Research Article

Effect of Abiotic Stress on Endogenous Phytohormones Profile in Some Seaweeds

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ABSTRACT

Endogenous phytohormones profile of auxins, Abscisic acid, Gibberellins were identified qualitatively and quantitatively in two different habitats as marine red alga *sarconema filiformae*, green alga *Ulva rigida* and brackish green alga *Ulva lactuca*. The analysis was carried out by using GC/MS technique. The algal samples were harvested in May 2015 from Suez Canal and Timsah Lake. Identification of isolated hormones based on data obtained of their mass spectrum and fragmentation pattern. *Ulva rigida* registered the highest concentration of total phytohormones, than *Sarconema filiforme* and *Ulva lactuca*. *Ulva rigida* recorded high concentrations of auxins followed by *Sarconema filiforme and then Ulva lactuca*. *Sarconema filiforme* and *Ulva rigida* were characterized by the same auxin constituents of (Indole-3-acetyl-L-isoleucine, Indole-3-acetyl-L-leucine methyl ester and Indole-3-butyryl-L-valine). 4-chloro indol-3-acetic acid and 3-indoxyl-β-D-Galacto pyronoside methyl ester were recorded in *Ulva lactuca*. *Ulva lactuca* contained the highest concentration of ABA than *Ulva rigida* and *Sarconema filiforme*. In ABA profile, 3 compounds were detected of the selected species (cis,trans-Abscisic acid-L-Alanine methyl ester, cis, trans-Abscisic acid-L-Valine and Cis, trans-Abscisic acid-L-Alanine). GAs constituent of *Sarconema filiforme* revealed high concentration than *Ulva lactuca* and finally *Ulva rigida*. At least 14 well known gibberellins (GA_1, GA_1 methyl ester, GA_4, GA_7 methyl ester, GA_8, GA_{13}, GA_{19}, GA_{23}, GA_{29}, GA_{44}, GA_{75}, Kaurene, Kaurenoic acid and Kaurenal methyl ester). GA_{13} and GA_{23} only shared at the three studied algal species. GA_8 shared at two seaweed species (*Ulva lactuca* and *Ulva rigida*) and Kaurene (*Sarconema filiforme* and *Ulva lactuca*). Generally, the bioactivity of phytohormones attributed to the methods of seaweed extraction and according to the types of seaweed, particularly due to differences in their chemical components, and environmental conditions stress.

Keywords: Phytohormones profile, *Sarconema filiformae, Ulva rigida, Ulva lactuca,* abiotic stress, GC/MS

INTRODUCTION

Plant hormones play an important role in mediating plant growth as well as signaling environmental changes, initiating stress responses and indicator molecules in the regulation of almost all phases of plant development from embryogenesis to senescence[1,2]. Auxins in seaweed extracts were shown to initiate root formation, inhibit its elongation, differentiation of phloem elements, apical dominance and tropisms. Plants are able to synthesize these compounds from tryptophan or indole[3]. The concentrations of auxins in seaweed extracts are different and strongly depend on the species. Auxins and their inactive analogs were present in brown (*Macrocystis* and *Laminaria*), red (*Botryocladia*), and green (*Enteromorpha, Chlorella*, and *Cladophora*) algae and also in Cyanobacteria (*Oscillatoria*)[4]. Recently, using modern methodologies, it was demonstrated the presence of auxins (IAA in particular) in green and characean algae and in the extracts from brown algae (*Fucus* and *Ascophyllum*)[5-7], *Caulerpa paspaloides*[8], *Ecklonia maxima*[9] and *Undaria pinnatifida*[10]. Other indole derivatives, such as indole-3-carboxylic acid (ICA), have been identified in *Botryocladia leptopoda*[11], *Prionitis lanceolata*[12]. In various groups of algae, some compounds were repeatedly

detected, which suppressed plant growth in bioassays as abscisic acids[13]. Abscisic acid (ABA) synthesized from carotenoids by more than 60 species of algae (e.g. *Chlorella* spp., *Haemato-coccus pluvialis*). ABA is mostly responsible for synthesis of proteins required for the response to drought[14]. It is supposed that algae produce a complex responsible for growth inhibition consisting of several components which act as ABA in algae[3], in addition, ABA plays an important role in controlling of the stomatal apparatus function and seed dormancy[15]. This hormone was found in green microalgae (*Chlorella* sp., *Dunaliella salina*, and *Haematococcus pluvialis*) and also in the thalli of brown macrophytes from the genus *Ascophyllum nodosum*[16] and some species of *Laminaria*[17-19], Chlorophyta species such as *Chara foetida*, *Draparnaldia mutabilis*, *Ulva lactuca*[20]. Among plant hormones, gibberellins were also isolated from seaweed extracts. Gibberellins (GAs) were identified in extracts from *Fucus vesiculosus*, *Fucus spiralis*[3] and *Ascophyllum nodosum*[14]. The main role of gibberellins is to initiate seeds germination, stem elongation, leaf expansion, flower development and fruit maturity[21]. Active gibberellins, GA_1 and GA_3, and inactive GA_6 were isolated chromatographically from the tissue extracts of *Fucus*

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vesiculosus and *F. spiralis*[22]. The substances with gibberellin activity were also isolated from the green Alga *Caulerpa paspaloides*, but they were not precisely identified[23]. Seaweed extracts are used commercially as growth stimulants in agricultural crops due to the plant hormones present[24]. El Shoubaky and Salem [25] used brackish green algae *Ulva lactuca* and *Enteromorpha clathrata* from Timsah Lake as biofertilizers due to high content of inorganic nutrients, organic matters and the plant growth hormones. *Ulva* with its worldwide distribution, wider adaptability to diverse environmental conditions, higher growth rates and amenability for depolymerization makes it an attractive feedstock for developing fertilizer and bio-refinery products[26]. Several methods have been developed for the analysis of phytohormones[27]. Gas chromatography–mass spectrometry (GC-MS) is a well recognized technique for the determination of phytohormones[28]. There are still difficulties in the analysis of some plant hormones by GC because certain phytohormones are thermally unstable[29]. It has been known for a long time that mutual interactions amongst the hormones profoundly affect their biosynthesis, metabolism and catabolism, as well as the sensitivity of specific tissues to them. Therefore, increasing interest has arisen in analyzing several hormones in the same sample[30]. Hence, the objective of this work was to analysis and study the influences of marine and brackish habitats on the composition endogenous phytohormones profile in some seaweed as marine red alga *Sarconema filiforme* and green alga *Ulva rigida*, in addition to the brackish green alga *Ulva lactuca* by using Gas chromatography–mass spectrometry (GC/MS) technique.

MATERIALS AND METHODS
Algae material: Seaweeds were varied from the red alga *Sarconema filiforme* (Sonder) Kylin, the green algae *Ulva rigida* C. Agardh and *Ulva Lactuca* Linnaeus. The marine *Sarconema filiforme* and *Ulva rigida* collected from El Qantara area, Suez Canal (salinity =39‰) (30.828248°N 32.317572°E), whereas the brackish *Ulva Lactuca* collected from Timsah Lake area, Suez Canal (salinity =25‰) (30.56667°N 32.28333°E). These species collected in May 2015. The selected species were identified by[31-33]. The algae samples were gently washed firstly with seawater then by tap water to remove dirt, sand and then kept in refrigerator until endogenous hormones analysis. Extraction of plant hormones: The method of extraction was adopted by[34]. 30 g of each fresh seaweeds tissue were collected and ground in cold 80% methanol. The macerated tissue was adjusted to 20 ml of methanol for each g fresh weight of sample. The tissues were extracted for 2 hour at 0°C, and then filtered. The residue was returned with fresh volume of methanol and stirred for 30 minutes with magnetic stirrer then filtered again. The procedure was repeated once more and the combined extracts were evaporated to the aqueous phase. The aqueous phase (10-30 ml) was adjusted to pH 8.6 with 1% (w/v) NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate

fraction was evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with 1% HCI (v/v) and re-partitioned three times with equal volumes of ethyl acetate. The remaining aqueous phase was discarded and the combined acidic ethyl acetate was reduced to 5ml (fraction I) to determine the acidic phytohormones. The acidic hormones (fraction I) were methylated according to Vogel[35] to use for GC/MS. The dried basic ethyl acetate fraction was dissolved in 80% methanol. The methanol was evaporated under vacuum leaving an aqueous phase which was adjusted to pH 2.8 with 1% HCI and partitioned three times with equal volumes (25-50 ml) of ethyl acetate. The ethyl acetate phase were combined (fraction II) reduced to 5 ml, stored at -20°C until GC/MS ana1ysis. GC/MS analysis: The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280 °C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C as a final temperature at an increasing rate of 5°C /min (hold 5 min). The quantification of all the identified components was investigated using a percent relative peak area. Identification of the endogenous phytohormones compounds was based on the comparison of their molecular weight and fragmentation pattern of their mass spectra with those of the NIST, WILLY library data of the GC/MS system.

RESULTS
The characteristic isolated phytohormones content (auxins, ABA and GAs) in the studied species *Sarconema filiforme*, *Ulva rigida* and *Ulva lactuca* were determined by using GC-MS technique. Figs. 1, 2 and 3 represented GC spectrum of the isolated phytohormones in the studied species respectively. Retention time (Rt) and the relative concentration (%) of the separated compounds were determined. The phytohormones analysis of the studied species showed that the marine red alga *Sarconema filiforme* contains 13 compounds, the marine green alga *Ulva rigida* 12 compounds while the brackish green alga *Ulva lactuca* 12 compounds. Fig. 1 showed that auxins of *Sarconema filiforme* were grouped at interval ranged between 5.29min to 10.73min whereas ABA were grouped at Rt 14.12 and 14.47min, finally GAs were grouped at high Rt interval limiting between 37.24min to 46.40min. Rt of the isolated auxins in *Ulva rigida* spreaded in the interval 5.9min to 7.16min, followed by ABA appeared in early Rt interval from 8.19 & 8.48min to 9.50min, finally GAs were at 21.96min to 47.12min (Fig. 2). Separated auxins of *Ulva lactuca* were recorded at Rt between 5.8min and 6.5min, ABA were at 13.56+14.29min followed by GAs at interval between 20.91min to 55.73min (Fig. 3). The Total concentration (%) of endogenous hormones was recorded in the studied seaweeds (Fig. 4). *Ulva rigida* registered the highest total concentration (84.78%),
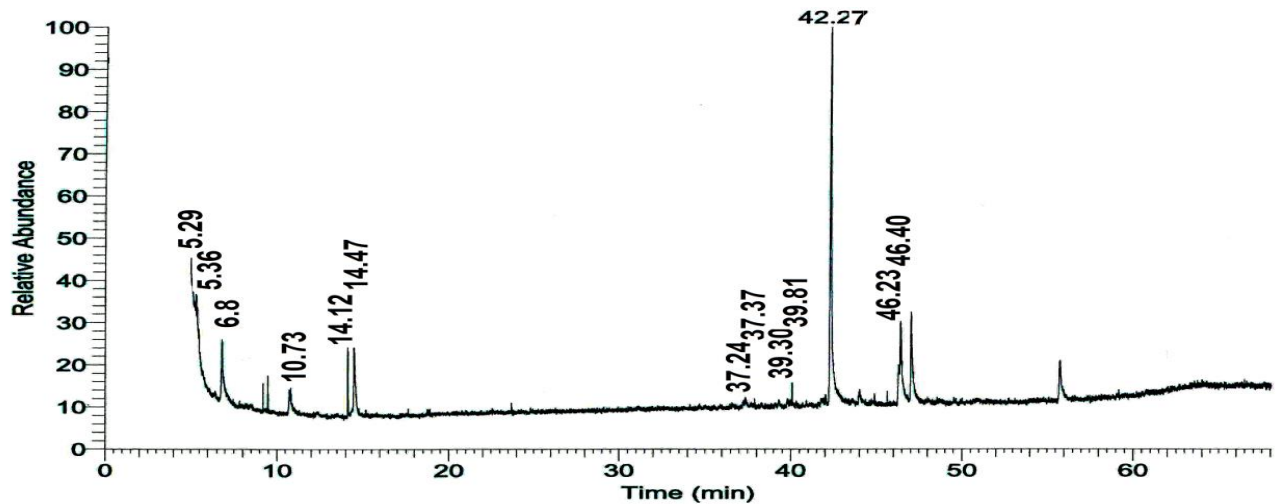
Figure 1. GC/MS showing the separated of phytohormones in *Sarconema filiforme*
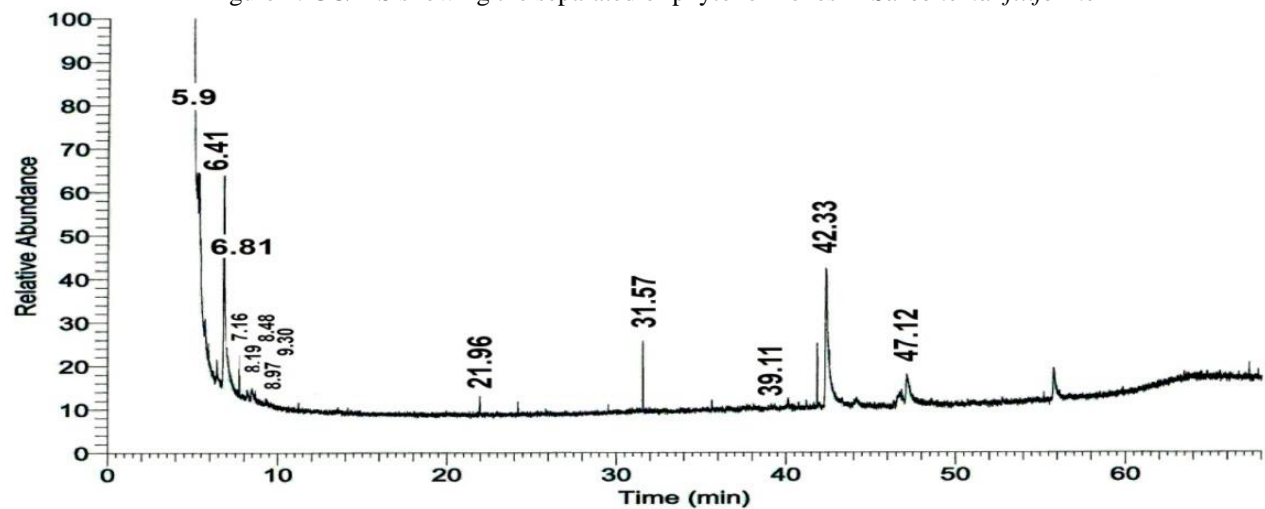

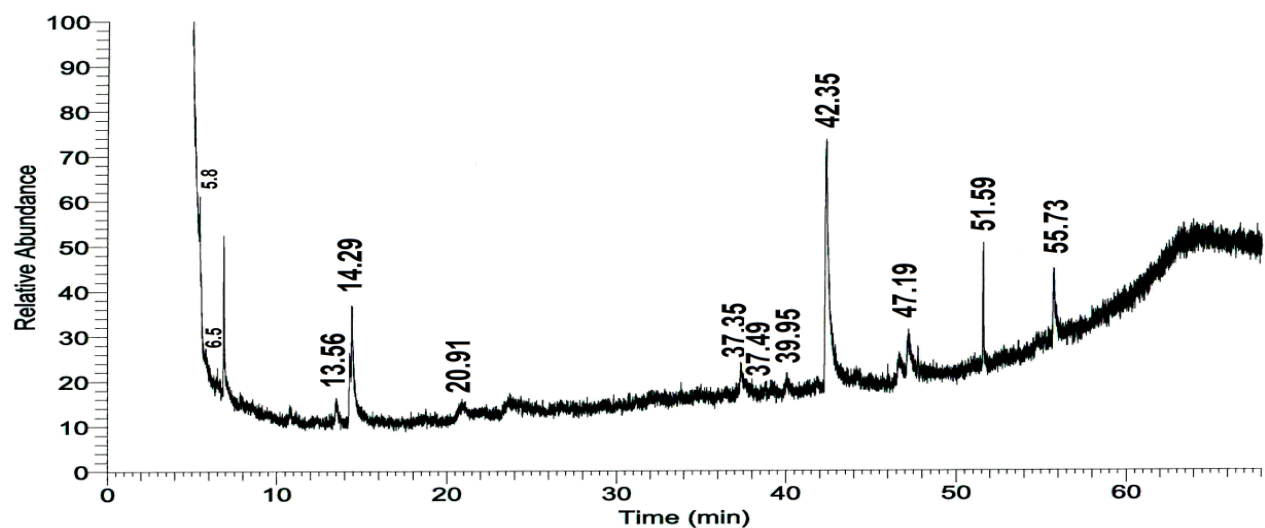Figure 2. GC/MS showing the separated of phytohormones in *Ulva rigida*

Figure 3. GC/MS showing the separated of phytohormones in *Ulva lactuca*

followed by *Sarconema filiforme* (76.78%) and *Ulva lactuca* (75.92%). The fluctuation in the total concentration percentage of each phytohormones types (auxins, ABA and GAs) was summarized in the studied species as in Figure 5, where the total concentration percentage of auxins in *Ulva rigida* registered the highest record (36.44%) followed by *Sarconema filiforme* (13.21%) *and the lowest one Ulva lactuca* (0.95%). *Sarconema filiforme* was characterized by poorly content of total ABA (2.91%), followed by *Ulva rigida* registered the medium record (9.31%), while *Ulva lactuca* gained the highest one (19.77). On the other hand, *Sarconema*
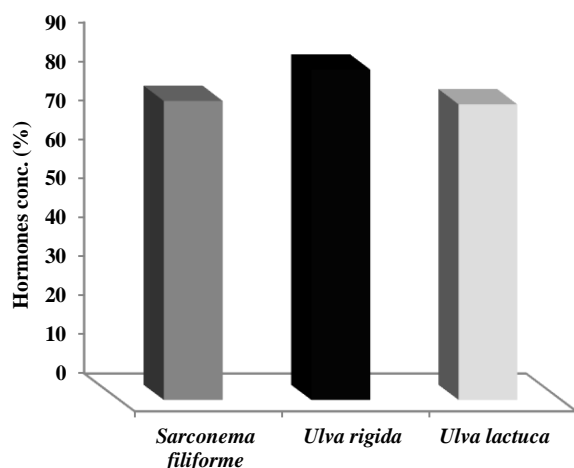
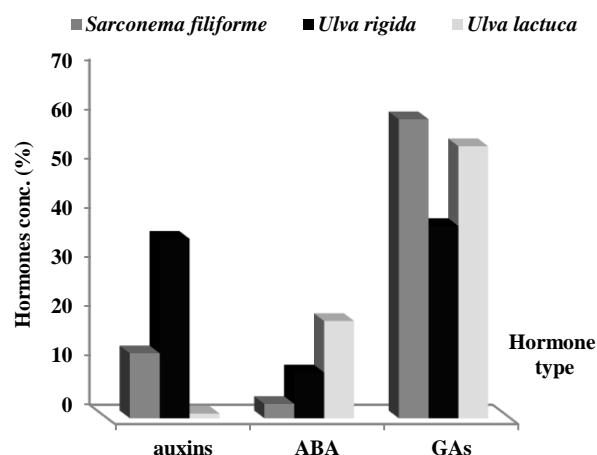Figure 4. Total Endogenous hormones concentration (%) in the studied seaweeds

Figure 5. Total Auxins, ABA and Gas concentration (%) in the studied seaweeds

filiforme regesterd the highest total concentration of GAs (60.66%), *Ulva lactuca* (55.20%) and lowest one was *Ulva rigida* (38.83%). Table 1 summarized the kinds of endogenous phytohormone compounds, retention time (Rt), molecular weight (M.W.), molecular formula (M.F.) and concentration (%).

Auxins profile
Auxins profile characterized in the marine algae *Sarconema filiforme* and *Ulva* concentration in *Ulva rigida* (34.42%). Indole-3-acetyl-L-leucine methyl ester and Indole-3-butyryl-L-valine were the main components of auxins profile in *Sarconema filiforme* recording 7.78 % and 4.44 % correspondingly. The brackish green alga *Ulva lactuca* recorded two different auxin compounds in lower concentration than the other marine species as 4-chloro indol-3-acetic acid (0.38%) and 3-indoxyl-β-D-Galacato pyronoside methyl ester (0.57%). Mass spectrum and fragmentation patterns of auxins for the characteristic compounds analyzed in the studied algal species are shown in Figure 6. The mass spectrum of Indole-3-acetyl-L-isoleucine, 4-chloro indol-3-acetic acid and 3-indoxyl-β-D-Galacto pyronoside methyl ester appeared their base beak for molecular at *m/z* 288, 209 and 310 correspondingly. Other auxin compounds shared the same molecular weight at *m/z* 302 as Indole-3-acetyl-L-isoleucine methyl ester, Indole-3-acetyl-L-leucine methyl ester and Indole-3-butyryl-L-valine.

ABA profile
In the ABA profile (Table 1), *cis,trans*-Abscisic acid-L-Alanine methyl ester were found in the marine algae *Sarconema filiforme* in concentration percentage (0.54 & 2.37%) and *Ulva rigida* (3.76 & 4.41%), while *cis, trans*-Abscisic acid-L-Valine recorded only in *Ulva rigida* (0.10 & 1.04%). *cis, trans*- Abscisic acid-L-Alanine singly registered in the brackish green alga *Ulva lactuca* in high concentration (1.29+18.48%). Figure 7 showed mass spectrum and fragmentation patterns for the isolated abscisic acids in each of studied algal species. The mass spectrum for *cis, trans*- Abscisic acid-L-Alanine methyl ester; *cis, trans*- Abscisic acid-L-Alanine and *cis, trans*-

Abscisic acid-L-Valine recorded molecular base peak at *m/z*349, 335 and 363 respectively.

Gibberellins profile
Gibberellins profile of the studied algal species (Table 1) contained 14 compounds of Gibberellin derivatives: GA_1, GA_1 methyl ester, GA_4, GA_7 methyl ester, GA_8, GA_{13}, GA_{19}, GA_{23}, GA_{29}, GA_{44}, GA_{75}, Kaurene, Kaurenoic acid and Kaurenal methyl ester. GC_mass analysis showed that, GAs content of the marine red alga *Sarconema filiforme* contains 7 Gibberellin derivatives GA_4, GA_{13}, GA_{23}, GA_{29}, Kaurene, Kaurenoic acid and Kaurenal methyl ester. Kaurene recorded the highest concentration (53.93%) in *Sarconema filiforme* followed by GA_{23} (2.28%), then GA_{13} (1.51%). GAs content of the marine green alga *Ulva rigida* involved on 5 Gibberellin derivatives GA_1, GA_1 methyl ester, GA_8, $GA_{13}$and GA_{23}. GA_{13} (31.63%) was the main concentration in *Ulva rigida*, followed by GA_{23} (6.62%). *Ulva lactuca* was represented by 8 Gibberellin derivatives like GA_7 methyl ester, GA_8, GA_{13}, GA_{19}, GA_{23}, GA_{44}, GA_{75} and Kaurene. The highest concentrations of GAs in *Ulva lactuca* were Kaurene (34.63%), then GA_{23} (11.33%), GA_{19} (3.40%), GA_{75} (2.66%) and GA_7 methyl ester (1.15%). This study demonstrated the mass spectrum and their fragmentation patterns of gibberellin derivatives for each algal species as in Figure 8. The mass spectrum recorded the molecular base peaks of the gibberellin derivatives at *m/z* 348, 362, 332, 344, 364, 378, 362, 378, 348, 346, 380, 272 for GA_1, GA_1 methyl ester, GA_4, GA_7 methyl ester, GA_8, GA_{13}, GA_{19}, GA_{23}, GA_{29}, GA_{44}, GA_{75} and Kaurene in that order. The main molecular base peak at *m/z* 302 was for both of Kaurenoic acid and Kaurenal methyl ester. In comparison, we found the studied algal species only shared in GA_{13} and GA_{23} and the rest GAs was differ. *Ulva rigida* registered the maximum concentration of GA_{13} (31.63%), then *Sarconema filiforme* (1.51%), and the lowest one *Ulva lactuca* (0.56%). GA_{23} recorded high concentration in *Ulva lactuca* (11.33%), followed by *Ulva rigida* (6.62%), and finally *Sarconema filiforme* (2.28%). On the other hand, there was further GAs shared between two seaweed species as GA_8 and Kaurene. GA_8 was confirmed in *Ulva lactuca* (0.53%) and *Ulva rigida*

Table 1. The types of phytohormone compounds, retention time (Rt), molecular weight (M.W.), molecular formula (M.F.) and concentration percentage (%) in the studied species

Compound name	M.W.	M. Formula	*Sarconema filiforme*		*Ulva rigida*		*Ulva lactuca*	
			Conc.(%)	R_t (min)	Conc.(%)	R_t (min)	Conc.(%)	R_t (min)
Auxins profile								
Indole-3-acetyl-L-isoleucine	288	$C_{16}H_{20}N_2O_3$	0.45	5.29	34.42	6.81	-	-
Indole-3-acetyl-L-isoleucine methyl ester	302	$C_{17}H_{22}N_2O_3$	0.54	5.36	1.67	6.41	-	-
Indole-3-acetyl-L-leucine methyl ester	302	$C_{17}H_{22}N_2O_3$	7.78	6.8	0.13	7.16	-	-
Indole-3-butyryl-L-valine	302	$C_{17}H_{22}N_2O$	4.44	10.73	0.22	5.9	-	-
4-chloro indol-3-acetic acid	209	$C_{10}H_8ClNO_2$	-	-	-	-	0.38	5.8
3-indoxyl-β-D-Galacto pyronoside methyl ester	310	$C_{15}H_{22}NO_6$	-	-	-	-	0.57	6.5
Abscisic acids profile								
cis, trans- Abscisic acid-L-Alanine methyl ester	349	$C_{18}H_{27}NO_5$	0.54 2.37	14.12+ 14.47	3.76 4.41	8.19+ 8.48	-	-
cis, trans- Abscisic acid-L-Alanine	335	$C_{18}H_{25}NO_5$	-	-	-	-	1.29 18.48	13.56+ 14.29
cis, trans- Abscisic acid-L-Valine	363	$C_{20}H_{29}NO_2$	-	-	0.10 1.04	8.67+ 9.30	-	-
Gibberellins profile								
GA_1	348	$C_{19}H_{24}O_6$	-	-	0.27	21.96	-	-
GA_1 methyl ester	362	$C_{20}H_{26}O_6$	-	-	0.20	39.11	-	-
GA_4	332	$C_{19}H_{24}O_5$	0.77	39.30	-	-	-	-
GA_7 methyl ester	344	$C_{20}H_{24}O_5$	-	-		-	1.15	51.59
GA_8	364	$C_{19}H_{24}O_7$	-	-	0.31	31.57	0.53	37.49
GA_{13}	378	$C_{20}H_{26}O_7$	1.51	46.23	31.63	42.33	0.56	39.95
GA_{19}	362	$C_{20}H_{26}O_6$	-	-	-	-	3.40	37.35
GA_{23}	378	$C_{20}H_{26}O_7$	2.28	46.40	6.62	47.12	11.33	55.73
GA_{29}	348	$C_{19}H_{24}O_6$	0.99	39.81	-	-	-	-
GA_{44}	346	$C_{20}H_{26}O_5$	-	-	-	-	0.94	20.91
GA_{75}	380	$C_{19}H_{24}O_8$	-	-	-	-	2.66	47.19
Kaurene	272	$C_{20}H_{32}$	53.93	42.27	-	-	34.63	42.35
Kaurenoic acid	302	$C_{20}H_{30}O_2$	0.58	37.24	-	-	-	-
Kaurenal methyl ester	302	$C_{21}H_{34}O$	0.60	37.37	-	-	-	-

(0.31%). Kaurene recoded high levels in *Sarconema filiforme* (53.93%) and *Ulva lactuca* (34.63%).

DISCUSSION
The physiological activities of plant growth hormones found in seaweed extracts are mainly responsible for plant growth stimulation and increase of the photosynthesis intensity[36,3]. In the present study, endogenous phytohormones profile (auxin, gibberellins and abscisic acids) was studied in detail. The order of hormonal content was markedly dependent on the algal species and abiotic stress in this study. GC/MS technique was used as a method to determine the separated phytohormones and their concentrations percentage, followed by mass spectrometry to determine their molecular weights and fragmentation pattern. The phytohormones analysis of the studied species showed that the marine green alga *Ulva rigida* registered the highest concentration, followed by the marine red alga *Sarconema filiforme*. The brackish green alga *Ulva lactuca* recorded very low total concentration of auxins. The brackish green alga *Ulva lactuca* showed widely potential differences between low content of total auxins and its high content of gibberellins. So *Ulva lactuca* takes large sizes in brackish water of Timsah Lake. This may attributed to eutrophication environmental condition of the brackish green alga *Ulva lactuca* . In this regard, El Shoubaky and Salem[25] mentioned that eutrophication process in Timsah Lake which its nutrient enrichment stimulates the algal growth and increases the sediment fertility at spring season. The macroalgae in Timsah Lake reached to be more than one meter in length[37-39]. Tarakhovskaya *et al.*[3] mentioned that other plant hormones
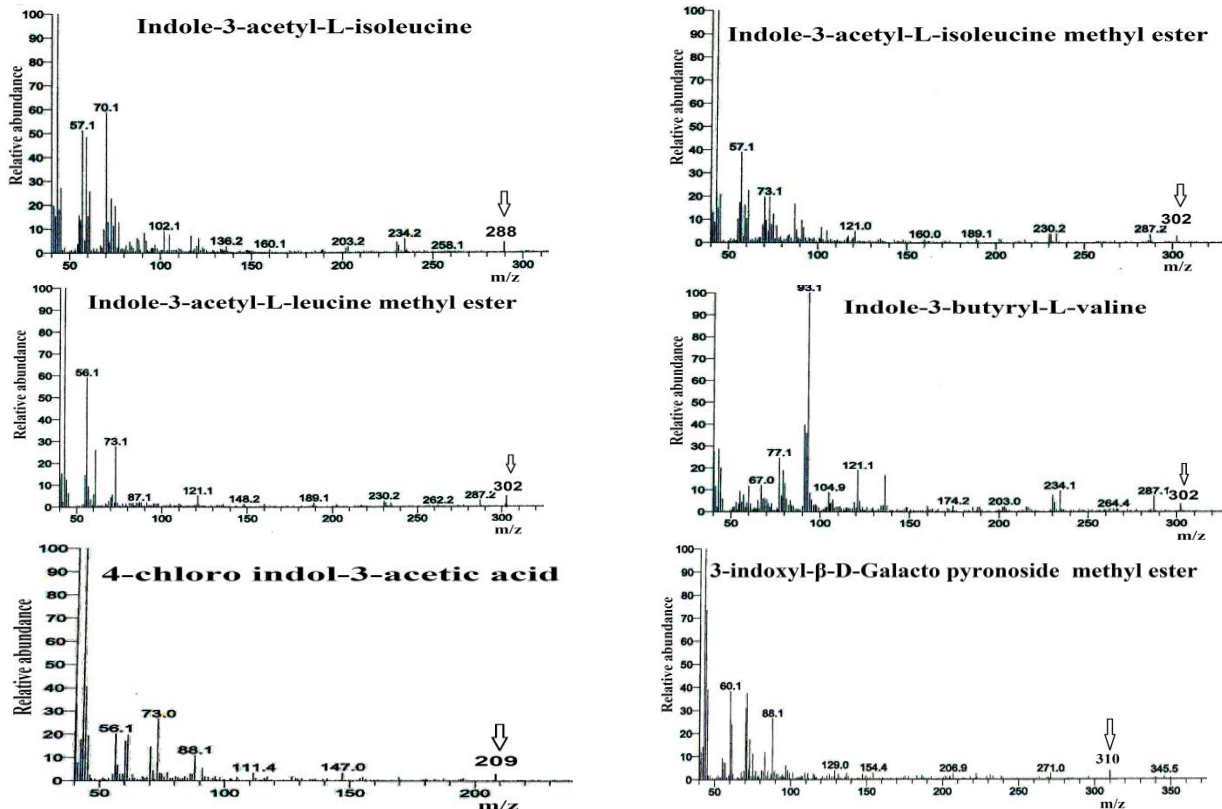
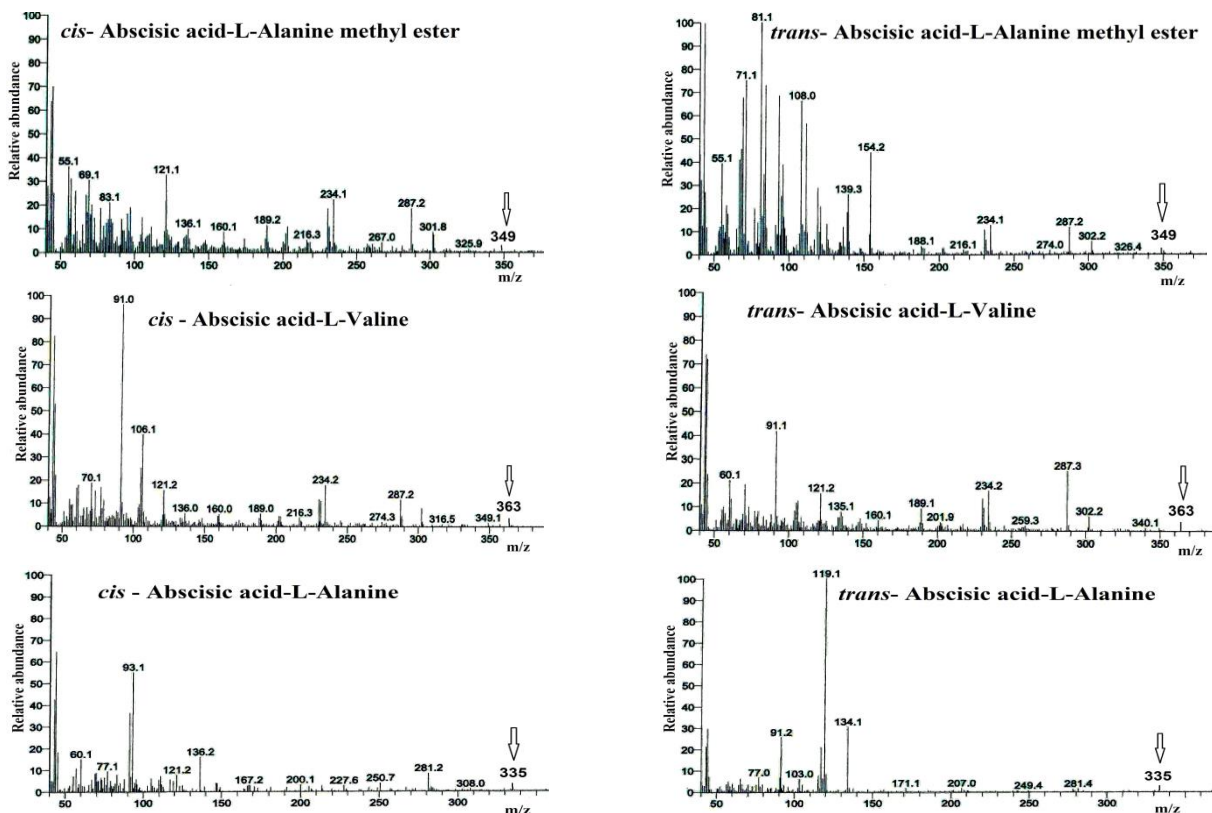Figure 6. Mass spectrum and fragmentation patterns of auxins in the studied algal species

Figure 7. Mass spectrum and fragmentation patterns of Abscisic acids in the studied algal species

present in seaweed extracts auxins, were shown to initiate root formation and inhibit its elongation. Some data indicate that auxin controls thallus branching of red and brown macrophytes on the principle of apical dominance[40], plays an important role in the formation of *Fucus* reproductive structures [41] and stimulate rhizoid formation in the green alga *Bryopsis plumose*[3]. In red macrophytes, treatment with natural or synthetic auxins accelerated
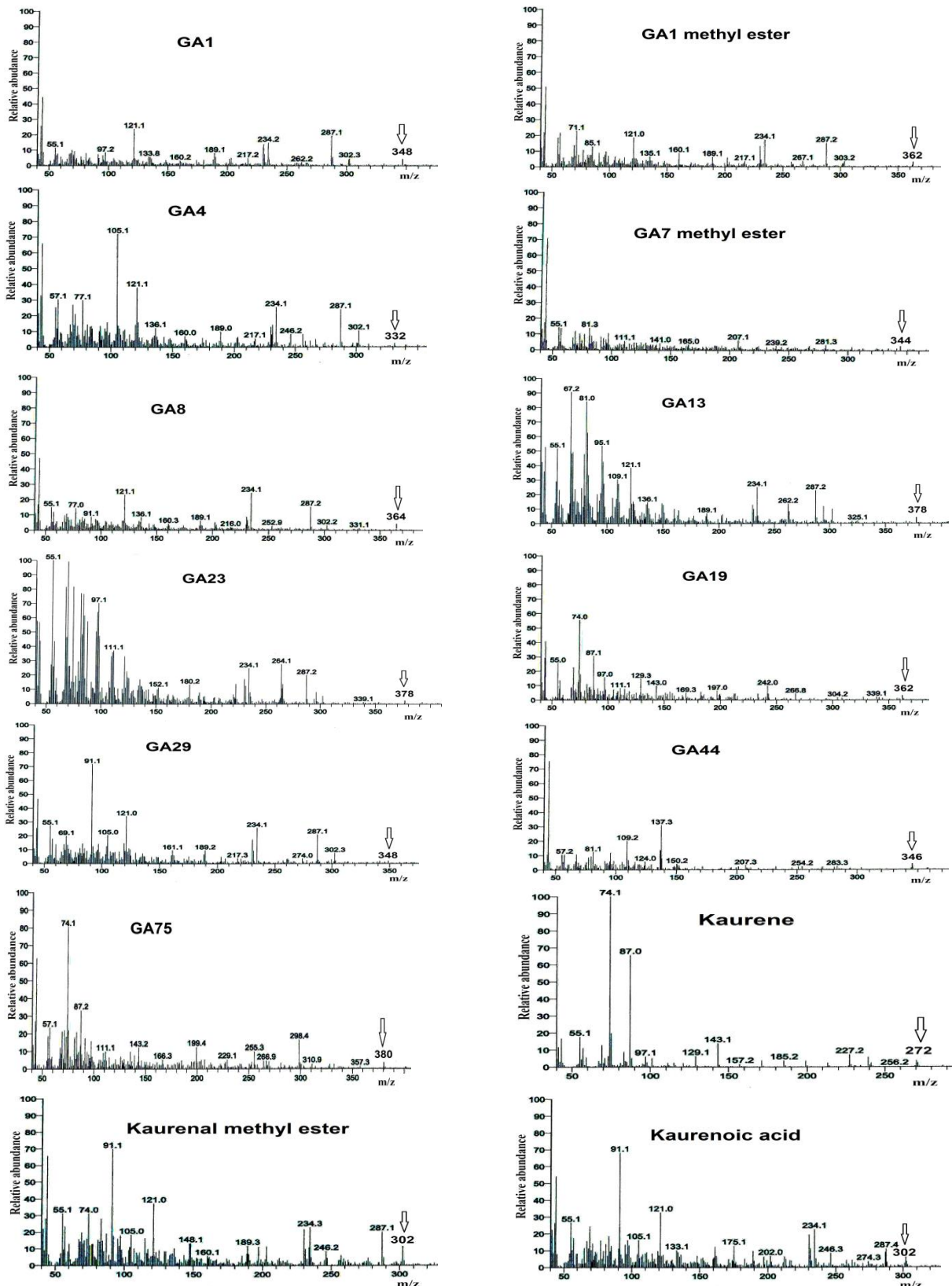
Figure 8. Mass spectrum and fragmentation patterns of gibberellins in the studied algal species

tissue growth in the culture and callus development[42]. Shanab *et al.*[43] showed that the hormonal function of auxins appears in the structurally more complex land plants, the highly differentiated marine algae and in structurally simple Prokaryotic Cyanobacteria. Auxins

profile in this study shared the same compounds in the marine algae *Sarconema filiforme* and *Ulva rigida*. Indole-3-acetyl-L-isoleucine was the highest concentration in *Ulva rigida* whereas Indole-3-acetyl-L-leucine methyl ester and Indole-3-butyryl-L-valine were the main

concentration in *Sarconema filiforme*. The brackish green alga *Ulva lactuca* recorded two different auxin compounds in low concentration than the other marine species as 4-chloro indol-3-acetic acid and 3-indoxyl-β-D-Galacto pyronoside methyl ester. This may attributed to eutophication environmental condition of the brackish green alga *Ulva lactuca*. Yokoya *et al.*[44] mentioned that IAA is the most frequently detected auxin in plants, and its concentration is regulated by a balance between biosynthesis, conjugation, and degradation[45]. IAA conjugation facilitates storage, transport and protection from peroxidation and catabolism and free IAA is formed by the hydrolysis of these conjugates[46]. Ester conjugates are formed when IAA links with sugars, and amide conjugates are formed by IAA links with amino acids and small peptides[45]. Our study recorded IAA linked with sugars as 3-indoxyl-β-D-Galacto pyronoside methyl ester in *Ulva lactuca*.[47,36] revealed the presence indole-3-acetic acid (IAA) in the extract from *Ascophyllum nodosum*, *Porphyra perforata*, *Botryocladia* spp., *Enteromorpha* sp. and in Cyanobacteria. In the green alga *Caulerpa paspaloides*, IAA and the product of its catabolism, dioxyindole-3-acetic acid, were detected[23]. In contrary, *Ulva lactuca* in our study registered high levels of ABA than *Ulva rigida* while *Sarconema filiforme* recorded the minimum one. Increasing of ABA concentration level in brackish green alga *Ulva lactuca* may be due to eutrophication conditions stress in Timsah Lake. This is agreement with Taylor *et al.*[48]. They mentioned that ABA concentrations increase under stressful environmental conditions in vascular plants and involved in a number of reactions to protect the plant from abiotic stress. Stirk *et al.*[49] showed that the microalgae *Dunaliella parva* and *Draparnaldia mutabilis* were grown in conditions of salt stress and increased pH, endogenous ABA levels increased[20] whereas ABA levels were generally higher in *Ulva fasciata* compared with *D. humifusa*. This may be due to *U. fasciata* being collected from a rock pool situated in the upper intertidal zone where it would be exposed to more extreme environmental changes compared to *D. humifusa* that was collected from a gully in the mid-intertidal zone. Stirk and Tarkowská[50] reported that in favourable conditions, ABA acts as a negative growth regulator inhibiting seed germination and post-germination growth[51]. However, ABA is an important stress hormone, increasing in concentration in response to aabiotic stress to trigger various physiological processes to improve the plants'chances of survival[51]. The ABA profile in the present study composed of *cis,trans*-Abscisic acid-L-Alanine methyl ester which found in the marine algae *Sarconema filiforme* and *Ulva rigida*, in addition to *cis, trans*- Abscisic acid-L-Valine recorded only in *Ulva rigida*. High concentration of *cis, trans*- Abscisic acid-L-Alanine only characterized in the brackish green alga *Ulva lactuca*. On the other hand, GAs in our study recorded high concentration in *Sarconema filiforme* more than *Ulva lactuca* and finally *Ulva rigida*. We identified at least 14 types of gibberellins. Endogenous Gibberellins profile in the studied algal species was created by GA_1, GA_1 methyl ester, GA_4, GA_7 methyl ester, GA_8, GA_{13}, $GA_{1\ 9}$, GA_{23},

GA_{29}, GA_{44}, GA_{75}, Kaurene, Kaurenoic acid and Kaurenal methyl ester. Actually, the bioactive GAs of them was GA_1, GA_4 and may be GA_7 methyl ester. Stirk and Tarkowská[50] mentioned that over 100 GAs have been identified in plants, but only a few are biologically active, e.g. GA_1, GA_3, GA_4, GA_5, GA_6 and GA_7. The remainder is either biosynthetic precursors or deactivation metabolites[52]. Tarakhovskaya *et al.*[3] and Stirk *et al.*[53] reported that in algae of GA-like activity based on bioassays. The structures of the biologically active GAs, those capable of evoking a physiological response, vary greatly [54]. Generally, the C19- GAs has a higher activity than the C20-GAs[55]. Of the C19-GAs, those which are characterized by 3p-hydroxylation, 3(3, 13-dihydroxylation and 1, 2-unsaturation tend to exhibit maximal biological activity[55]. This study showed that the marine red alga *Sarconema filiforme* recorded seven GAs. Kaurene was the highest concentration. GAs in the marine green alga *Ulva rigida* were characterized by five GAs and GA_{13} was the main concentration level. *Ulva lactuca* recorded eight GAs and Kaurene was the highest concentration. We found that GA_{13} and GA_{23} only shared between the three studied algal species. Another GAs shared between two seaweed species as GA_8 and Kaurene. GA_8 was confirmed in *Ulva lactuca* and *Ulva rigida* while Kaurene recoded high levels in *Sarconema filiforme* and *Ulva lactuca*. The activity of each compound increases with its presumed position in a biosynthetic pathway leading from kaurene to GA_3[56]. The latest our Kaurene results may be agree with Stirk *et al.*[57]. They explained that in plants, GAs are formed from geranylgeranyl diphosphate via a number of intermediates such as ent-kaurene, which are oxidized to form GA_{12}ald that is then converted to GA_{12}. GA_{12} is modified by various oxidation steps to produce biologically active forms with these final steps in the GA biosynthetic pathways being species specific. The main pathways in plants are either $GA_{12}{\rightarrow}GA_{15}{\rightarrow} GA_{24} \rightarrow GA_9 \rightarrow GA_4 \rightarrow GA_{34}$ or $GA_{12} \rightarrow GA_{53}{\rightarrow} GA_{44} \rightarrow GA_{19} {\rightarrow}GA_{20}{\rightarrow}GA_1$. As an alternative regulatory pathway, GA_{19} may be hydroxylated to produce the inactive GA_{13}[58]. The present study was observed that there was an increase in total GAs than auxins concentration in marine studied species. This may attributed to water salinity stress. Also, in the brackish green alga *Ulva lactuca*, there was wide differentiation between auxins and GAs. This may be due to eutrophication stress in Timsah Lake. In this regard Weiss and Ori[58], Yamaguchi[52] mentioned that there is cross talk in most cases between GAs and other hormones such as positive interactions with auxin to promote cell expansion and differentiation and root elongation; antagonistic interactions with ABA to promote germination, growth and flowering; positive or negative interactions depending on developmental and environmental conditions with the stress-related ethylene and negative feedback with cytokinins. In fact, there is evidence that seaweed extract-induced, aabiotic stress tolerance is due in part, to the cumulative effect of plant growth regulator (PGR)- like activities, biostimulants (or biological elicitors), osmo-protectants and other compounds that can elicit antioxidant

activity[60]. The concentration of growth-stimulating compounds in different seaweed extracts was variable, which could also have variable effects on the plant responses[25,61].

CONCLUSION

The characteristic isolated phytohormones content (auxins, ABA and GAs) in the studied species *Sarcodnema filiforme*, *Ulva rigida* and *Ulva lactuca* were determined by using GC-MS technique. The phytohormones analysis of the studied species showed that the marine green alga *Ulva rigida* registered the highest concentration, followed by the marine red alga *Sarconema filiforme*, while brackish green alga *Ulva lactuca* recoded very low total concentration of auxins and high ABA constituents. There was increasing in total GAs than auxins concentration in marine studied species. Also, in the brackish green alga *Ulva lactuca*, there was wide differentiation between auxins and GAs. The overall bioactivity of endogenous phytohormones attributed to seaweed extracts varied widely according to the type of seaweeds and their environmental condition stress.

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