was adopted to test the antimicrobial activity of these fresh and dried extract (methanol, ethanol, acetone, diethyl ether). The fresh-algae extracts were more influential against pathogens of the current study compared to dried-algae extracts. Abd El - Baky *et al*. (2009) quantified the carotenoids, phenolics and total chlorophyll contents in *U. lactuca*. Extracts were tested for antibacterial activity for six bacteria where the minimal inhibitory concentrations (MIC) were ranged between 0.35-0.40 mg/mL.

Recent study revealed the seasonal variation of antimicrobial activity, which were extracted by eight types of solvents from *U. lactuca*, and were concentrated using column chromatography to enhance their potential against the tested microbes namely, *Bacillus subtilis, Staphylococcus aureus*, and methicillin-resistant *S.*

aureus (Tan *et al*., 2012). Likewise, Kandhasamy and Arunachalam (2008) also reported the *in vitro* antibacterial potential of various solvent-extracts of *U. lactuca*. Various model pathogenic bacteria (Gram positive and negative) were selected for study, and was found that the methanolic extract is more potent in inhibiting these pathogens. A bioactive steroid 3-O-B-D-glucopyranosyl clerosterol was extracted from *U. lactuca*. Antimicrobial activity was tested against (Awad, 2000) by disk diffusion method against 10 different microbes including, Gram positive and negative bacteria, yeast and a fungus. Significantly, positive results were found in this study strengthening the use of this green macro-algae in the production of natural antimicrobials (Table 5). .

Alghazeer *et al*. (2013) also attempted to evaluate the antibacterial activity of crude aqueous and methanolic extracts from *U. lactuca* obtained from the Libyan coast. Antibacterial potential of crude extract was tested against 8 pathogenic bacteria including, Gram positive (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus* spp.,) and negative (*Escherichia coli, Klebsiella* spp*., Pseudomonas aeruginosa* and *Salmonella typhi*). However, methanolic extract was observed better in some cases against these pathogenic bacteria. So, there are stronger evidences of developing natural products with antimicrobial capacity that can help in developing medicine of great interest.

Antiparasitic and larvicidal activity

Culex pipiens, a blood-sucking mosquito belong to the family *Culicidae*, is a vector of diseases, like Japanese encephalitis, meningitis and urticaria. Insecticidal properties of *U. lactuca* was evaluated using various organic solvents; acetone, chloroform, ethanol, methanol and petroleum ether). Acetonic extract was found to be the best against the inhibition of the mosquito population by killing the larvae of the *C. pipiens* (Abbassy *et al*., 2014). In accordance, a study was designed to test the antiprotozoal effects of crude extract of *U. lactuca* (Spavieri *et al*., 2010). Model protozoa used in this *in vitro* assay were *Leishmania donovani*, *Trypanosoma cruzi*, and *Trypanosoma brucei rhodesiense*. The crude extarcts' cytotoxicity was assessed using cells from mammalian skeletal myoblast (L6). *Ulva* extract indicated only a moderate (IC_{50} = 34.9 µg/ml) antiprotozoal (specifically trypanocidal) activity against *Trypanosoma cruzi* but good leishmanicidal activity (IC_{50} = 12 µg/ml). While there was no proof of cytotoxicity for L6 myoblast cells, confirming that the antiprotozoal activity of *U. lactuca* is very specific.

Antiviral property

In humans, acute encephalitis is mainly caused by Japanese encephalitis virus. The symptoms of the attack involves some inflammatory reactions and neurological problems. Ulvan a sulfated polysaccharide extracted from *U. lactuca* was found to have antiviral activity. Chiu *et al*. (2012) described the activity of *U. lactuca* extract, which could inhibit Japanese encephalitis virus infection in *Vero* cells by blocking the viral adsorption and entry. Additionally, the ulvan reduced the pro-inflammatory cytokines production in virus-infected primary mixed neuroglia cells. According to *in vivo* model, C3H/HeN mice were infected by virus. The mice presented some abnormal neuro-behavioral symptoms and were died within seven days of infection, while the mice pretreated with ulvan polysaccharides were remained resistant to paralysis and death, suggesting the possible antiviral potentials of the extract.

Antioxidant potential

Antioxidants are very important in regulating the oxidative stress related diseases in human. In this instance marine green algae have been explored and found to possess a broad spectrum of strong antioxidant activity. Qi *et al*. (2005) evaluated the native and modified ulvan's antioxidant activity. Ulvan are the sulfated hetero-polysaccharides obtained from *Ulva* sp. of Chlorophyta. Sulfate contents of ulvan was modified (19.5-32.8%) using sulfur trioxide/N, N-dimethyl formamide. Antioxidant power was assessed in terms of scavenging activity of hydroxyl and superoxide radicals, reducing power assay and metal chelating potential. All the studied parameters of antioxidant characterization were better indicted after improving the sulfate groups, i.e. better scavenging activity, stronger reducing power and more pronounced chelating ability. It was suggested that the natural ulvan already have sulfates but by increasing the number of sulfate radicals in the polymers stronger antioxidant potential was observed.

Another study was conducted by Qi *et al*. (2006) to authenticate *in vitro* antioxidant potential of natural and chemical derived (acetylated and benzoylated) ulvans. Various antioxidant assays, like scavenging activity (against superoxide and hydroxyl radicals), chelating ability and reducing power, were adopted to confirm the antioxidant potential. It was observed that the chemically modified ulvan presented stronger antioxindant ability compared to native ulvan. Similarly, is a study Qi *et al*. (2010) determining the antioxidant potential of polysaccharides extracted from *Ulva*. The extracted fractions indicated significant inhibitory effect on hydroxyl and superoxide radicals' activity. The half maximal inhibitory (IC_{50}) value were 2.8 and 22.1 µg mL^{-1}, respectively. Abd El - Baky *et al*. (2009) quantified the carotenoids, phenolics and total chlorophyll contents in *U. lactuca*, and their antioxidant activities very tested. HPLC (High Performance Liquid Chromatography) and

TLC (Thin Layer Chromatography) of organic extract indicated the presence of 34 bioactive compounds. Major fractions were: chlorophyll-a (15.60-30.90%), chlorophyll-b (12.20-14.89 %), α-carotene (11.44-11.47 %), cis β-carotene (13.12-14.47%), and all-trans β-carotene (6.16-29.70%). Remarkable antioxidant potential, with IC_{50} (in terms of DPPH radical scavenging activity) ranging from 16.5-18.7 µg/mL. Comparing to this IC_{50}, the synthetic antioxidants including α-tocopherol, butylated hydroxyanisol (BHA) and butylated hydroxyltoluene (BHT) presented 14.4, 13.1 and 13.1 µg/mL, respectively. Khairy and El-Sheikh (2015) reported the seasonal effect on antioxidant activity of *U. lactuca*. β-carotene, DPPH (2,2-diphenyl-1-picrylhydrazyl) and total phenolic contents were taken into consideration for estimating the antioxidant potential. In summer season *U. lactuca* presented the maximum free radical scavenging activity. These studies suggest *U. lactuca* extract a stronger potential antioxidant agent for food preservation.

Table 5: Some studies related the biological activity of *Ulva* sp.

Biological activity	References
Antioxidant	Hassan et al. (2011); Khairy and El-Sheikh (2015); Qi et al. (2010); Qi et al. (2005); Qi et al. (2006)
Anticoagulant	Mao et al. (2006)
Antibacterial	Alghazeer et al. (2013); Awad (2000); Kandhasamy and Arunachalam (2008); Kim et al. (2005); Tan et al. (2012); Tuney et al. (2006); Vallinayagam et al. (2009)
Anti-inflammatory	Awad (2000); Margret et al. (2009)
Antitumor	Lee et al. (2004)
Anti-hyperlipidemic	Pengzhan et al. (2003)
Hypocholesterolemic	Hassan et al. (2011)
Hepatoprotective	Devaki et al. (2009); Sathivel et al. (2008)
Cytotoxic	Alves et al. (2013)
Antifungal	Kim et al. (2005); Tuney et al. (2006)
Antiviral	Chiu et al. (2012)
Anti-parasitic	Spavieri et al. (2010)
Insecticidal	Abbassy et al. (2014)

Anti-inflammatory activity

Various drugs are present in pharmaceutical market having the anti-inflammatory activities. Neutropenia is a common side effect of chemotherapeutic drugs. Moreover, drug administration result injury to blood forming cells and other cells that are essential for maintenance of the defense mechanism resulting the enhanced chances of tumor metastasis. Immune cells like macrophages that play major critical role in immunity by regulating the phagocytosis or by producing cytokines (Robertson and Ritz, 1990). Recently, nominal chemical compounds have been identified and extracted or isolated for various biologically activities. Awad (2000) isolated a biologically active steroid (3-O-β-D-glucopyranosyl clerosterol) from *U. lactuca*. The isolated extract was tested against anti-inflammatory assay namely, mouse ear oedema, where the phorbol derivatives were used to induce oedema (5 mg/ ear). A significant ($p < 0.05$) reduction in oedema (62.2 and 72.2%) at the doses of 1000 and 1500 mg/ear indicated this extract could be a promising anti-inflammatory agent. Similarly, green macroalgae *U. lactuca* was tested in rat model to protect the oedema by reducing the inflammation. Moreover, the elite organs were examined microscopically, and no alteration was seen in the positive control and treated ones. Additionally, the hematological assays were found normal, suggesting that the anti-inflammatory properties of the natural marine sources should be taken into consideration (Margret *et al*., 2009).

Antitumor activity

The term tumor and cancer are mostly used interchangeably but not necessarily the same. Tumor refers to a mass and it has two main types, benign and malignant tumors. In cancer the tumors are second type and are dangerous but malignant are sometimes mimicked by benign and are treated accordingly. Lee *et al*. (2004) evaluated the methonolic extract of *U. lactuca* in regulating the tumor genesis. Water soluble portion of extract was concentrated up to 140µg/ml and human leukemia cells (U 937) were treated). Interestingly, 50% growth inhibition was observed after treatment. On the other hand stimulated growth of splenocytes was observed at a concentration of 100µg/ml. Moreover, the nitric oxide production was improved by macrophage cell lineage (RAW 264.7), as nitric oxide is also thought to mediate cytokines functionality. Similarly, in mouse splenocytes, alkaline phosphatase activity was stimulated with a concentration of 10µg/ml of the water soluble fraction. In three cell lines, dose dependent response was observed, indicating a useful natural immune-stimulating and antitumor agent. In a study, cytotoxicity of ulvan extracted from green algae *U. lactuca* was assessed *in vitro* (Alves *et al*., 2013b). Fibroblast-like cells (Mouse C3H/An, L929 cells) were incubated in the presence of ulvan and their viability was evaluated by tetrazolium test. Moreover, to assess the cell number, total protein and double stranded DNA were quantified. Hyaluronic acid (HA) was compared as a non-cytotoxic control in terms of cell number and cellular metabolic activity (MTS assay). The results suggested that ulvan (polysaccharide) a non-cytotoxic and biocompatible, within the given test conditions.

Anti-hyperlipidemic activity

Hyperlipidemia is considered one of the risk factor in cardiovascular diseases. Hot water extraction was made to get ulvan from *Ulva* sp. and precipitated using ethanol. A group of 50 ICR (a kind of albino mice) were treated with ulvan by oral feeding and their plasma lipid level was noticed. For comparison inositol niacinate was used as positive control. The mice treated with ulvan dose were significantly ($p<0.01$) lower in the total cholesterol, low-density lipoprotein and triglycerides, while an increase in serum high-density lipoproteins was observed. Additionally, the ulvan feed also lowered the atherogenic index in plasma, suggesting ulvan a potential protector against cerebrovascular and ischemic cardiovascular diseases (Pengzhan *et al*., 2003).

Hypercholesterolaemia (dyslipidemia) is the presence of elevated levels of blood cholesterol. It is also a somewhat miscellaneously tested as a possible risk factor for cardiac ailments. Hassan *et al*. (2011) also studied the *U. lactuca* ulvan's (sulfated polysaccharides) anti-hypercholesterolemic potential. Albino rats were used as *in vivo* model and were fed with ethnolic-precipitated polysaccharides for 21 days. Control group was treated with a reference drug named as Lapitor (Atorvastatine Ca). Ulvan treated rats were found lower in serum total lipids and triglycerides, total cholesterol, LDL (low-density lipoprotien) and VLDL (very low density lipoprotein) levels. Improved level of atherogenic index, HDL (high density lipoprotein) (180% rise), creatine kinase and lactate dehydrogenase enzymes' activities were also observed. In addition, improved liver enzymatic (glutathione peroxidase, superoxide dismutase and catalase) activities was seen. On other hand, reduced glutathione and total thiol was also observed, suggesting ulvan as a potent agent against induced hypercholesterolemic conditions.

Hepatoprotective effects

Hepatitis is one of the major problems of liver which disturbs its normal functioning. It could be induced by some microorganisms, or viruses or by chemical intoxicants. *U. lactuca* polysaccharide extract was tested against a chemo-induced hepatitis in rats where Galactosamine was used @ 500 mg/kg body weight (Sathivel *et al*., 2008). Spectophotometric analysis was conducted for the following parameters: free fatty acid (FFA), triglycerides (TG), phospholipids (PL), serum total cholesterol (TC), HDL, LDL, VLDL, hepatic protein thiols, tissue lipoperoxides (LPO) and anti-oxidants (glutathione (GSH) and vitamins (C, E). Electron microscopy (EM) was used to track the ultrastructural alterations in liver tissues after treating with galactosamine and ulvan. Liver damage in galactosamine induced hepatitis was observed by significant changes in serum lipid profile, hepatic protein thiols contents and non-enzymatic anti-oxidants of tissues. Moreover, lipid deposits in the form of assorted droplets and deformation in mitochondria was observed through EM. The rats, pretreated with ulvan for three weeks (30mg/kg body weight) indicated a significant ($p<0.05$) retardation in any type of abnormality and provided the liver protection possibly by exhibiting a free-radical quenching activity. In line to the above study, Devaki *et al*. (2009) also conducted an experiment of the similar nature but the ulvan dose was increased to 200 and 500 mg/kg body weight. However, the dose of 500mg/kg of body weight was given intraperitoneally, and the changes in liver microsomal and mitochondrial fractions were noticed based on the urea cycle, microsomal enzymes and trichloro-acetic acid (TCA) status. Ultra-structural anomalies in mitochondria (swelling, loss in mitochondrial structural cristae) and microsomes were seen. In addition, altered functionality of energy-metabolism enzymes was also a consequence. Ulvan treated rats were observed to be saved from any abnormality of the microsomal enzymes and TCA, and less severe changes in mitochondria were found. In case of urea cycle enzymes, no effective prevention was seen in the pretreated rats. It was suggested that the presence of sulfated polysaccharides might have protected the functionality of microsomal membrane and mitochondria that normalize the galactosamine induced oxidative stress.

Anticoagulant property

Anticoagulants are especially used in medication to prevent the clotting of blood. Many oral coagulants are available these days. Various anticoagulant substances are also used in blood sampling equipment. Since the start of 21st century numerous new agents have been recognized and introduced, and referred as the directly acting oral anticoagulants. A similar attempt was made by Mao *et al*. (2006), and found that the anti-coagulant property of the purified polysaccharides from *Ulva* sp. Hot water extraction and ethanol was used in extraction process while for purification ion exchange and HPLC were used. Anticoagulant properties were tested *in vitro* by determining the thromboplastin-activation time of human plasma, and was compared with heparin. A considerable anticoagulant activity was observed. It was suggested that sulfated polysaccharides inhibited the thrombin activity directly and also by the potentiation of heparin cofactor II (HCII).

Biomedical applications

Considering the advantages of cheaper origin, abundant availability and low toxicity with cytocompatibility suggests to broaden the ulvan's applications. Other than antioxidant, anti-inflammation, immune-modulation, antitumor and anticoagulant potential, attempts have been made to extrapolate ulvans in biomedical applications (Table 6). In this respect Toskas *et al*. (2011) first fabricated the electrospun nanofibers from ulvan obtained from *Ulva*. Blends were prepared by various ratios of ulvan: polyvinyl alcohol (PVA), and the composites were electrospun. The least diameter observed was 84 nm. Nanofibers were highly ordered and crystalline structures as observed by transmission electron microscopy (TEM). A non-woven membrane was used to deposit these nanofibers without any type of interconnections. It was observed that at lower viscosity the spinnability of ulvan solution was much improved, as seen by rheological and scanning electron microscopy (SEM). IR analysis (infrared) suggested that ulvan are in compatibility with the polyvinyl alcohol (PVA) chains. It was suggested the in this new type of fibres, the integration of ulvan and PVA was resulted by ionic assembly in the presence of divalent cations and borate esters. So, this ordered crystalline structure of interesting physicochemical and biological properties could be used in drug delivery and wound dressings etc. In another study nanofibrous membrane for osteoblasts cultivation was developed. In this membrane the ulvans were complexed with chitosan polyelectrolytes. Three model membranes were developed using ulvan and chitosan alone and in combination in presence of some polyelectrolytes. Oppositely charged backbones of the two polymers resulted in supramolecular structural formation through electrostatic forces. It was noticed that the membrane porosity was alteration with changing the polyelectrolytic ions and by varying the ratio of both the mixing molecules. FTIR analysis authenticated the presence of both polysaccharides in the membranes. Membranes cytocompatibility was evaluated by fluorescence microscopic technique. Murine osteoblast-like cell (7F2) line was tested for attachment and proliferation on the membranes. It was found that ulvan and ulvan: chitosan membranes supported the osteoblasts' attachment and replication without altering the morphology and viability of cells. Better attachment of osteoblasts was hypothesized due to the mimicking of these nanofibrous constructs to that of extracellular matrix like structures (Toskas *et al*., 2012).

In another study, 2-D membranes of ulvan (sulfated polysaccharides) were tested for possible use in drug delivery (Alves *et al*., 2013b). Crosslinked water-insoluble membranes were made using 1,4-Butanediol diglycidyl ether. The crosslinking success of ulvan and 1,4-Butanediol diglycidyl ether was authenticated using FTIR. Membranes mechanical properties were investigated by tensile testing (quasi-static model). Hydration behavior of membranes was tested using swelling characteristics. Crosslinked ulvans' presented a remarkable water uptake capacity (~1800% to initial dry weight) and mechanical strength (1.76 MPa). Using the dexamethasone as a model drug, an initial steady drug release (of 49%) was found followed by a slower but sustained release until two weeks. Ulvan crosslinked membranes designed to use as wound dressings in medicated wound management, can also demonstrate a potential in therapeutic agents delivery devices. Likewise, ulvan based scaffold was made for bone tissue engineering (Alves *et al*., 2013b). Three dimensional scaffold was produced using poly-(D,L)-lactide (PDLLA) loaded with ulvan particles, that were prepared through sub-critical fluid sintering using carbon dioxide gas at temperature and pressure of 40°C and 50 bar, respectively. The prepared matrix was tested for suitability of being a successful scaffold in bone tissue regeneration. Mechanical compression testing, degradation water uptake, micro-computed tomography and cytotoxicity potential assay were used to characterize the 3-D structure. A model drug (dexamethasone) was applied on ulvan particles, and the particles were dispersed in PDLLA matrix. Then localized sustained drug release was evaluated. It was found that the scaffolds possess suitable morphometric features, with appropriate mechanical performance and cytocompatibility. Furthermore, the sustained release of dexamethasone loaded ulvan particles embedded within the PDLLA matrix may enhance the applicability of this 3-D model scaffold for localized delivery of relevant bioactive mediators. The option is still there to extrapolate the applications and use of this natural polysaccharides with other biomaterials to enhance the value and potentiated application in unexplored field of study.

Table 6. Biomedical applications of ulvan extracted from *Ulva* sp.

Constructs	Processing technique	Reference
Nanofibers	Electrospinning of ulvan and PVA	Toskas et al. (2011)
3-D scaffold	Freeze dried ulvan crosslinked 1,4-butanediol diglycidyl ether	Alves et al. (2012a)
Particles	Extrusion dipping method	Alves et al. (2012b)
Membranes	Ulvan crosslinked 1,4-butanediol diglycidyl ether	Alves et al. (2013)

Miscellaneous

Heavy metal pollution is a major concern of the industrialized world where water is considered a one of the major source of these pollutants. Various synthetic and natural sorbents, and filter materials have been tested for removal of this type of toxicants. Like other natural sources, *Ulva* sp. based biosorbent was tested for its suitability. Freeze-dried mass of thalli and cell walls of *Ulva* sp. was evaluated (Webster *et al*., 1997). It was found that the adsorption of Cd, Co, Cu and Zn is concentration dependent, and it followed Freundlich and Langmuir adsorption-isotherm model. Various adsorption models suggested different binding affinities for various metals in the study. NMR experiments for Cd indicated two binding sites availability on the powdered thallus, while only one on cell walls. It was suggested that the oxygen containing functional groups (OH, SO_4) in *Ulva* powder are responsible for metal binding ability. To test the metal absorption behaviour of *U. lactuca,* fresh and dehydrated samples were placed in sodium chloride solutions (0.01-5.0 M) (Schijf and Ebling, 2010). To estimate the stability of acid functional groups manual titration was done and the P*ka* values were found. Acid functional groups were stable at the tested range of ionic strength. The better stability of these functional groups advocates the utility of seaweed as biosorbent and biomonitor not just for seawater, but for freshwaters to brines. Additionally, highly acidic esters sulfate of *U. lactuca*, might also play a part in adsorption of trace metals.

Seaweed biofilters have already been tested and found useful in treating the fishpond effluents. To extend its utility, Neori *et al*. (2003) tested the efficiency of *U. lactuca* by taking into consideration a three-stage filter design. The fresh *U. lactuca* was stocked at a density of 1 kg/m^2 in the pond. Total ammonia nitrogen (TAN) was efficiently removed (85-90%), with production of high protein by *U. lactuca* (44% dry weight) in all the three stages. It was suggested that the three-stage design could provide a significant functional and economic benefits in biofiltration of fishpond water.

Ulva sp. has found much higher in biomass production compared to terrestrial crops. Higher photosynthetic efficiency of this weed is found much higher (45 T ha^{-1} y^{-1}). Anaerobic digestion of wet biomass for methane production deemed more promising, suggesting the eco-sustainable and eco-friendly production of bioenergy (Bruhn *et al*., 2011). To test the possible feed alternative of *U. lactuca* meal was added in the diet of African catfish (*Clarias gariepinus*) (Abdel-Warith *et al*., 2015) and body composition, growth performance, feed utilization were tested. Control feed was found similar to 10% *Ulva* meal in terms of body weight, growth rate and feed utilization, indicating a possible substitute for other feeds.

Conclusion

Promising potential applications of *Ulva* sp. with reference to *U. lactuca* has been discussed. Like other green algae, *U. lactuca* is also a rich source of many essential bioactive fractions ranging from antioxidants and essential minerals to the highly signified and complete profiles of amino acids and fatty acids. Other than that, higher level of soluble dietary fibres make it a potential source for healthy foods and medicinal implications. The polysaccharides (ulvan) of this species have been tested for drug release, wound dressing to tissue engineering in composite with other natural or synthetic polymers. Interestingly, ulvan based structures were found cytocompatible and non-toxic in nature. So, sustainable and wise valorization of marine resources presents a highly fascinating platform for developing novel biomaterials, which are economical and eco-friendly. In line with the current applications, further considerations should be given to develop some nutraceutical supplements.

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