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# The Marine Macroalgae of the Genus *Ulva*: Chemistry, Biological Activities and Potential Applications

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## Abstract

This review summarizes a literature survey of the marine macroalgae of the genus *Ulva* (Phylum Chlorophyta), covering the period of 1985 to 2012. The secondary metabolites isolated from members of this genus and biological activities of the organic extracts of some *Ulva* species as well as of the isolated metabolites are discussed. The emphasis on their application in food industry and their potential uses as biofilters are also addressed.

**Keywords:** Chlorophyta; *Ulva;* Macroalgae; Secondary metabolites; Biological activities; Food; Biofilters

## Introduction

The genus *Ulva* (Phylum Chlorophyta, Class Ulvophyceae, Order Ulvales, Family Ulvaceae) was first identified by Linnaeus in 1753 [1]. Since then many taxonomists and phycologists have been involved in the identification of *Ulva* species [2] which are notoriously difficult to classify due to the morphological plasticity expressed by many members as well as the few reliable characters available for differentiating taxa [2,3]. Its morphology resembles bright green sheets [4], heavily influenced by environmental conditions, age of the thallus, and life style, making difficult the delineation of species by morphological features alone [2]. There is an important relationship between morphological features of *Ulva* and salinity and nutrient concentrations in these freshwater habitats; however, the most essential physical factor positively correlated with mats development was water depth [3,5].

At present, there are 562 species names in the Algaebase and 99 of which have been flagged as currently accepted taxonomically [6]. These organisms have a potential for rapid and proliferous growth [3] with a ubiquitous distribution with species living in a wide range of habitats and environments [2,3,5,7]. Although *Ulva* species are primarily marine taxa found in saline and salty waters, they can also proliferate in freshwater habitats [6].

## Secondary Metabolites

Although the members of the phylum Chlorophyta are abundant and relatively easy to collect, their chemistry continues to be underrepresented [8]. Among all macroalgae, the green algae with less than 300 known compounds are the least producers of natural compounds when compared to the red (Rhodophyta) and brown algae (Phaeophyta) [9,10]. Anyhow, a wide range of compounds, predominantly terpenes, polyphenols and steroids, have been reported in various marine green algae [11,12]. The chemical composition of these macroalgae was found to vary depending on geographical distribution and seasons and the principal environmental factors affecting the composition being water temperature, salinity, light, nutrients and minerals availability [5].

The samples of *U. lactuca*, collected from different geographical locations, were investigated for their chemical constituents. By using gas chromatography coupled to mass spectrometry (GC-MS), Flodin and Whitfield were able to detect 2,4,6-tribromophenol (1) (Figure 1) from the crude extract of *U. lactuca* [13], collected in Turimetta Head, North of Sydney, on the Eastern coast of Australia. Later, 3-*O*-β-D-glucopyranosylstigmasta-5,25-diene (**2**) (Figure 1) was isolated from the methanol extract of *U. lactuca*, collected from the coast of Alexandria, Egypt [12,14,15]. Using High Performance Liquid Chromatography (HPLC), the Chinese group was able to isolate five *nor*-isoprenoids: (3*S*,5*R*,6*S*,7*E*) 3,5,6-trihydroxy-7-megastigmen-9-one (**3**), (3*R*,5*R*,6*R*,7*E*) 3,5,6-trihydroxy-7-megastigmen-9-one (4), (3*S*,6*R*) 3,6-dihydroxy-4,7-megastigmadien-9-one (**5**), grasshopper ketone (**6**), and isololiolide (**7**) (Figure 1), from the methanol extract of *U. lactuca*, collected off the coast of Bohai in China [16].

This has also happened to *U. fasciata*, a derivative of sphingosine (2-amino-4-octadecene-1,3-diol) and *N*-palmitoyl-2-amino-1,3,4,5-tetrahydroxy-octadecane (8) (Figure 2) were reported from *U. fasciata,* collected in India [15]. The extract of this alga, collected in Asbu-Qir, Egypt, was also found to contain three unsaturated fatty acids: 8-hydroxy-9-decenoic acid (9), 3,3-diol-4-decenoic acid (10), and 3-oxo-4-decenoic acid (11) [8] (Figure 2).

From the dichloromethane extract of *U. fasciata* Delile, collected in the intertidal zone in Vizhinjam harbor - Kerala at the Southwest coast of India, seven diterpenes: labda-14-ene-8-ol (12), labda-14-ene-3α,8α-diol (13), labda-14-ene-8α-9α-diol (14), labda-14-ene-8α-hydroxy-3-one (15), *ent*-labda-13(16)14-diene-3-one (16), *ent*-labda-13(16),14-diene (17), and *ent*-labda-13(16),14-diene-3α-ol (18) (Figure 3) were isolated and purified by column chromatography and Thin Layer Chromatography (TLC) [11].

Additionally, guai-2-en-10α-methanol (19) and guai-2-en-10α-ol (20) (Figure 4) were isolated and identified from the methanol extract

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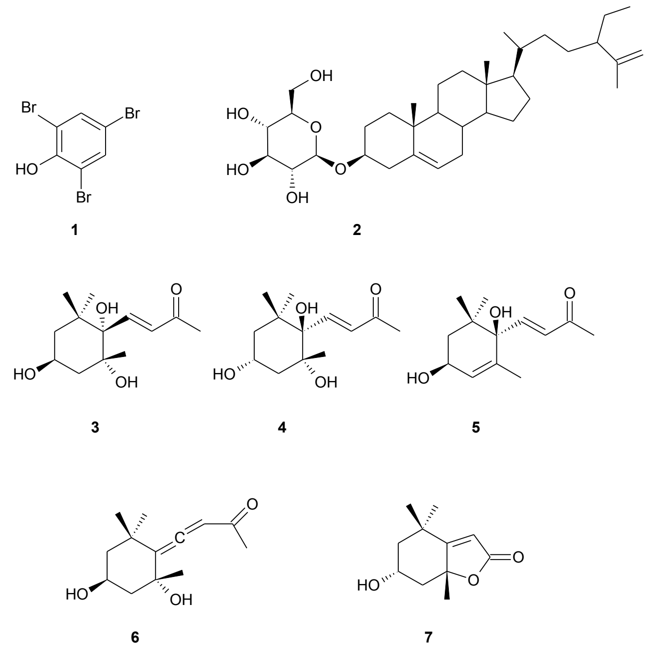


**Figure 1:** Structures of 2,4,6-tribromophenol (1), 3-*O*-*β*-D-glucopyranosylstigmasta-5,25-diene (2), (3*S*,5*R*,6*S*,7*E*) 3,5,6-trihydroxy-7-megastigmen-9-one (3), (3*R*,5*R*,6*R*,7*E*) 3,5,6-trihydroxy-7-megastigmen-9-one (4), (3*S*,6*R*) 3,6-dihydroxy-4,7-megastigmadien-9-one (5), grasshopper ketone (6), and isololiolide (7).

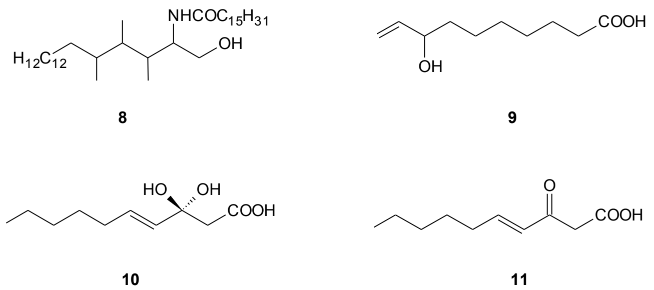


**Figure 2:** Structures of *N*-palmitoyl-2-amino-1,3,4,5-tetrahydroxyoctadecane (8), 8-hydroxy-9-decenoic acid (9), 3,3-diol-4-decenoic acid (10), and 3-oxo-4-decenoic acid (11).

of *U. fasciata* Delile, collected in Vizhinjam and Mullur in Kerala, India [12]. From the methanol extract of the same species collected in the same local, Chakraborty and Paulraj have reported isolation of five sesquiterpenes: 2,5,5-trimethyl-4-(4´-methyl-3´-pentenyl)-2-cyclohexen-1-ol (21), 4-isopentyl-3,4,5,5-tetramethyl-2-cyclohexen-1-ol (22, two diastereoisomeric compounds), 6-isopentyl-1,5,5,6-tetramethyl-1-cyclohexene (23), and 3,4,5,5-tetramethyl-4-(3´-oxopentyl)-2-cyclohexen-1-one (24) (Figure 4) [17].

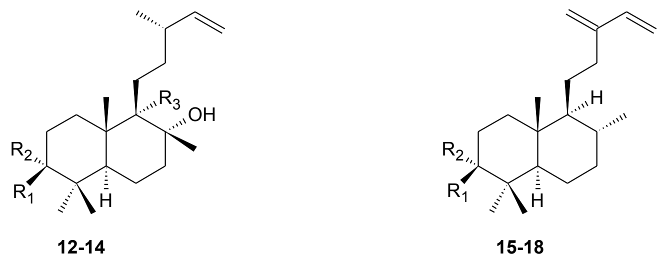
The only report on chemical constituents of *U. pertusa* by Garson described isolation of two fatty acids: 2(*R*)-hydroxy-hexadecanoic acid (25), and 2-oxo-hexadecanoic acid (26) [18] (Figure 5).

Yildiz et al., reported isolation of gallic acid (27) from the methanol extract of *U. rigida,* collected at the Marmara Sea-Turkey [19]. Additionally, 24-*nor*-22-dehydrocholesterol (28) [20] and isofucosterol (29) [21] were also isolated from this specie (Figure 6).

Roussis et al. have investigated the production and release of volatile metabolites from *U. rigida* during light and darkness, under natural conditions and after the biological death of the plant [22]. Using GC and GC-MS methods to analyze the chemical constituents released from live *U. rigida* to the atmosphere, they have found that oxygenated metabolites, mainly aldehydes and alcohols, were the major components and aliphatic and aromatic hydrocarbons were present in significant amounts; however vestigial quantities of the halogenated and sulfated compounds were also detected. In order to determine the chemical load of the volatile compounds that would be liberated in the atmosphere at the end of the biological cycle, the volatile oil obtained from steam distillation of *U. rigida* was analyzed by GC-MS. The results showed that dimethyl sulfide, acetaldehyde and dichloromethane were the major compounds. Furthermore, dichloromethane was found to be a major metabolite during the light period [22].

## Biological Activities

In the recent years, the importance of marine algae as a supply for new bioactive substances has been growing very rapidly [15,23-27] owing to their capacity to produce metabolites that exhibit various biological activities such as antibacterial, anti-inflammatory, antiproliferative,



| | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|
| $R_1$ | H | OH | H | =O | =O | H | H |
| $R_2$ | H | H | H | - | - | H | OH |
| $R_3$ | H | H | OH | - | - | - | - |

**Figure 3:** Structures of labda-14-ene-8-ol (12), labda-14-ene-3α,8α-diol (13), labda-14-ene-8α-9α-diol (14), labda-14-ene-8α-hydroxy-3-one (15), ent-labda-13(16)14-diene-3-one (16), ent-labda-13(16),14-diene (17), and ent-labda-13(16),14-diene-3α-ol (18).

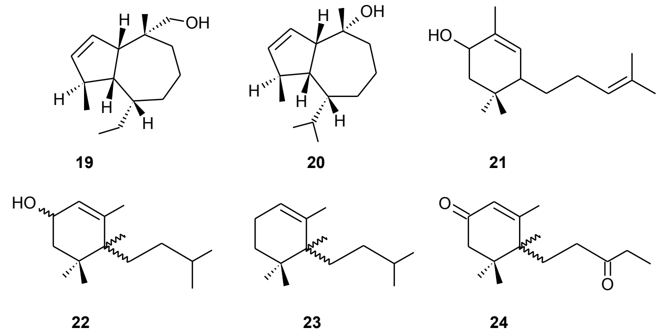
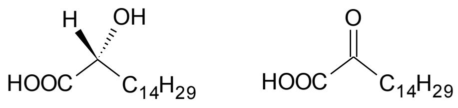
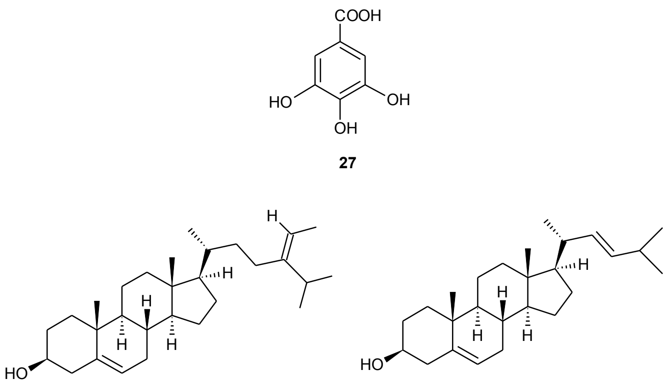


**Figure 4:** Structures of guai-2-en-10α-methanol (19) and guai-2-en-10α-ol (20), 2,5,5–trimethyl-4-(4´-methyl-3´-pentenyl)-2-cyclohexen-1-ol (21), 4-isopentyl-3,4,5,5-tetramethyl-2-cyclohexen-1-ol (22, two diastereoisomeric compounds), 6-isopentyl-1,5,5,6-tetramethyl-1-cyclohexene (23), and 3,4,5,5-tetramethyl-4-(3´-oxopentyl)-2-cyclohexen-1-one (24).



**25** **26**

**Figure 5:** Structures of 2(*R*)-hydroxy-hexadecanoic acid (25) and 2-oxo-hexadecanoic acid (26).



**27** **28** **29**

**Figure 6:** Structures of gallic acid (27), 24-*nor*-22-dehydrocholesterol (28) and isofucosterol (29).

antiviral [28,29], antifungal [27], antineoplastic [28], anticancer, anti-obesity, antidiabetic, antihypertensive, antihyperlipidemic, and antioxidant [30]. Although not many members of the genus *Ulva* have been extensively investigated, there are some interesting aspects of biological activities of the extracts and isolated metabolites. Table 1 lists biological activities of extracts and the secondary metabolites isolated from some *Ulva* species.

It is important to mention also that ulvan, a major Sulfated Polysaccharide (SP) found in the cell wall of green algae, composed mainly of rhamnose, glucuronic acid, iduronic acid, xylose, glucose, sulphate with small amounts of iduronic acid and traces of galactose and represents 8-29% of the algae dry weight [25,26,44-51]. The presence of the sulfate groups and the unusual chemical composition and structure of ulvan render it different biological properties [26,52,53]. This complex SP can also be of potential interest for food, pharmaceutical and agricultural applications [26,51-54]. In the recent years, ulvan has been extensively investigated for development of novel drugs and functional foods [26,49,53]. Because of its peculiar composition and structure [50], it represents a potential source of new functional biopolymer and it is widely used as biomaterial for tissue engineering in regenerative medicine. Furthermore, studies showed that sulfated polysaccharides can also exhibit beneficial biological activities such as anticoagulant, antiviral, antioxidant, anti-inflammatory [54] and antiproliferative [27]. As attention has been focused on natural antioxidants in the past years, since synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) were found to implicate in liver damage and carcinogenesis, several natural SPs, including ulvan, were evaluated for their antioxidant activity. Interestingly, ulvan from *U. pertusa* as well as acetylated and benzoylated ulvans were found to have antioxidant activity including scavenging activity against superoxide and hydroxyl radicals, reducing power and chelating ability [55]. Qi et al., in their study of the antioxidant activities of different molecular weight ulvans from *U. pertusa* (Chlorophyceae), obtained by $H_2O_2$ degradation, have found that low molecular weight ulvans exhibited a strong antioxidant activity, and their rationale for this was that low molecular weight SPs may incorporate into the cells more efficiently and donate proton more effectively when compared to high molecular weight SPs [56]. Ulvan, from *U. pertusa,* was also found to be a potential antihyperlipidemic agent and had significantly reduced serum Triglyceride (TG), total and Low Density Lipoprotein Cholesterol (LDL-cholesterol) and elevated High Density Lipoprotein Cholesterol (HDL-cholesterol) in mice [26]. The antihyperlipidemic activity of ulvan was found to depend on the molecular weight of ulvan fractions; high molecular weight fraction was more effective on total serum and LDL-cholesterol whereas low molecular weight fractions were more effective on TG and HDL-cholesterol [26,57].

Toskas et al. were able to fabricate defect free nanofibers by electrospinning ulvan and Polyvinyl Alcohol (PVA) solutions with appropriate ulvan to PVA mass ratios and polymer concentrations [58]. The spinnability of ulvan, in combination with its interesting biological and physicochemical properties, can lead to new biomedical applications such as drug release system. Recently, Toskas et al. have also succeeded in synthesizing ulvan/chitosan polyelectrolyte membrane which can promote the attachment and proliferation of 7F2 osteoblasts and maintain the cell morphology and viability. This new generation of biomaterials composed of ulvan and chitosan might have high impact in biomedical applications as potential scaffold materials [59].

Another class of secondary metabolites of green algae is sterols. Sterols from green algae have been reported to be non-toxic and capable of reducing blood cholesterol level and were found to be able to reduce the tendency to form a greasy liver and excessive fat deposition in the heart [60].

Finally, it is worth mentioning green algae as an important source of Polyunsaturated Fatty Acids (PUFAs). Due to nutritional values and beneficial effects of PUFAs, many researchers have investigated the suitability of using macroalgae as novel dietary sources of PUFAs. Pereira et al. have detected higher percentages (16%) of α-Linolenic Acid (ALA), in comparison to Linoleic Acid (LA), in *Ulva* sp., collected at the Algarve coast, Portugal [61]. Moreover, PUFAs can be considered also as having an important ecological role. Alamsjah et al. have found that Hexadeca-4, 7, 10, 13-Tetraenoic Acid (HDTA), Octadeca-6,9,12,15-Tetraenoic Acid (ODTA), and α-Linolenic Acid, isolated from the methanol extract of *U. fasciata* by bioassay-guided fractionation, exhibited a potent algicidal activity against the red-tide phytoplankton *Heterosigma akashiwo* (with $LC_{50}$ 1.35 μg/ml, 0.83 μg/ml and 1.13 μg/ml, respectively) and this result demonstrated the potential of these PUFAs for practical harmful algal bloom control [62].

## Use of *Ulva spp.* as Food

Although algae are known to contain a multitude of bioactive compounds, the interest in these organisms is also due to their nutritional proprieties [30,31,63]. Edible marine algae, sometimes referred as seaweeds, have attracted a special interest as good sources of nutrients [26] since they are an excellent source of vitamins, carbohydrates, dietary fiber and minerals [14,31,64,65] that could be potentially exploited as functional ingredient for both human and animal health [23,25]. Interestingly, many types of seaweeds used as traditional food in many cultures are known not only to cure various diseases but also to maintain health and were found to have immunomodulating and antitumor activities [36].

| Species | Extracts or Metabolites | Biological activities | References |
|---|---|---|---|
| ***U. compressa*** | Methanol | Antioxidant | [31] |
| ***U. conglobata*** | Sulfated polysaccharides | Anticoagulant | [32] |
| ***U. lactuca*** | Dichloromethane/methanol | Antimicrobial | [33] |
| | Ethanol | Antimicrobial, antiparasitic | [34,35] |
| | Dichloromethane/methanol | Antioxidant | [33] |
| | Methanol | Antitumor, antimicrobial, antiviral, and immune stimulating | [36] |
| | Sulfated polysaccharide | Inhibits Japanese Encephalitis Virus (JEV) | [37] |
| | 3-*O*-β–D–glucopyranosylstigmasta-5,25-diene (**6**) | Anti-inflammatory | [14,15] |
| | Methanol | Anti-inflammatory | [38] |
| ***U. fasciata*** | Dichloromethane/methanol | Antiviral | [39] |
| | *N*-palmitoyl-2-amino-1,3,4,5-tetyrahydroxyoctadecane (**8**) | Antiviral | [15] |
| | Dichloromethane/methanol | Antibacterial | [40] |
| ***U. fasciata* Delile** | Ethanol | Antiproliferative | [41] |
| | Labda-14-ene-3α,8α-diol(**13**) and Labda-14-ene-8α-hydroxy-3-one(**15**) | Antimicrobial | [11] |
| | Methanol | Antioxidant | [17] |
| ***U. rigida*** | Diethyl ether and methanol | Antimicrobial | [42] |
| | Dichloromethane and ethanol | Cytotoxicity | [43] |
| | Ethanol | Antimutagenic, antioxidant | [10] |

**Table 1:** Biological activities of the genus *Ulva.*

The green algae belonging to the genus *Ulva* are a group of edible algae that are widely distributed along the coasts of the world's oceans [7,63], and they have an interesting chemical composition that makes their commercial exploitation attractive to produce functional or health promoting food [7,66]. The macroalgae of the order Ulvales are already used in Asia as a food condiment and as a nutritional supplement in Japan, China and other Southeast Asian countries as well as in North and South America and Oceania. For instance, they are consumed as part of the traditional Hawaiian cuisine [67], in Japan, they are included in a variety of dishes such as salads, soups, cookies, meals and condiments as well as a mixed product with other green seaweeds [7,68]. Interestingly, the interest in these algae as a novelty food is expanding in the West [7], and especially in France where they were authorized for human consumption as vegetables [69].

In the recent years, ulvan has been investigated in order to develop functional foods [26] since they cannot be digested in the human gastrointestinal tract [45,47,67,70] and therefore may be regarded as a good source of dietary fiber and a potential source of prebiotics [67]. It is interesting to note that only few species of *Ulva* have been studied for their application in food industry. These include *U. lactuca, U. pertusa, U. compressa* and *U. clathrata.* These macroalgae exhibited a broad spectrum of nutritional composition which makes them excellent candidates for a healthy food for human nutrition [68]. With high levels of protein (between 10 and 25% of dry mass), dietary fiber, low total lipid contents, and relatively high levels of essential amino acids, they constitute a good alternative source of amino acids and of some essential polyunsaturated fatty acids such as Oleic, Linoleic and Linolenic Acids, vitamins and minerals, especially iron [38,59,68,71]. While *U. lactuca* is used in salads, cookies and soups [62,68], *U. pertusa*, which is frequently consumed under the name of "aonori" in Japan, has a high level of protein (between 20 and 26% dry product) [63], and abundant with vitamins, trace elements and dietary fibers but with low calorie [55]. The dried *U. compressa* is used in cooking, particularly with eggs. It is also used as an ingredient in the preparation of high fiber snack "Pakoda", a common Indian product made from chickpea flour. The crude protein levels of this alga are found to be ranging from 21 to 32% [63]. On the other hand, as *U. clathrata* is a good source of soluble dietary fiber, high-quality polyunsaturated fatty acids, carotenoids and some minerals, it can be a valuable source of protein and may be efficiently used as an ingredient in human and animal foods [7].

## Application of *Ulva spp.* as Biofilters

Besides their use as food, the macroalgae of the genus *Ulva* can also have applications in the removal of nutrients from effluent waters of sewage, industry and mariculture. Studies showed that some *Ulva* species have been tagged as pollution indicator due to their biomass accumulation when they inhabited in highly polluted waters [2,48,72]. For instance, *U. lactuca* has proven to be a good seaweed biofilter in the treatment of fishpond effluents [73]. The opportunistic growth ability of these seaweeds makes them good candidates for water recycling in integrated invertebrates or fish aquaculture systems and of urban waters [54]. Msuya and Neori [74] have demonstrated that *U. reticulata*, as well as *Gracillaria crassa,* could be cultured and used to remove nutrients from mariculture fishpond effluents by simple facilities and using only water tidal flow [74].

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