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LC-ESI-MS/MS Analysis of Bioactive Compounds From the Seaweed *Ulva lactuca* Extract and Their Anti-Obesity Effects

Omnia Aly[a*], Alyaa Aly [b], Yasser Diab [b], Amr Mahmoud [b]

[a]*Department of Medical Biochemistry, National Research Centre, Cairo 12622, Egypt.*
[b]*Department of Biochemistry, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt.*

Abstract

An excessive accumulation of body fat to the point where a person's health is adversely affected is referred to as obesity. Obesity is associated to numerous health problems, including metabolic syndrome, type 2 diabetes, hypertension, and cardiovascular dysfunction. Additionally, the World Health Organization (WHO) classified obesity as an epidemic pathophysiologic condition due to its high prevalence rate. Also, if the obesity incidence kept rising in the present direction, WHO warning that by 2030, the percentage of overweight and obese cases would be 38% and 20%, respectively. As a result of the globally high obesity prevalence rates, concerns of its therapies including; synthetic molecules and surgery, side effects of used synthetic materials, long time treatment and significant financial cost depletion, alternatives with naturally origin are required for their expected role in pharmacotherapy. So, plants' natural products have been shown to be promising safe anti-obesity agents. Marine life showed to cover a wide range of potential therapeutic treatments. The green macroalgae *Ulva lactuca* showed variable bioactivities especially regulating blood glucose. Therefore, the aim of this work is to investigate the potential anti-obesity activity of *Ulva lactuca* extract through *in Vivo*. Also, to evaluate the biochemical and histological effects in obese rats fed high-carbohydrate and high-fat diets. This study was conducted using 40 albino male rats that were randomly divided into 4 groups (10 each): 1st control group, the 2nd rats fed on a high carbohydrate, high-fat diet (HCHFD), the 3rd rats fed on standard diet + the methanolic *Ulva lactuca* extract, and the 4th rats were fed on HCHFD + the methanolic *Ulva lactuca* extract. Using the *Ulva lactuca extract in Vivo* mitigates the HCHFD effects for blood glucose level, insulin resistance, total cholesterol, LDL, triglyceride and HDL-cholesterol**.** Also, this extract overcame the negative effects the HCHFD effects for leptin, irisin, resistin, and adiponectin in the *Ulva lactuca* + HCHFD group. Also, the liquid-chromatography–electrospray onization–tandem mass spectrometry (LC-ESI-MS/MS) analysis of bioactive compounds of *Ulva lactuca* extract resulted in identification of ten known bioactive compounds including; Bromophenol derivative, Kojic acid derivative, Redoxcitrinin, sesquiterpenoid, fucoxanthin, aspergilsmin E, aldobiuronic acid, furaltadone, stigmasterol and arachidonic acid. These compounds are known to have many therapeutic efficacies such as: anti-obesity activity, anti-hyperglycemic effects, recognized as inhibitors of protein tyrosine phosphatase 1B, protect β-pancreatic cells, improving insulin resistance, they also have antioxidant, antimicrobial, anti-inflammatory activities, regulate hyperlipidemia, regulating the absorption of triglyceride pancreatic lipase and cardiovascular diseases.

Keywords: Macroalgae *Ulva lactuca*, anti-obesity, serum glucose, lipid profile, leptin, irisin, resistin, adiponectin and LC-ESI-MS/MS

1. Introduction

Obesity is known as an excessive build-up of body fat to the point where an individual's health is negatively impacted **[1]**. There are many factors are associated with obesity such as: hypertension, type 2 diabetes, metabolic syndrome and cardiovascular dysfunction **[2]**. However, obesity is depending on many other interactions including: environmental, nutritional, lifestyle, and genetic variables **[3]**. Also, as a result of the massive prevalence rate of obesity WHO considered it as an epidemic pathophysiologic disease worldwide **[1,4]**. Hruby and Hu 2015 **[5]**. stated that if the obesity prevalence continued in the same manner, the estimated overweight cases will be 38% and 20% for obese by 2030. The primary treatments for obesity that are now in use include synthetic molecules and surgery, both of which have a high risk of severe recurrence and side effects **[6]**. Hence, due to the high rate of obesity prevalence, global health concerns about its treatments, long treatment period and high economic cost depletion, alternatives with naturally origin are required **[7]**. Historically, natural products and their structural analogs have played a significant role in pharmacotherapy. This helped the world to overcome the toxicity, other side effects, economic concerns, and governmental approval of synthetic medications. So, with the globally vast growing demand of natural alternatives, plants showed to be more promising to overcome these concerns **[8,9]**. There are tremendous number of natural products derived from plants, that have been shown to be involved in the anti-obesity effects via numerous mechanisms, such as appetite regulators, pancreatic lipase and amylase inhibitors, insulin sensitivity enhancers, adipogenesis inhibitors, and adipocyte apoptosis inducers. **[10,11,12]**. **Chandrasekhar *et al* and Rathod *et al*. [13,14]** stated that marine life showed to be considered as a wide source of potential novel compounds that isolated from its organisms as promising therapeutic agents. A

*Corresponding author e-mail: **onnyashraf@live.com** ; **(**Omnia Aly).
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large group of organisms known as marine algae were specifically studied in order to identify their metabolites. Marin algae showed a wide range of naturally occurring compounds, such as polysaccharides, peptides, fatty acids, polyunsaturated fatty acids, minerals, vitamins, and pigments such as phycobilins, carotenoids, carotene, xanthophylls, chlorophylls, phenolics, etc **[9,13,14]**. These marine algae's bioactive substances have prospective use in the areas of anti-obesity, anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, and anticancer therapies **[13,14,15,16]**. Various studies have shown that green macroalgae *Ulva lactuca* show variable bioactivities such as: antimicrobial, antioxidant, anticancer, anti-inflammatory, antiviral, antifungal, antidiabetic and much more therapeutic effects **[17,18,19,20,21]**. Therefore, the aim of this work is to investigate the potential anti-obesity activity of the green macroalgae *Ulva lactuca* extract through *in Vivo*. Also, to evaluate the biochemical and histological effects in obese rats fed high-carbohydrate and high-fat diets. Use liquid-chromatography–electrospray onization–tandem mass spectrometry (LC-ESI-MS/MS) to the chemical analysis of the bioactive extract.

2. Materials and methods

2.1. Biological material

The green macroalgae *Ulva lactuca* was collected from Abu Qir beach, Alexandria coast, Egypt. The algae sample was brought frozen to the laboratory in ice box, where it was kept at -20°C awaiting additional analyses.

2.2. Algae sample extraction

Methyl alcohol was used to extract 600 grams of the *Ulva lactuca* sample at room temperature (rtm) (28 ±2° C). The obtained fifty-five grams of residue was desalted with chloroform. The desalted extract (50g) was retained for phytochemical screening, LC-Mass analysis to identify the bioactive components, and assessment of its anti-obesity capabilities.

2.3. Experimental animals

2.3.1. The *in Vivo* experimental analysis of obesity

This study was conducted using forty albino male rats (age: 4-6 weeks, weighting: 95-106 g), that were obtained from Animal House of the National Research Centre. These rats were randomly divided into 4 groups (10 each) in separated cages and were kept in rtm of (22 ± 2° C) with half day light and half day dark cycle. All rats were left for two weeks for accommodation. The trial lasted 20 weeks, and during the final 8 weeks, the methanolic Ulva lactuca extract was administered orally in addition to the diet, as described below. The control group: rats were fed standard diet, the second group: rats were fed on a high carbohydrate, high-fat (HCHFD) diet according to **Wilson *et al.* [22]** The 3rd group, rats were fed on standard diet + the methanolic *Ulva lactuca* extract was given orally (100 mg/kg/day) according to **Rinawati and Muhsin. [23]** The 4th group; rats were fed on HCHFD + the methanolic *Ulva lactuca* extract was given orally (100 mg/kg/day). The Ethics Committee of the National Research Centre, Dokki, Cairo, Egypt, has given the approval number (84125062023) for all the procedures used during this experiment.

2.3.2. Collection of samples

At the end for the experimental period, rats were fasted overnight and blood samples were collected from the tail's peripheral vein after rats aspirated formalin as anesthesia. Blood samples were centrifuged for ten minutes at 3000 rpm, and then the clear serum was collected and stored at -80°C till the analyses. Histopathological samples were kept in a preservative 10 % formalin-phosphate buffer till analysis. Finally, rats were killed according the ethical code approved by the National Research Centre's Ethics Committee, Dokki, Cairo, Egypt.

2.4. Biochemical analyses

Serum fasting glucose was determined according to **Passing and Badlok. [24]** using Biocon Diagnostic kit, Germany. Serum cholesterol quantification was done according to the procedure by **Allain *et al*. [25]** using the kit from DRG, USA. High density lipoprotein (HDL)-cholesterol and triglyceride were determined using the kit provided by Biocon Diagnostic, Germany according to **Lopes-Virella *et al* and Glick *et al*. [26,27]**. While, Low density lipoprotein (LDL)-cholesterol was calculated according to **Friedewald *et al*. [28]**. Serum insulin concentration was assayed using the kit by DRG diagnosic, USA, according to **Judzewitsch *et a.l* [29]** The homeostasis model assessment of insulin resistance (HOMA-IR) was utilized to calculate insulin resistance as described by **Matthews *et al.* [30]** using the following equation: insulin resistance= [fasting insulin (µIU/ml) X fasting glucose (mg/dl)]/405.

Serum Leptin was determined according to the method described by **Considine *et al.* [31]** using the kit by DRG, USA. The quantification of irisin, resistin and adiponectin were done according to **Pilz *et al.* [32]** and **Watanabe *et al.* [33]** using the kits provided by (R&D Systems, Minneapolis, MN, USA).

2.5. Histopathological Studies

Liver organ collected from each experimental group were washed many times using distilled water, alcohol and xylene before making section with thickness of 5 µm. Sections were stained with both of hematoxylin and eosin then sections were mounted in DPX, and then the histopathological changes were examined according to **Bancroft *et al al.* [34]**

2.6. Liquid Chromatography-Mass Spectrometry (LC-ESI-MS/MS) Analysis

Liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS) was used to analyze the methanolic extract of the macroalgae *Ulva lactuca* sample for the identification of compounds. An ExionLC AC system was used for separation, and a SCIEX Triple Quad 5500+ MS/MS system with electrospray ionization (ESI) for detection was used for detection. For the positive ionization mode, it was done with a Ascentis® Express 90 Å C18 Column (2.1×150 mm, 2.7 µm). The two eluents of the mobile phases (A: 5 mM ammonium formate pH 3, and B: acetonitrile (Liquid chromatographic grade). The gradient of mobile phase was as follows: eluent B 5% at 0-1 min, 5-100% starting from 1 to 20 min, from 20 to 25 min 100% of B, 5% at 25.01, 5% from 25.01-30 min. A 5 µL of the sample was used for the injection with follow rate of 0.3 ml/min.

A negative ionization mode was applied for MS/MS analysis with a scan (EMS-IDA-EPI) starting from 100 to 1000 Da (MS1) using the following parameters: IonSpray voltage: 5500; curtain gas: 25 psi; source temperature: 500°C; ion source gas 1 and 2 were 45 psi and from 50 to 1000 Da (MS2) using a collision energy spread: 15; collision energy: 35, and declustering potential: 80. However, the negative ionization mode was done using a Ascentis® Express 90 Å C18 Column (2.1×150 mm, 2.7 µm). The two eluents of the mobile phases (A: 5 mM ammonium formate pH 8, and B: acetonitrile (Liquid chromatographic grade). The gradient of mobile phase was as follows: eluent B 5% at 0-1 min, 5-100% starting from 1 to 20 min, from 20 to 25 min 100% of B, 5% at 25.01, 5% from 25.01-30 min. A 5 µL of the sample was used for the injection with follow rate of 0.3 ml/min A negative ionization mode was applied for MS/MS analysis with a scan (EMS-IDA-EPI) starting from 100 to 1000 Da (MS1) using the following parameters: IonSpray voltage: 4500; curtain gas: 25 psi; source temperature: 500°C; ion source gas 1 and 2 were 45 psi and from 50 to 1000 Da (MS2) using a collision energy spread: 15; collision energy: 35, and declustering potential: 80. MS-DIAL was used for the identification of compounds.

2.7. Statistical analysis

Data are presented as mean ± S.D. (standard deviation). SPSS was used foe the statistical analysis for Windows version 9.0. Also, the One-way ANOVA was used to estimate and compare the differences between groups. And then, it was followed with post hoc t-test (least significant difference, LSD). Statistics were defined as significant when $P < 0.05$.

3. Results and Discussion

3.1. *In Vivo* Anti-Obesity Effects of *Ulva lactuca*

Obesity is called a complex multifactorial disease because there are many factors are associated with it such as: hypertension, type 2 diabetes, metabolic syndrome and cardiovascular dysfunction **[2,35]**. It is known as an excessive build-up of body fat to the point where an individual's health is negatively impacted and in turns up to be a critical world wild health problem **[1,36]**. Over the past 50 years, there has been a notable rise in the obesity rate globally **[37]**. Also, as a result of the massive prevalence rate of obesity the WHO considered it as an epidemic pathophysiologic disease worldwide **[1,4]**.

There are massive number of studies indicated that, there are many biochemical parameters are associated with obesity such as: blood glucose concentration, insulin resistance and other lipid indices **[38,39,40,41]**. So, this study was done to figure out new alternative naturally origin as anti-obesity agents. Results in Table 1, showed that the fasting glucose levels in the control group were observed at 91.75±4.9 mg/dl. But, the fasting glucose level in HCHFD group exhibited a significant increase in glucose levels of 194±4.9 mg/dl. This is a clear impact of the HCHFD of inducing hyperglycemia which is in accordance with **Jin *et al.* [38]**. The macroalgae *Ulva lactuca* group showed to have fasting glucose levels almost similar to the control group (91.9±2.7 mg/dl). This result is indicating that *Ulva lactuca* is effective in preserving normoglycemic conditions. However, by comparing the four groups fasting glucose levels, the *Ulva lactuca* + HCHFD group showed an intermediate value of (149±6.9 mg/dl), which is significantly lower than the HCHFD group, but higher than the control and macroalgae groups. That is indicating a partial counteraction role of the macroalgae *Ulva lactuca* against HCHFD-induced hyperglycemia.

Table 1. The effect of the macroalgae Ulva lactuca on fasting glucose, insulin, and insulin resistance in macroalgae different groups

Parameters	Groups (10 rats each)			
	Control	HCHFD	*Ulva lactuca*	*Ulva lactuca* + HCHFD
Glucose(mg/dl)	91.75±4.9	194±4.90[a]	91.90±2.70[b]	149±6.90[a,b,c]
Insulin(µIU/ml)	12.30±1.40	7.80±0.90[a]	12.9±2.50[b]	8.80±0.14[a,b,c]
Insulin Resistance	2.80±0.25	3.74±0.23 [a]	2.90±0.29 [b]	3.23±0.17 [a,c]

Values are expressed as mean ± standard error (S.E.), value of p <0.05 was considered statistically significant, (a) significant when compared to control group, (b) significant when compared to HCHFD group, and (c) significant when compared to *Ulva lactuca* group.

The insulin level results showed in table (1) indicated that the control group and *Ulva lactuca* group have almost results closed to each other. The control group insulin level was 12.3±1.4 µIU/ml and the macroalgae *Ulva lactuca* group showed insulin levels of (12.9±2.5 µIU/ml). However, the results showed a decrease to 7.8±0.9 µIU/ml in the HCHFD group. In the macroalgae *Ulva lactuca* + HCHFD group, the recorded results was 8.8±0.14 µIU/ml, which is higher than the HCHFD group and lower than the control and macroalgae groups.

One of the most important parameter of obesity is the insulin resistance. The finding indicated that the control group was 2.8±0.25. While, the HCHFD group results showed a slightly increased level of 3.74±0.23. The *Ulva lactuca* group showed an insulin resistance level of 2.9±0.29, which is closely to the control. The *Ulva lactuca* + HCHFD group showed a value of 3.23±0.17. That was lower than the HCHFD group, and higher than both the control and *Ulva lactuca* groups.

These results imply that *Ulva lactuca* can successfully sustain normal insulin and glucose levels while it could be used to stop the rise in insulin resistance linked to HCHFD. So, this mitigative effect of *Ulva lactuca* is clearly showed that it could be used potentially anti-obesity agent. These results are in agreement with those of **AbouZid *et al*; Labbaci and Boukortt, and Chen *et al*. [42,43,44]** where it was found that the green alga *Ulva lactuca* showed anti-hyperglycemic by lowering blood glucose level and prevent insulin resistance. These previous effects may be due to the extract inhibits satietogenic effect of the HCHFD [**43]**. Also, the polar extract of this alga contains high flavonoids and phenolic compounds which have the ability to regulate genes expression that control insulin signaling **[42,45]**. Also, the ability of this alga extract could manage glucose level through its high content of polyphenols and flavonoids which have their anti-diabetic effects via many mechanisms such as: activation of peroxisome proliferator-activated receptor (PPAR), α glucosidase/α amylase inhibition, adenosine monophosphate-activated protein kinase (AMPK) pathway activation, glucose uptake and insulin sensitivity improvement **[45,46]**.

3.2. Lipid profile

Table 2. The effect of the macroalgae Ulva lactuca extract on lipid profile in different groups

Parameters	Groups (10 rats each)			
	Control	HCHFD	*Ulva lactuca*	*Ulva lactuca* + HCHFD
Cholesterol (mg/dl)	102.18±4.50	175.78±5.60 [a]	103.93±5.90 [b]	140.45±2.90 [a,b,c]
Triglyceride (mg/dl)	74.54±2.39	166.78±4.59 [a]	75.01±3.40 [b]	143.26±4.30 [a,b,c]
HDL-cholesterol (mg/dl)	38.21±0.70	18.41±1.10 [a]	37.91±1.60 [b]	23.82±2.020 [a,b,c]
LDL-cholesterol (mg/dl)	49.06±1.60	124.01±2.90 [a]	51.01±1.50 [b]	87.97±3.20 [a,b,c]

Values are expressed as mean ± standard error (S.E.), value of p <0.05 was considered statistically significant, (a) significant when compared to control group, (b) significant when compared to HCHFD group, and (c) significant when compared to *Ulva lactuca* group.

The results in (Table 2) indicated that, in comparison to the control group (102.18±4.5 mg/dl), the HCHFD group had considerably higher cholesterol levels (175.78 ± 5.6 mg/dl). The cholesterol levels of the *Ulva lactuca* group were 103.93±5.9 mg/dl, which was similar to the control group. This suggests that macroalgae have the ability to sustain normal cholesterol levels. The cholesterol levels of the *Ulva lactuca* + HCHFD group were intermediate at 140.45±2.9 mg/dl, which was greater than both of the control and *Ulva lactuca* groups, but lower than those of the HCHFD group.

In the same manner, the HCHFD group had significantly higher triglyceride levels (166.78±4.59 mg/dl) than the control group (74.54±2.39 mg/dl). Triglyceride levels in the *Ulva lactuca* group (75.01±3.4 mg/dl) were nearly identical to those in the control group, but in the *Ulva lactuca* + HCHFD group, was intermediate (143.26±4.3 mg/dl). That was greater than both of the control and *Ulva lactuca* groups, but lower than those of the HCHFD group. However, the HCHFD group's HDL-cholesterol levels dropped considerably (18.41±1.1 mg/dl) when compared to the control group's (38.21±0.7 mg/dl). There was a protective effect as the HDL-cholesterol level (37.91±1.6 mg/dl) of the *Ulva lactuca* group was similar to those of the control group. The HDL value in the *Ulva lactuca* + HCHFD group was intermediate at 23.82±2.02 mg/dl.

Conversely, the HCHFD group had significantly higher LDL-cholesterol level (124.01±2.9 mg/dl) thanthe control group (49.06±1.6 mg/dl). LDL-cholesterol level in the *Ulva lactuca* group (51.01±1.5 mg/dl) was nearly identical to those in the control group, but in the *Ulva lactuca* + HCHFD group, was intermediate (87.97±3.2 mg/dl mg/dl). That was greater than both of the control and *Ulva lactuca* groups, but lower than those of the HCHFD group.

So far, results in both tables (1&2) revealed that using *Ulva lactuca extract in Vivo* mitigate the HCHFD effects for blood glucose level, insulin resistance, total cholesterol, LDL, triglyceride and HDL-cholesterol **[47,48]**. These results showed that *Ulva lactuca* has the applicable potential as natural anti-obesity agent.

Many studies have proven that insulin is recognized as lipogenesis stimulator which increases triacylglycerols and fatty acid synthesis **[49]**. Also, triglycerides and LDL-cholesterol are positively linked with blood glucose level resulting in high obesity risk and cardiac illnesses **[50,51]**. The altered lipid profile associated with obesity is primarily caused by the increased discharge of free fatty acids from adipose tissue, which increases the synthesis of triglycerides in the liver. Insulin resistance exacerbates this process **[52,53]**. However, the HDL-cholesterol concentration is decreased in obesity, which is correlated with both the distribution and the degree of obesity **[54]**.

Table 3. The effect of the macroalgae *Ulva lactuca* extract on serum leptin, irisin, resistin, and adiponectin in different groups

Parameters	Groups (10 rats each)			
	Control	HCHFD	*Ulva lactuca*	*Ulva lactuca* + HCHFD
Leptin (ng/ml)	5.30±2.4	8.22±2.3 [a]	5.80±1.9[b]	6.80±2.8 [a,b,c]
Irisin (pg/ml)	35.47±2.5	10.83±2.2 [a]	37.01±2.4[b]	18.99±2.6[a,b,c]
Resistin (pg/ml)	37.70 ±3.2	58.70±3.5[a]	37.50±3.8[b]	45.30±2.9[a,b,c]
Adiponectin (ng/ml)	17.89±2.8	7.76±2.4 [a]	18.10±3.1	12.01±2.2 [a,b,c]

Values are expressed as mean ± standard error (S.E.), value of p <0.05 was considered statistically significant, (a) significant when compared to control group, (b) significant when compared to HCHFD group, and (c) significant when compared to *Ulva lactuca* group.

3.3. Serum levels of obesity-related biomarkers (leptin, irisin, resistin, and adiponectin)

Results in table 3, indicated that the HCHFD group had significantly higher leptin levels (8.22±2.30 ng/ml) than the control group (5.30±2.40 ng/ml), suggesting a rise in leptin associated to obesity. The administration of *Ulva lactuca* treatment attenuated this increase to 5.80±1.90 ng/ml, while the *Ulva lactuca* + HCHFD group displayed an even lower level at 6.80±2.80 ng/ml, indicating the *Ulva lactuca*'s ability to control leptin levels. That was greater than both of the control and *Ulva lactuca* groups, but lower than those of the HCHFD group.

The HCHFD group had significantly lower levels of irisin (10.83±2.20 pg/ml) than the control group (35.47±2.50 pg/ml). Irisin levels were nearly equal to the control after treatment with *Ulva lactuca*, returning to 37.01±2.40 pg/ml. With a level of 18.99±2.60 pg/ml, the *Ulva lactuca* + HCHFD group showed some improvement. That was lower than both of the control and *Ulva lactuca* groups, but greater than those of the HCHFD group. This suggests that *Ulva lactuca* may be useful in restoring irisin levels that have been impacted by a high-fat diet.

Compared to the control group (37.70 ±3.20 pg/ml), resistin levels was raised in the HCHFD group to (58.7±3.50 pg/ml). However, the *Ulva lactuca* treatment showed a result of (37.5±3.80 pg/ml) that is closed to the control group. The *Ulva lactuca* + HCHFD group treatment displayed moderated levels at 45.3 ±2.90 pg/ml, that displayed showed some improvement compared to the HCHFD. That was higher than both of the control and *Ulva lactuca* groups, but lower than those of the HCHFD group. Ultimately, the HCHFD group's adiponectin levels (7.76±2.40 ng/ml) were considerably lower than those of the control group (17.89±2.8 ng/ml). Adiponectin levels were successfully brought to 18.1±3.10 ng/ml, which is near control levels, by the *Ulva lactuca* treatment. Adiponectin levels increased to 12.01±2.2 ng/ml in the *Ulva lactuca* + HCHFD group, further supporting the health benefits of macroalgae *Ulva lactuca.*

Results in Table 3, showed that all the results of Ulva lactuca showed to be similar to control group. While, these were significant (P<0.05) increase in leptin, and resistin levels in HCHFD compared to the control group. However, the results of the Ulva lactuca + HCHFD group showed significant decrease (P<0.05) in irisin and adiponectin when compared to HCHFD group. By comparing all the results together, it is clear that using *Ulva lactuca extract in Vivo* overcame the negative effects the HCHFD effects for leptin, irisin, resistin, and adiponectin in the *Ulva lactuca* + HCHFD group. These results showed that *Ulva lactuca* has the applicable potential as natural anti-obesity agent. **Nimptsch *et al.* [55]** indicated that the biomarkers leptin, irisin, resistin, and adiponectin are crucial important in obesity and metabolic syndrome because their unique function in the complex metabolic pathways connected to these diseases. **Liu *et al.* [56]** indicated that individuals with obesity have shown high leptin and low adiponectin when compared to control. Leptin exhibits a range of metabolic activities, including controlling body weight and energy homeostasis, lowering glucagon and hepatic glucose synthesis, and enhancing insulin sensitivity **[57]**.Serum irisin levels in the HCHFD group were lower but increased after receiving *Ulva lactuca* treatment. This implies the function of irisin in enhancing fat metabolism and energy uptake in adipose tissues **[58]**. Mice studies indicated that resistin hormone is correlated with insulin sensitivity level and obesity in obese mice.

Also, HCHFD has a negative impact on resistin level in rats which was significantly higher in the obese rats compared with control **[59,60,61]**.

3.4. Histopathological Examination of Macrolagae *Ulva lactuca*

Rats in the control group had liver sections examined under a light microscope, and this revealed the typical histological-architecture of the hepatic lobule (A1-A2). However, rats from the HFCHD group, had cholangitis, fibroplasia in the portal triad, portal infiltration with inflammatory cells, and hepatocytes' vacuolar degeneration in their livers (B1-B2). Some rat liver sections from the *Ulva lactuca* group showed solely Kupffer cell multiplication (C2), while other sections appeared to have apparent histologically normal hepatic tissue (C1). Conversely Rats in the HFCHD+ *Ulva lactuca* group showed mild fibroplasia in the portal triad (D2), vacuolar degeneration of some hepatocytes, and proliferation of Kupffer cells (D1).

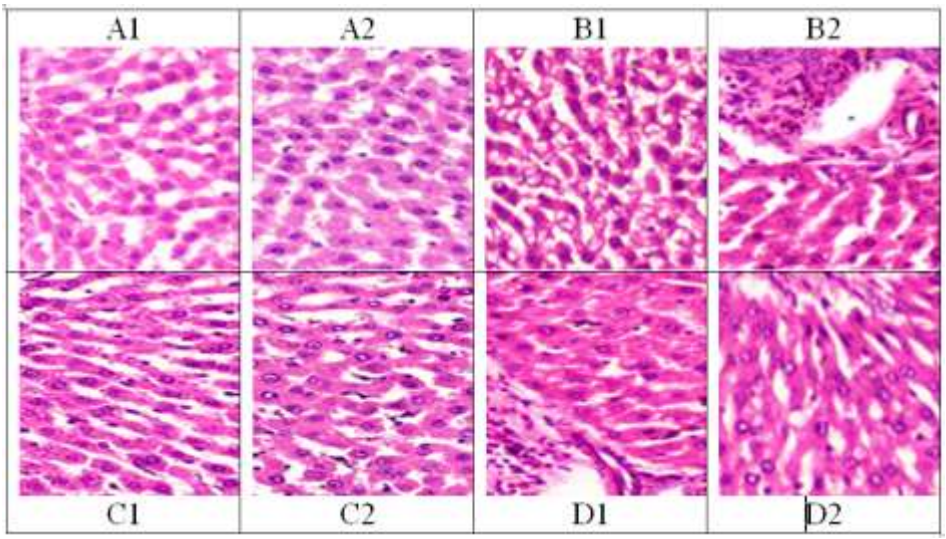


Figure 1. A photomicrograph of the rats' liver of control group (A1-A2), HCHFD group (B1-B2), *Ulva lactuca* group (C1-C2) and HFCHD + *Ulva lactuca* group (D1-D2).

3.5. LC-ESI-MS/MS analysis of bioactive Compounds from the seaweed *Ulva lactuca* extract

The objective of the current study was to discover and characterize different chemicals found in *Ulva lactuca's* crude extract in order to investigate any potential therapeutic uses, especially with regard to managing obesity. Utilizing liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (Figure 2 and 3), a wide range of metabolites was discovered from the seaweed *Ulva lactuca* extract. Table 4, lists these as terpenoids, bromophenols, sterols, fatty acids, alkaloids, pyrone derivatives, and polyketide derivatives. These metabolites were identified by cross-referencing their precise mass data with entries in many databases, including PubChem, the Metabolite and Chemical Entity (METLIN) database, and the Dictionary of Natural Products (DNP). The annotation of ten different compounds was made easier by this quick identification technique.

Table 4. Detection of compounds obtained from a crude defatted methanolic extract of *Ulva lactuca* by using LC-ESI-MS/MS) analysis.

No	RT min	Precursor mass (*m/z*)	MS/MS (*m/z*)	Adduct	Name	Molecular formula	Molecular wieght	Source	References
1	0.54	171.08	171,143	$[M-H]^-$	4-bromophenol	C_6H_5BrO	173.01	Pterocladiella capillacea	(Whitfield et al., 1999)
2	16.15	156.09	156,138,125	$[M-H]^+$	kojic acid monomethyl ether	$C_7H_8O_4$	170	*Ulva pertusa*	(X. Li et al., 2003)
3	2.80	236.09	236, 218, 176	$[M-H]^+$	Redoxcitrinin	$C_{13}H_{16}O_4$	236.26	Fungus *Penicillium sp*	(D. Zhang et al., 2007)
4	14.78	209.11	210, 150, 138	$[M-H]^+$	6-Isopenteny-1,5,5,6-tetramethyl-1-cyclohexen	$C_{15}H_{28}$	208	*Ulva fasciata*	(Chakraborty & Paulraj, 2010)
5	27.60	659.64	660.43, 659.43, 641.42,149.09, 109	$[M-H]^+$	Fucoxanthin	$C_{42}H_{58}O_6$	658.9	*Hijikia fusiformi*	(YAN et al., 1999)
6	3.52	215.08	182,132	$[M-H]^+$	Aspergilsmin E	$C_{10}H_{15}O_5$	214.21	Alga-Derived Fungus Aspergillus giganteus	(J. J. Chen et al., 2020)
7	22.17	339.10	163, 339	$[M-H]^-$	Aldobiuronic acid	$C_{11}H_{18}O_{11}$	326.25	*chlorella vulgaris*	(OGAWA et al., 1998)
8	7.63	325.11	127, 252, 281	$[M-H]^+$	Furaltadone	$C_{13}H_{16}N_4O_6$	324.29	*Ulva lactuca*	(Leston et al., 2011)
9	27.56	413.26	147,159,255	$[M-H]^+$	Stigmasterol	$C_{29}H_{48}O$	412.7	*Navicula incerta*	(Y.-S. Kim et al., 2014)
10	4.24	305.06	221,235,287	$[M-H]^+$	Arachidonic acid	$C_{20}H_{32}O_2$	304.5	*Leptomitus lacteus*	(Fox et al., 2000)

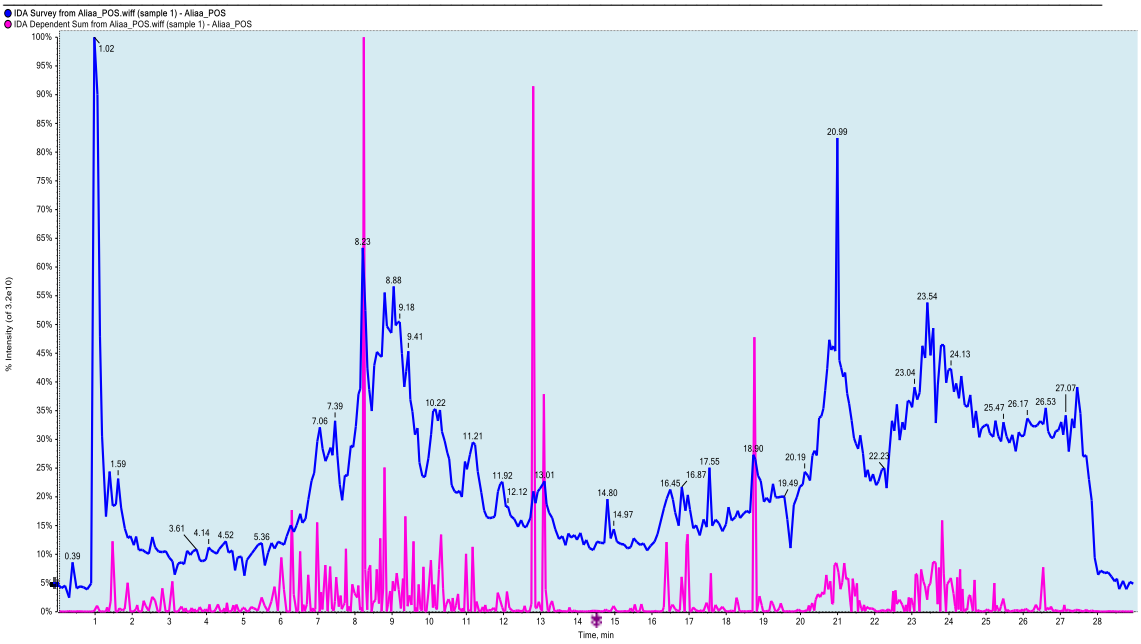


Figure 2. The LC-ESI-mass spectrum of crude extract of *Ulva lactuca* in positive mode (The pink color showed fragments of compounds, while the blue color showed patterns for compounds)

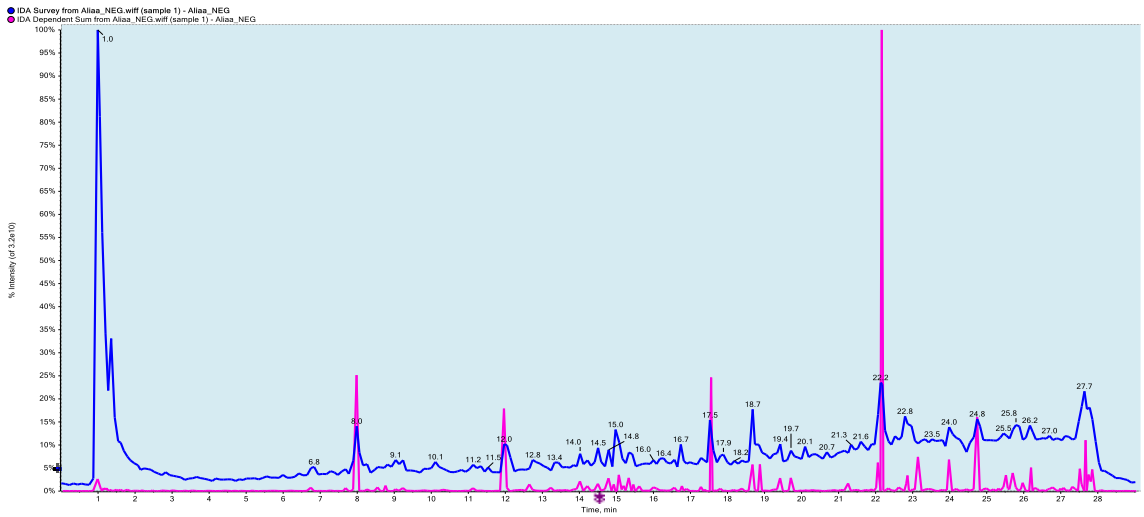


Figure 3. The LC-ESI-mass spectrum of crude extract of *Ulva lactuca* in Negative mode (The pink color showed fragments of compounds, while the blue color showed patterns for compounds)

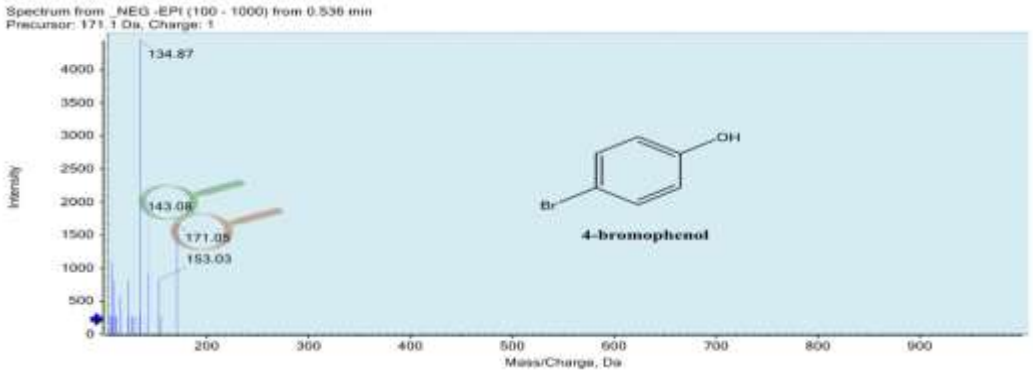


Figure 4. The LC-ESI-mass spectrum of compound (1) 4-bromophenol.

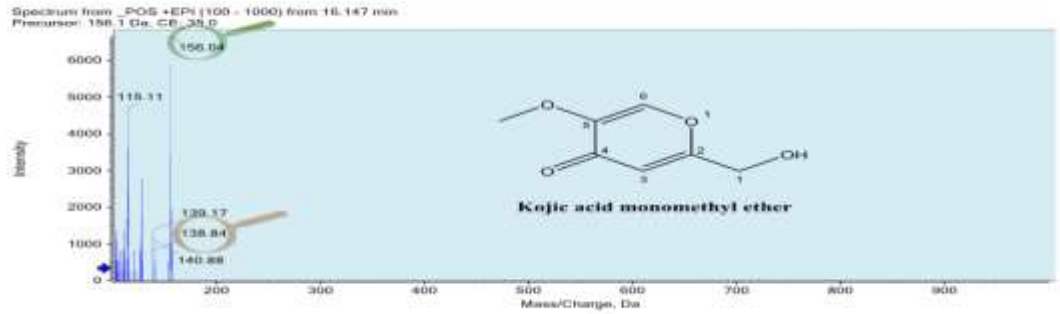


Figure 5. The LC-ESI-mass spectrum of compound (2) kojic acid monomethyl ether.

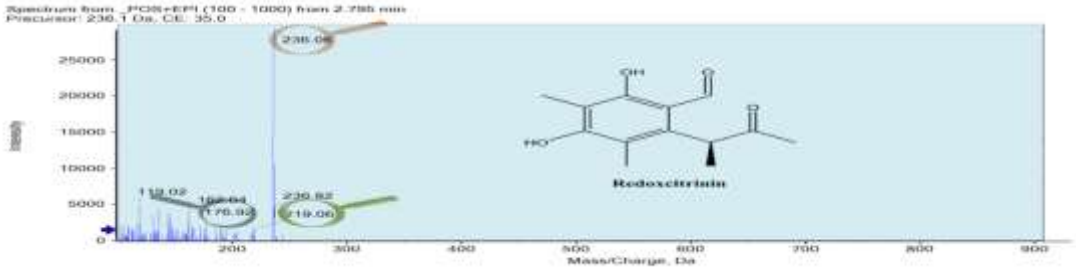


Figure 6. The LC-ESI-mass spectrum of compound (3) Redoxcitrinin.

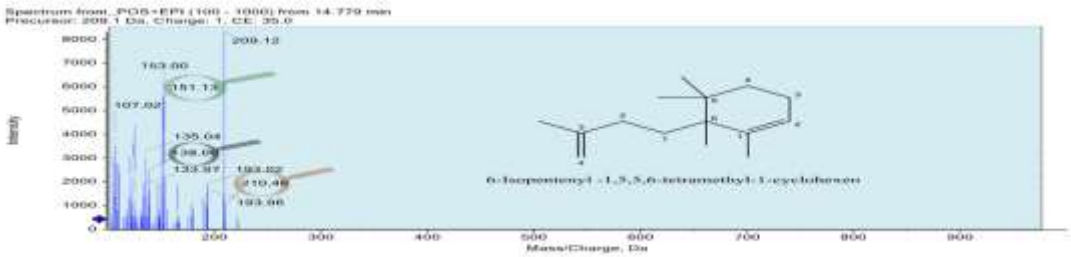
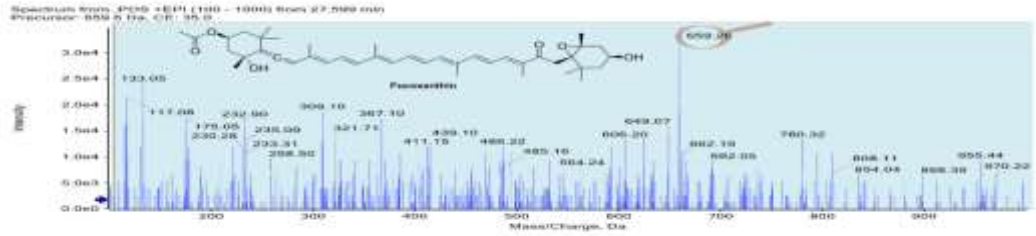


Figure 7. The LC-ESI-mass spectrum of compound (4) 6-Isopenteny-1,5,5,6-tetramethyl-1-cyclohexen.

Figure



8. The LC-ESI-mass spectrum of compound (5) Fucoxanthin.

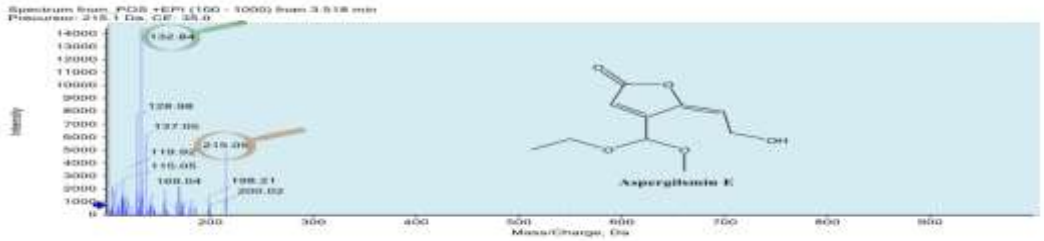


Figure 9. The LC-ESI-mass spectrum of compound (6) Aspergilsmin E.

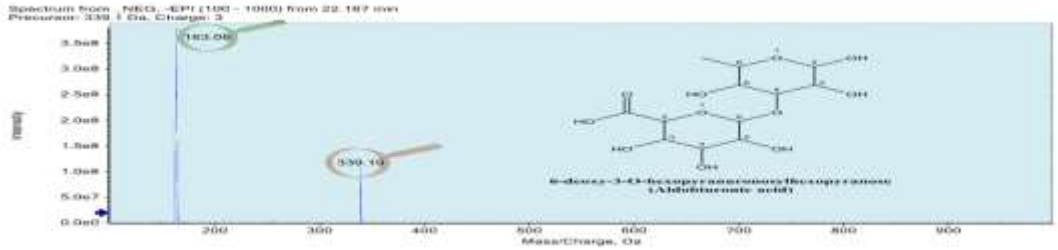


Figure 10. The LC-ESI-mass spectrum of compound (7) Aldobiuronic acid.

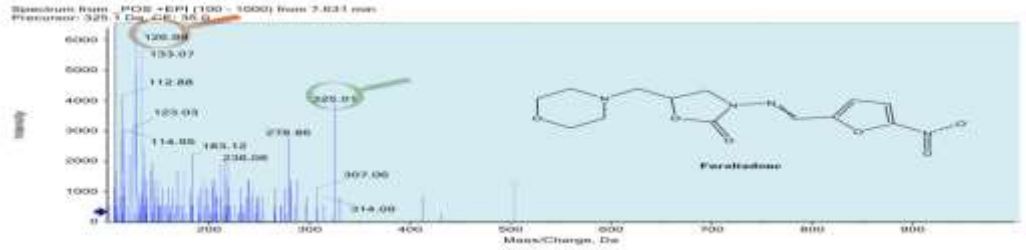


Figure 11. The LC-ESI-mass spectrum of compound (8) Furaltadone.

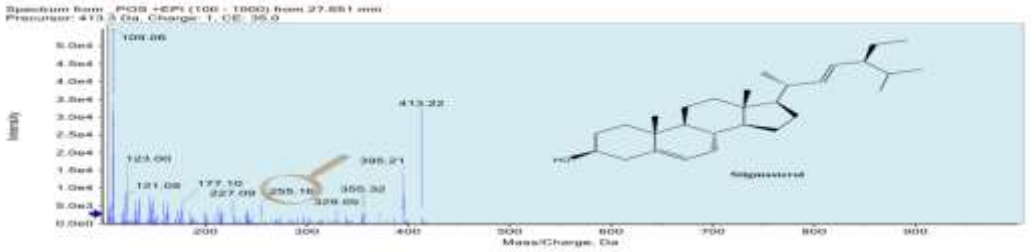


Figure 12. The LC-ESI-mass spectrum of compound (9) Stigmasterol.

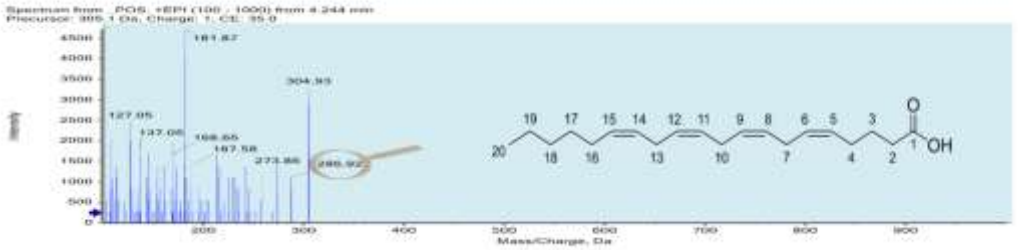


Figure 13. The LC-ESI-mass spectrum of compound (10) Arachidonic acid.

Compound (1), a prior detection of this chemical was made in *Pterocladiella capillacea* **[62]**. Additionally, this spectrum data (Figure 4) was nearly identical to those reported earlier in MSBNK-CASMI_2016-SM865051, PubChem CID 7808 **[63]**. Because of its regulatory function in insulin and leptin signaling, protein tyrosine phosphatase 1B, a validated target for type 2 diabetes and obesity therapy, has been shown to be inhibited by bromophenol derivatives **[64]**.

Compound 2, kojic acid monomethyl ether (Figure 5), was discovered in the *Ulva lactuca* extract earlier and identified on in the *Ulva pertusa* [**65]**. Compound 2**,** has shown potential as antioxidant, antimicrobial, anti-inflammatory, and obesity management agents by inhibiting pancreatic lipase enzymes **[66]**.

Compound (3) Redoxcitrinin (Figure 6) was identically virtualized on the reported in the literature of **Zhang *et al.* [67]**. This compound is recognized as a biogenetic precursor of citrinin, which inhibits both ATP-Citrate Lyase and α-Glucosidase **[44]**. Particularly useful in lowering blood plasma glucose levels, inhibition of α-glucosidase slows down the breakdown of carbohydrates, which in turn postpones the absorption of dietary lipids and carbs. The prevention and treatment of diabetes, obesity, and overweight are greatly aided by this mechanism **[68]**.

While, the following sesquiterpenoid compound (4) Figure 7, (6-Isopenteny - 1, 5, 5, 6 – tetramethyl - 1 – cyclohexen) was previously identified by **Chakraborty and Paulraj [69]**. Sesquiterpenoids have anti-diabetic effects because they protect β-pancreatic cells and increase insulin production. By controlling glucose transport, altering important proteins in the insulin

signaling cascade, and lowering cholesterol levels, they improve insulin sensitivity in organs such the liver, adipose tissue, and skeletal muscle **[70]**. And, the fucoxanthin Compound 5, a carotenoid-based on the precursor (Figure 8) was discovered previously in seaweeds *Hijikia fusiformi* **[71]**. Due to a variety of processes, this chemical has lately drawn interest for its anti-obesity benefits. These include the liver's uptake of cholesterol through the down-regulation of low-density lipoprotein (LDL) and the decrease of plasmatic and hepatic triglyceride concentrations **[72]**.

Additionally, by inhibiting adipocytokines, it raises blood glucose levels and improves insulin resistance. This impact is made possible by the elevation of GLUT4 expression and the enhancement of glucose transporter 4 (GLUT4) translocation from the cytosol to the cell membrane **[73,74]**.

Compound 6 is known as a polyketide Aspergilsmin E (Figure 9) was before categorized from the marine Alga *Aspergillus giganteus* **[75]**. (Compound 7), Aldobiuronic acid (Figure 10), this disaccharide, was detected from *chlorella vulgaris* **[76]**. Compound 8, Furaltadone (Figure 11), a nitrofuran compound was previously detected in *Ulva lactuca* **[63,77]**.

Compound 9 the Stigmasterol (Figure 12), was identified previously from the marine *Navicula incerta* **[78]**. It has been demonstrated that stigmasterol significantly improves glucose absorption. This improvement is attained by reducing insulin resistance, which is done by increasing the expression of the GLUT4 glucose transporter and its translocation, which exhibits anti-hyperglycemic activity mainly by encouraging the pancreatic β-cells of Langerhans to regenerate. Increased insulin release as a result of this regeneration regulates and lowers blood glucose levels **[55,79]**. And, polyunsaturated fatty acid was before detected as arachidonic acid (Figure 13) (Compound 10) as described in **[80]**. Some of the most important biological activities of arachidonic acid are its ability to inhibit fat accumulation in adipose tissue, lowering muscle's insulin sensitivity, and early preventing insulin resistance **[81,82]**.

4. In conclusion, this study emphasizes the important therapeutic potential of marine-derived materials, particularly the extract from *Ulva lactuca* in the treatment of obesity and related metabolic disorders. The in *Vivo* study proved how effective this extract was in preserving healthy insulin and glucose levels, lowering insulin resistance, and improving lipid profiles, all of which helped to lessen the negative consequences of a HCHFD.

Furthermore, the extract's capacity to control important obesity-related biomarkers like adiponectin, resistin, irisin, and leptin emphasizes their potential as natural substitutes for traditional pharmaceutical therapies for obesity. *Ulva lactuca* is easily extracted from the sea or rocks, making it a useful ingredient for scientific studies as well as the cosmeceutical and nutraceutical sectors.

Many natural substances found in macroalgae work as anti-obesity agents in different ways. The extraction and identification of bioactive components from this extract place the basis for further investigation into their mechanisms of action and possible uses in the treatment and prevention of conditions linked to obesity metabolic disorders.

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