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-butryl-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 (GA1, GA4, GA1 methyl ester, GA3, GA7, methyl ester, GA4, GA7, methyl ester, GA8, GA19, GA23, GA25, GA41, GA75, Kaurene, Kaurenoic acid and Kaurenal methyl ester). GA13 and GA23 only shared at the three studied algal species. GA8 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 senescence1,2. 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 indole3. 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)5,6, Caulerpa paspaloideis8, Ecklonia maxima9 and Undaria pinnatifida10. Other indole derivatives, such as indole-3-carboxylic acid (ICA), have been identified in Botryocladia leptopoda11, Prioniopsis lanceolata12. In various groups of algae, some compounds were repeatedly detected, which suppressed plant growth in bioassays as abscisic acids13. Abscisic acid (ABA) synthesized from carotenoids by more than 60 species of algae (e.g. Chlorella spp., Haemat- cococcus pluvialis). ABA is mostly responsible for synthesis of proteins required for the response to drought14. It is supposed that algae produce a complex responsible for growth inhibition consisting of several components which act as ABA in algae3, in addition, ABA plays an important role in controlling of the stomatal apparatus function and seed dormancy15. This hormone was found in green microalgae (Chlorella sp., Dunaliella salina, and Haematococcus pluvialis) and also in the thallus of brown macrophytes from the genus Ascophyllum nodosum16 and some species of Laminaria17,19. Chlorophyta species such as Chara foetida, Draparnaldia mutabilis, Ulva lactuca20. Among plant hormones, gibberellins were also isolated from seaweed extracts. Gibberellins (GAs) were identified in extracts from Fucus vesiculosus, Fucus spiralis and Ascophyllum nodosum14. The main role of gibberellins is to initiate seeds germination, stem elongation, leaf expansion, flower development and fruit maturity21. Active gibberellins, GA1 and GA3, and inactive GA13 were isolated chromatographically from the tissue extracts of Fucus.

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vesiculosus and F. spiralis. The substances with gibberellin activity were also isolated from the green Alga Caulerpa pascaloides, but they were not precisely identified. Seaweed extracts are used commercially as growth stimulants in agricultural crops due to the plant hormones present. El Shoubaky and Salem 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. Several methods have been developed for the analysis of phytohormones. Gas chromatography–mass spectrometry (GC–MS) is a well recognized technique for the determination of phytohormones. There are still difficulties in the analysis of some plant hormones by GC because certain phytohormones are thermally unstable. 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. 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‰) (30.5667°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. 30 g of each fresh seaweed 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% HCl (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 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% HCl 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 analysis. 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/MS analysis. Figs. 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 interval ranged between 5.9min to 7.16min, followed by ABA and GAs at interval between 20.91min to 55.73min. The Total concentration (%) of endogenous hormones was recorded in the studied seaweeds (Fig. 4). Ulva rigida registered the highest total concentration (84.78%).
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*
Ulva lactuca

Ulva rigida

ABA
GAs

Ulva rigida

ies as

+ 0.57

ester;

spectrum

abscisic acids in each of studied algal species.

spectrum

registered in & 1.04

2.37

Alanine methyl ester were found in the marine algae

In the ABA profile (Table 1),

ABA profile

Auxins profile

Gibberellins 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-butryl-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-Galacto pyroneside 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 pyroneside methyl ester appeared their base peak 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-butryl-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/z349, 335 and 363 respectively.

Gibberellins profile

Gibberellins profile of the studied algal species (Table 1) contained 14 compounds of Gibberellin derivatives: GA1, GA1 methyl ester, GA4, GA7 methyl ester, GA8, GA13, GA19, GA23, GA26, GA44, GA75, Kaurene, Kaurenolic acid and Kaurenoic acid and Kaurenoic acid esters. 

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

Figure 4. Total Endogenous hormones concentration (%) in the studied seaweeds

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

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

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

<table>
<thead>
<tr>
<th>Compound name</th>
<th>M.W.</th>
<th>M. Formula</th>
<th>Sarconema filiforme</th>
<th>Ulva rigida</th>
<th>Ulva lactuca</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Conc.(%)</td>
<td>Rt(min)</td>
<td>Conc.(%)</td>
<td>Rt(min)</td>
<td>Conc.(%)</td>
</tr>
<tr>
<td><strong>Auxins profile</strong></td>
<td></td>
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<tr>
<td>Indole-3-acetylL-</td>
<td>288</td>
<td>C_{18}H_{26}N_{2}O_{3}</td>
<td>0.45</td>
<td>5.29</td>
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<td>isoalleucine methyl ester</td>
<td>302</td>
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<td>5.36</td>
<td>1.67</td>
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<tr>
<td>Indole-3-acetylL-</td>
<td>302</td>
<td>C_{17}H_{22}N_{2}O_{3}</td>
<td>7.78</td>
<td>6.8</td>
<td>0.13</td>
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<tr>
<td>leucine methyl ester</td>
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<td></td>
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<tr>
<td>Indole-3-butyrylL-</td>
<td>302</td>
<td>C_{17}H_{22}N_{2}O</td>
<td>4.44</td>
<td>10.73</td>
<td>0.22</td>
</tr>
<tr>
<td>Valine</td>
<td>209</td>
<td>C_{10}H_{19}ClN_{2}O_{3}</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>3-Indolyl-β-D-Galactopyranoside methyl ester</td>
<td>310</td>
<td>C_{12}H_{22}NO_{6}</td>
<td>-</td>
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<td>-</td>
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<tr>
<td><strong>Abscisic acids profile</strong></td>
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<tr>
<td>cis, trans- Abscisic acid-L-Alanine methyl ester</td>
<td>349</td>
<td>C_{18}H_{27}NO_{3}</td>
<td>0.54</td>
<td>14.12+</td>
<td>3.76</td>
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<tr>
<td>cis, trans- Abscisic acid-L-Alanine</td>
<td>335</td>
<td>C_{18}H_{26}NO_{3}</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>cis, trans- Abscisic acid-L-Valine</td>
<td>363</td>
<td>C_{20}H_{28}NO_{2}</td>
<td>-</td>
<td>-</td>
<td>0.10</td>
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<td><strong>Gibberellins profile</strong></td>
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<tr>
<td>GA_{1}</td>
<td>348</td>
<td>C_{19}H_{24}O_{6}</td>
<td>-</td>
<td>-</td>
<td>0.27</td>
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<tr>
<td>GA_{1} methyl ester</td>
<td>362</td>
<td>C_{20}H_{26}O_{6}</td>
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<td>0.20</td>
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<tr>
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<td>GA_{8}</td>
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<td>C_{19}H_{23}O_{7}</td>
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<td>-</td>
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<tr>
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<td>46.23</td>
<td>31.63</td>
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<tr>
<td>GA_{19}</td>
<td>362</td>
<td>C_{20}H_{26}O_{6}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GA_{23}</td>
<td>378</td>
<td>C_{20}H_{26}O_{7}</td>
<td>2.28</td>
<td>46.40</td>
<td>6.62</td>
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<td>GA_{29}</td>
<td>348</td>
<td>C_{19}H_{23}O_{6}</td>
<td>0.99</td>
<td>39.81</td>
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<tr>
<td>GA_{44}</td>
<td>346</td>
<td>C_{20}H_{25}O_{5}</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GA_{77}</td>
<td>380</td>
<td>C_{19}H_{23}O_{8}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kaurene</td>
<td>272</td>
<td>C_{20}H_{12}</td>
<td>53.93</td>
<td>42.27</td>
<td>-</td>
</tr>
<tr>
<td>Kaurenoic acid</td>
<td>302</td>
<td>C_{20}H_{12}O_{2}</td>
<td>0.58</td>
<td>37.24</td>
<td>-</td>
</tr>
<tr>
<td>Kaurenoic methyl ester</td>
<td>302</td>
<td>C_{21}H_{12}O</td>
<td>0.60</td>
<td>37.37</td>
<td>-</td>
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</table>

(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. 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 mentioned that eutrophication process in Timsah Lake which its nutrient enrichment stimulates the algal growth and increases the sediment fertility at spring season. The macroalga in Timsah Lake reached to be more than one meter in length. Tarakhovskaya et al. mentioned that other plant hormones...
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\textsuperscript{40}. plays an important role in the formation of \textit{Fucus} reproductive structures \textsuperscript{41} and stimulate rhizoid formation in the green alga \textit{Bryopsis plumose}\textsuperscript{3}. In red macrophytes, treatment with natural or synthetic auxins accelerated
tissue growth in the culture and callus development. Shanab et al. 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

Figure 8. Mass spectrum and fragmentation patterns of gibberellins in the studied algal species
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-[beta]-D-Galactopyranoside methyl ester. This may attributed to eutrophication environmental condition of the brackish green alga *Ulva lactuca*. Yokoya et al. mentioned that IAA is the most frequently detected auxin in plants, and its concentration is regulated by a balance between biosynthesis, conjugation, and degradation. IAA conjugation facilitates storage, transport and protection from peroxidation and catabolism and free IAA is formed by the hydrolysis of these conjugates. Ester conjugates are formed when IAA links with sugars, and amide conjugates are formed by IAA links with amino acids and small peptides. Our study recorded IAA linked with sugars as 3-indoxyl-[beta]-D-Galactopyranoside methyl ester in *Ulva lactuca*. Revealed the presence indole-3-acetic acid (IAA) in the extract from *Ascoscypha nodosum*, *Porphyra perforata*, *Botryocladia spp.*, *Enteromorpha sp.* and in Cyanobacteria. In the green alga *Caulerpa paspaloides*, IAA and the product of its catabolism, dioxygenol-3-acetic acid, were detected. 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 eutrophic conditions stress in Timsah Lake. This is agreement with Taylor et al. 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. showed that the microalgae *Dunaliella parva* and *Draparnaldia mutabilis* were grown in conditions of salt stress and increased pH, endogenous ABA levels increased 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 with *D. humifusa* that was collected from a gully in the mid-intertidal zone. Stirk and Tarkowska reported that in favourable conditions, ABA acts as a negative growth regulator inhibiting seed germination and post-germination growth. However, ABA is an important stress hormone, increasing in concentration in response to abiotic stress to trigger various physiological processes to improve the plants’ chances of survival. 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 GA1, GA3 methyl ester, GA4, GA7 methyl ester, GA8, GA13, GA9, GA23, GA29, GA44, GA53, Kaurene, Kaurenoic acid and Kaurenal methyl ester. Actually, the bioactive GAs of them was GA1, GA4 and may be GA1 methyl ester. Stirk and Tarkowska mentioned that over 100 GAs have been identified in plants, but only a few are biologically active, e.g. GA1, GA3, GA4, GA5, GA6, and GA7. The remainder is either biosynthetic precursors or deactivation metabolites. Tarakhovskaya et al. and Stirk et al. 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. Generally, the C19-GAs has a higher activity than the C20-GAs. 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. 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 GA13 was the main concentration level. *Ulva lactuca* recorded eight GAs and Kaurene was the highest concentration. We found that GA13 and GA23 only shared between the three studied algal species. Another GAs shared between two seaweed species as GA1 and Kaurene. GA1 was confirmed in *Ulva lactuca* and *Ulva rigida* while Kaurene recorded 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 GA3. The latest our Kaurene results may be agree with Stirk et al. 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 GA12 and that is then converted to GA12. GA12 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 GA12 → GA15 → GA34 → GA5 → GA3 → GA12 or GA12 → GA53 → GA44 → GA10 → GA30 → GA1. As an alternative regulatory pathway, GA19 may be hydroxylated to produce the inactive GA13. 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, Yamaguchi 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, abiotic 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. The concentration of growth-stimulating compounds in different seaweed extracts was variable, which could also have variable effects on the plant responses.

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 Sarcodnema filiforme, while brackish green alga Ulva lactuca recorded 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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REFERENCES


