Hypoglycemic and Cytotoxic Effects of the Brown Alga Cystoseira Trinodis and the Blue-Green Alga Microcystis Aeruginosa Extracts

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ABSTRACT
The present study aimed to determine the hypoglycemic, cytotoxic effects and phytochemical constituents of Cystoseira trinodis and Microcystis aeruginosa extracts. The GC-MS and 1H NMR analysis of C. trinodis and M. aeruginosa extracts revealed the presence of fatty acids, steroids, terpenoids and vitamins which may play an antidiabetic role. The cytotoxic effects of those extracts were tested on the tumor cell lines (HepG2) and the hypoglycemic effects were investigated in alloxan-induced hyperglycemic mice. Fasting blood glucose level showed significant decreased on days 1, 3, 10, 17, 24 and 31 groups treated with C. trinodis extract, compared to diabetic groups. The cytotoxic effect of C. trinodis petroleum ether extract displayed LD₅₀ value of 112 mg/ml, while chloroform extract displayed LD₅₀ value of 77 mg/ml. The two fractions of Microcystis aeruginosa had no cytotoxic effect on HepG2 cells.

Keywords: Antidiabetic, Cyanophyta, Cystoseira trinodis, Cytotoxicity, Microcystis aeruginosa, Natural products, Phaeophyta, Phytochemical constituents.

INTRODUCTION
The growth of human population leads to intensification of agriculture and promotes, through eutrophication, development of cyanobacteria. Cyanobacteria contribute significantly to primary productivity of some compounds that have potential biotechnological application and drug production (Muruga et al., 2014). Increased outbreaks of toxic cyanobacteria blooms events resulting from elevated water temperatures and extreme hydrologic events attributable to climate change and increased nutrient loadings associated with intensive agricultural practices (Lunetta et al., 2015). One of the most widespread and bloom-forming species in freshwater is toxic Microcystis aeruginosa (Rzymski et al., 2014). Marine algae are one of the largest producers of biomass in the marine environments (Bhadury and Wright, 2004). Various bioactive compounds are discovered, isolated and used from different classes of algae to develop novel pharmaceuticals (Parsaeimehr and Feng Chen, 2013). Algae contain useful substances including pigments (β carotene, astaxanthin, zeaxanthin, lutein, canthaxanthin, chlorophyll, phycocerythrin, fucoxanthin and phycocyanin), polyunsaturated fatty acids (docosahexaenoic acid, eicosapentaneoic acid and arachidonic acid), antioxidants (catalases, polyphenols, superoxide, dismutase and tocopherols), essential amino acids (leucine, aspartic acid, glutamic acid, phenylalanine, asparagine and glycine) and antimicrobials (tropolidithic acid, labdane diterpenes, brominated hydroquinones and phlorotannins) (Elena et al., 2012).

Phenolic-rich extracts from edible marine macro-algae had a potential antidiabetic effect (Nwosu et al., 2011). Brown algae were found to have secondary metabolites most of which are phenolic compound which had medicinal potentials (Abou-Elela et al., 2009) and fucoxanthin which exhibited antidiabetic activities (Maeda et al., 2007). Some species of Cystoseira had been studied for its hypoglycemic efficacy (Guiry et al., 2009). Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both (Loghmani, 2005). People with diabetes may develop serious complications such as heart disease, stroke, kidney failure, blindness and premature death (National Diabetes Statistics Report, 2014). Diabetes can be classified into type 1, type 2, gestational diabetes mellitus and specific types of diabetes due to other causes (Diabetes Care, 2015). It is considered a major health problem all over the world. Globally, the number of people who has been diagnosed with diabetes has exploded in the past two decades with 6% per annum increase and by 2025, about 324 million people will be diabetic (Mohammed et al., 2007). Diabetes is associated with increased risk of cancers of the liver, pancreas, endometrium, colon, breast and bladder (Suh and Kim, 2011). As a result, there is a need to search for effective, safe and better antidiabetic agents.

The aim of this study is to screen the phytochemical components, cytotoxicity and antidiabetic effect of the brown alga Cystoseira trinodis and the blue-green alga Microcystis aeruginosa extracts.

MATERIALS AND METHODS
Algal bloom of Microcystis aeruginosa with thick gelatinous massive growth layer (3-5 cm) was collected during autumn from El-Manzala Lake with plankton net of 10 μm mesh size. Cystoseira trinodis had been collected manually from the intertidal zone of Hurghada, Red Sea coast of Egypt. The algae were rinsed several time with saline water and finally with distilled water to remove epiphytes and sediments. The algal samples of both strains were then allowed to dry in air using string net. The dry samples were ground and the dry weight was evaluated and then stored in stoppered bottles in refrigerator (Perunnal et al. 2012).

Thin layer chromatography (TLC) was performed using pre-coated TLC plates (20x20cmx0.2mm) (Silica gel 60GF254). The material to be extracted was generally applied in appropriate solution of 0.1%
concentration by means of micropipette at starting point 1.5 cm apart from the lower edge of the plates. The plates were put in a previously cleaned and dried jar containing the suitable eluent. The plates were removed from the jars, allowed to dry spontaneously. The spots were viewed under 254 and 365 nm UV lamps.

The columns employed for this study were made of glass of different dimensions (100cm height and 5 cm radius) ending with tap to regulate the flow rates of liquids. A pad of cotton was inserted into the bottom of the tube to pack the column, the column was held vertically, the tap was closed and then filled with appropriate solvents, allowing it to drain through the pad to expel air bubbles. The column was filled with the silica gel 60, 70-230 mesh (E. Merck) and compacted then the extract was applied as a silica gel supported thin layer, another pad of cotton was inserted into the top of the column. The stationary phase (silica gel) was always covered with sufficient quantity of solvent and never allowed to dry. Then the column was eluted with different solvents of increasing polarities to isolate the fractions. It performed with an Agilent 6890 gas chromatograph (GC) equipped with an Agilent mass spectrometric (MS) detector, with a direct capillary interface and fused silica capillary column PAS 5ms (30m × 0.32mm ×0.25um film thickness). Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1 ml/min., pulsed split less mode. The solvent delay was 3min. and the injection size was 1.0ul. The mass spectrophotometric detector was operated in electron impact ionization mode with ioning energy of 70e.v. scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadruple temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually tuned using perfluorotributylamine (PFPTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C/min. and 10 min. hold at 280°C the detector and injector temperature were set at 280 and 250°C, respectively. Wiley and Nist 05 mass spectral data base was used in the identification of the separated peaks (Gomathi et al., 2013).

1H NMR (H-Proton Nuclear Magnetic Resonance) spectra were recorded on 300 MHz (BRUKER). Chemical shifts are given in δ (ppm) relative to TMS as internal slandered material at faculty of Science, Kafr El-Sheikh University. The albino mice Mus musculus used in this study had the weights between 20-30 grams and free of physical deformities. The mice were housed in cages (48x 35x 22 cm) with three mice per cage under 12hours light/ 12hours dark cycle (Caral, 2006). It was supplied with standard pellet diet and tap water ad libitum throughout the experimental period. They were acclimated for two weeks in the environment before the actual experiments (Jalal, 2007). All mice were randomly divided into six groups with six mice each, three males and three females. Group A was the non-diabetic control group and was untreated with alloxan. Group B was the negative control which was administrated with distilled water. Group C was the positive (+) control group which was treated with glibenclamide (5mg/kg body weight). Group D and E for C. trinodis petroleum ether (0.5g/kg B.W.) and chloroform (0.5g/kg B.W.) extracts respectively. Group F and G for M. aeruginosa petroleum ether (0.5g/kg B.W.) and chloroform extracts (0.5g/kg B.W.), respectively.

Processing of algal material
After collection, Microcystis aeruginosa (200g) and Cystoseira trinodis (1Kg) dried samples were overnight soaked in methanol at room temperature then filtered off. The marc was washed several times by methanol then the filtrate was evaporated by rotary evaporator to afford the crude extract (174.91g and 85.03g, respectively).

The algal extracts were packed in a continuous extraction apparatus. Successive extraction to exhaustion was done with solvents of increasing polarity in the following order: petroleum ether (60°C - 80°C) then chloroform. The marc after each extraction was dried until freed from the solvent, before extraction with the next solvent. The obtained two solvent fractions were separately concentrated under reduced pressure, dried to constant weight in vacuum desiccators, weighted and reserved in the refrigerator until further investigation. The petroleum ether extract of C. trinodis (6.32g) and M. aeruginosa (1.3g) were analyzed by GC/MS.

The chloroform extract of C. trinodis (6.5g) and M. aeruginosa (9.5g) was loaded on a silica gel-packed column (2Kg) and gradient eluted with a mixture of petroleum ether in ethyl acetate. The effluent was collected in 100 ml fractions. Fractions of C. trinodis (12-14), (15-17), (18-24), (26-28), (30-35) and (36-50) and M. aeruginosa (12-17), (22-32) and (5-7) were concentrated under reduced pressure at 40°C to a smaller volume (5ml) and monitored by silica gel GF254 TLC using the suitable solvent system and conc. H2SO4 and methanol (1:1) as a spray reagent. Similar fractions were pooled, concentrated to a small volume (5ml) and left in refrigerator and identified by 1H NMR.

Study of hyperglycemic activity of C. trinodis and M. aeruginosa
Administration of alloxan to induce diabetes
A freshly prepared solutions of alloxan (0.15g/kg B.W.) was used to induce diabetes (Wolfson and Wendy, 2007; Tanquilut et al., 2009; Kumar et al., 2009 and Rupasinghe et al., 2003). The administration of alloxan and treatments were done via intraperitoneal injection using 0.5 ml insulin syringe with 30 gauge needles on the lower right or left quadrant and lateral to the umbilicus. This was done by a standard one-hand restraint technique according to Fjellberg (2006).
Processing of experimental animals
Mice were left 2 weeks for acclimatization. The experiment ran for 31 days excluding the two weeks acclimatization period of the mice. On Day zero, mice were fasted for 12 hours. On Day 1, body weight, body temperature and blood samples were collected. Fasting blood glucose level was determined using the glucometer System - GlucoDrTM Plus. After the fasting blood glucose level was determined, alloxan was administered per intraperitoneal injection for the treatment groups B—to—G to induce hyperglycemia. Group a (six mice) was left untreated. After 48 hours, body temperature, weight and fasting blood glucose level were recorded. Those mice with >200mg/dl were considered diabetic according to Li et al. (2009). Those diabetic mice were randomly distributed to the different treatment groups (six mice per group) at a dose of 0.5 g/Kg. After 6 hours, Glibenclamide was administered to mice of Group C as positive control group. After the administration of different treatments, the blood glucose level was determined thrice initially after 1 hour with an interval of one hour each. The administration of respective treatments was continuous until day 30. Fasting blood glucose level and body temperature and weight were measured in days 10, 17, 24 and 31.

Oral glucose tolerance test (OGTT)
After 12-hr fasting, a blood sample was collected to measure the fasting blood glucose value. Mice were then provided per oral gavage with glucose solution (2g/Kg body weight). Blood glucose was measured every 30 min for 2 hours (Buse et al., 2011).

Cytotoxicity assay of C. trinodis and M. aeruginosa extracts using HepG2 cell line
Cytotoxic activity was measured by apoptotic cell death scored by cell surface evaluation and nuclear morphology. The neutral red method, as originally developed by Borenfreund and Puerner (1985) was applied. The cultures were removed from incubator into laminar flow hood and 0.33% neutral Red Solution was added in an amount equal to 10% of the culture medium volume. Cultures were returned to incubator for 2 hours. At the end of the incubation period, the medium was carefully removed and the cells quickly rinsed with PBS. The fixative or wash solution removed and the incorporated dye are then solubilized in a volume of Neutral Red Assay Solubilization Solution equal to the original volume of culture medium. The cultures were allowed to stand for 10 minutes at room temperature. The absorbance was measured spectrophotometrically at a wavelength of 540 nm. The background absorbance of multiwall plates was also measured at 690 nm and subtract from 540 nm measurement.

Statistical analysis
Results were expressed as means ± SEM. For the comparison among groups, data were analyzed with one-way Analysis of Variance (ANOVA) followed by the Tukey-Kramer post-hoc multiple comparison test. The significant level was set at p < 0.05 using SPSS version 18.

RESULTS
The petroleum ether extract of Microcystis aeruginosa contained 19 compounds which were identified of which 18 compounds were aliphatic and only one was aromatic. The aliphatic compounds were 10 fatty acids, 4 alkanes, fatty alcohol and 2 monoterpenoids. Hexadecanoic acid (palmitic acid) was found as major constituent with the peak area 48.43 % and retention time 21.71. In aromatic group, the compound 7-methoxy 2, 2-dimethyl-2H-1-benzoate with the peak area 0.13 % and retention time 15.41 (Table 1). In the chloroform extract of C. trinodis and M. aeruginosa fractions were analyzed by 1H NMR then GC/MS which indicated the presence of mixtures of fatty acids (Table 2, 3, 4 and 5).

Cystoseira trinodis petroleum ether extract was analyzed by GC/MS. About 50% of the peaks (20 compounds) were identified according to Nist library to afford compounds from (1) to (20). Totally 20 compounds were identified, of which 17 compounds were belonged to aliphatic group and 3 compounds were aromatic. The aliphatic group comprised 3 alkanes, 11 fatty acids, 2 diterpenes, one steroid and one carotenoid. Hexadecenoic acid methyl ester was found as major constituents with the peak area 6.82 % and retention time 25.06 followed by 4, 8, 12, 16-tetramethylheptadecane with the peak area 3.69 % and retention time 22.25. The aromatic group possessed benzaldehyde with 0.47 % and retention time 3.60.
**Hypoglycemic and Cytotoxic Effects of the Brown Alga**

Table (1): Identified compounds of *Cystoseira trinodis* and *Microcystis aeruginosa* petroleum ether fractions.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Rt</th>
<th>Area %</th>
<th>Compound name</th>
<th>Rt</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexadecane</td>
<td>7.82</td>
<td>0.36</td>
<td>n-heptadecane</td>
<td>16.94</td>
<td>3.25</td>
</tr>
<tr>
<td>Heptadecane</td>
<td>19.24</td>
<td>0.42</td>
<td>Octadecane</td>
<td>18.25</td>
<td>2.82</td>
</tr>
<tr>
<td>Eicosane</td>
<td>20.71</td>
<td>0.14</td>
<td>Nonadecane</td>
<td>19.49</td>
<td>0.53</td>
</tr>
<tr>
<td>Dodecanoic acid</td>
<td>17.51</td>
<td>0.18</td>
<td>Dodecanoic acid</td>
<td>15.09</td>
<td>0.13</td>
</tr>
<tr>
<td>Tetradecanoic acid</td>
<td>20.40</td>
<td>2.94</td>
<td>Hexadecanoic acid</td>
<td>21.71</td>
<td>48.43</td>
</tr>
<tr>
<td>n-hexadecanoic acid</td>
<td>22.47</td>
<td>2.08</td>
<td>Heptadecanoic acid</td>
<td>22.11</td>
<td>1.04</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td>25.24</td>
<td>1.83</td>
<td>Octadecanoic acid</td>
<td>23.08</td>
<td>2.26</td>
</tr>
<tr>
<td>Tetradecanoic acid methyl ester</td>
<td>19.61</td>
<td>0.45</td>
<td>Nonanoic acid, methyl ester</td>
<td>12.94</td>
<td>0.06</td>
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<td>Hexadecanoic acid methyl ester</td>
<td>21.98</td>
<td>0.75</td>
<td>Tetradecanoic acid methyl ester</td>
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<td>0.40</td>
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<tr>
<td>12-methyltetradecanoic acid</td>
<td>19.91</td>
<td>0.51</td>
<td>Hexadecanoic acid methyl ester</td>
<td>20.08</td>
<td>13.9</td>
</tr>
<tr>
<td>6,10,14-trimethyl pentadecanone</td>
<td>21.22</td>
<td>0.34</td>
<td>2-methyl-heptadecane</td>
<td>17.68</td>
<td>0.25</td>
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<tr>
<td>Neophytadiene</td>
<td>27.13</td>
<td>0.28</td>
<td>Iso-octadecanoic acid</td>
<td>22.82</td>
<td>1.02</td>
</tr>
<tr>
<td>4,8,12,16-tetra methyl heptadecane</td>
<td>22.25</td>
<td>3.69</td>
<td>Hexadecadiolic acid mono methyl ester</td>
<td>11.22</td>
<td>0.04</td>
</tr>
<tr>
<td>Hexadecenoic acid methyl ester</td>
<td>25.06</td>
<td>0.82</td>
<td>Nonanoic acid</td>
<td>10.46</td>
<td>0.08</td>
</tr>
<tr>
<td>9-octadecenoic acid methyl ester</td>
<td>24.35</td>
<td>1.44</td>
<td>6,10,14-trimethyl-2-pentadecanone</td>
<td>18.87</td>
<td>1.60</td>
</tr>
<tr>
<td>9-octadecenoic acid</td>
<td>26.12</td>
<td>0.22</td>
<td>Iso-octadecanoic acid</td>
<td>11.81</td>
<td>0.08</td>
</tr>
<tr>
<td>5,8,11,14-tetra ecosatetraenoic acid</td>
<td>21.11</td>
<td>0.21</td>
<td>Oleyl alcohol</td>
<td>13.55</td>
<td>0.14</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>3.60</td>
<td>0.47</td>
<td>3-oxo-ß-ionone</td>
<td>16.66</td>
<td>0.25</td>
</tr>
<tr>
<td>Fucosterol</td>
<td>35.97</td>
<td>0.15</td>
<td>2,2-dimethyl-7-methoxy-2,3-dihydro-2H-benzo[b]thiophene</td>
<td>15.41</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>40.09</td>
<td>1.73</td>
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</table>

Table (2): Identified compounds from *Cystoseira trinodis* fractions (1-3).

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Rt</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6, 10, 14-trimethyl-2-pentadecanone</td>
<td>37.77</td>
<td>1.46</td>
</tr>
<tr>
<td>Tetradecanoic acid, 3-hydroxy-methyl ester</td>
<td>38.64</td>
<td>0.94</td>
</tr>
<tr>
<td>Palmitic acid, methyl ester</td>
<td>39.80</td>
<td>5.54</td>
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<tr>
<td>Palmitic acid</td>
<td>41.90</td>
<td>47.03</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>44.07</td>
<td>4.85</td>
</tr>
</tbody>
</table>

Table (3): Identified compounds in *Microcystis aeruginosa* fraction (12-17).

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Rt</th>
<th>Area %</th>
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</thead>
<tbody>
<tr>
<td>Pleiocarpamine</td>
<td>5.14</td>
<td>0.88</td>
</tr>
<tr>
<td>Pentadecanoic acid 2-glyceride</td>
<td>35.89</td>
<td>1.81</td>
</tr>
<tr>
<td>Palmitic acid 2-tetradeoxyethyl ester</td>
<td>36.77</td>
<td>3.55</td>
</tr>
<tr>
<td>Octadecanoic acid 1-glyceride (1-Monostearin)</td>
<td>37.95</td>
<td>1.77</td>
</tr>
<tr>
<td>1,2-dipalmitin</td>
<td>39.83</td>
<td>1.16</td>
</tr>
<tr>
<td>Isochilpin B</td>
<td>40.84</td>
<td>6.47</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>41.40</td>
<td>2.17</td>
</tr>
<tr>
<td>1-Monopalmitin</td>
<td>45.33</td>
<td>5.98</td>
</tr>
<tr>
<td>Ethyl-isoalcholate</td>
<td>61.44</td>
<td>6.85</td>
</tr>
</tbody>
</table>
Effect of algal fractionized extracts on fasting blood glucose levels Short – term effect on blood glucose

Alloxan increased significantly blood glucose in all alloxan-treated groups. On the 3rd day, the acute effect of the algal extract on blood glucose was tested. Group C was treated with the anti-diabetic drug Glibenclamide, while groups D to G were exposed to different extracts.

Figure (1) shows that fasting blood glucose decreased gradually over 3h in groups A (non-diabetic control group), C (+ve control), D and E (treated groups by C. trinodis extracts). Blood glucose of groups B (-ve control) and G (treated group with chloroform extract of M. aeruginosa) remained elevated one hour after the administration of respective treatments for Groups C (+ve control), F and G (treated group with chloroform extract of M. aeruginosa) showed a significantly (p<0.0001) lower fasting blood glucose than Group B (diabetic control group).

One hour later, a significant drop of blood glucose level was observed (20% in Group C, 52% in Group D and 61% in Group E). The results were similar after 2 and 3 hours.

Table (4): Identified compounds from Microcystis aeruginosa fraction (22-32)

<table>
<thead>
<tr>
<th>Compound name</th>
<th>R_t</th>
<th>Area %</th>
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<tbody>
<tr>
<td>loliolide</td>
<td>34.06</td>
<td>0.74</td>
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<tr>
<td>2-Hydroxy-5,6-epoxy-β-ionone</td>
<td>36.57</td>
<td>0.24</td>
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<tr>
<td>2-pentadecanone, 6,10,14-trimethyl</td>
<td>37.77</td>
<td>0.45</td>
</tr>
<tr>
<td>Heptadecenoic acid</td>
<td>39.07</td>
<td>0.35</td>
</tr>
<tr>
<td>Palmitic acid, methyl ester</td>
<td>39.80</td>
<td>2.07</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>40.76</td>
<td>7.58</td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td>45.97</td>
<td>0.31</td>
</tr>
<tr>
<td>9-octadecanamide</td>
<td>46.10</td>
<td>1.34</td>
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Table (5): Identified compounds from Microcystis aeruginosa fraction (5-7)

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<tr>
<th>Compound name</th>
<th>R_t</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-pinene</td>
<td>10.40</td>
<td>0.97</td>
</tr>
<tr>
<td>Tetradecanoic acid, methyl ester</td>
<td>34.81</td>
<td>0.35</td>
</tr>
<tr>
<td>Tetradecanoic acid, 12-methyl-, methyl ester</td>
<td>36.40</td>
<td>0.54</td>
</tr>
<tr>
<td>Octadecan</td>
<td>36.60</td>
<td>0.28</td>
</tr>
<tr>
<td>2-pentadecanone 6,10,14-trimethyl</td>
<td>37.80</td>
<td>5.08</td>
</tr>
<tr>
<td>Heptadecenoic acid</td>
<td>39.09</td>
<td>0.24</td>
</tr>
<tr>
<td>Palmitic acid, methyl ester</td>
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<td>78.54</td>
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<tr>
<td>Palmitic acid, ethyl ester</td>
<td>41.48</td>
<td>0.71</td>
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<td>Heptadecanoic acid, methyl ester</td>
<td>42.25</td>
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<tr>
<td>Octadecanoic acid, methyl ester</td>
<td>44.49</td>
<td>2.59</td>
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</table>

Figure (1): Fasting blood glucose (mg/dl) after different treatments on day 3 showing acute glucose lowering effect of algal extracts. Data are means ± SEM. **p<0.001= significant.
**Long – term effect on blood glucose**

The results showed that on days 1, 3, 10, 17, 24 and 31 the fasting blood glucose of group A, C, D and E were decreased gradually with days. On the other hand, group B still increased at all the experiment days. Group F and G were highly increased until mice death (Table 6).

The fasting blood glucose level mean on day 1 showed no intra group significance (p 0.467). ANOVA showed significant difference among groups on days 3, 7, 10, 17, 24 and 31. On day 3, forty-eight hours after the administration of alloxan to the diabetic groups B, C, D, E, F and G which exhibited hyperglycemia and had significant fourfold (p<0.0001) increase in the fasting blood glucose level as compared to Group A (Normal non-diabetic group).

On day 10, fasting blood glucose mean of Group A was significantly different compared to Group B (p 0.000), while no significance between groups C and D (p 0.077) compared to Group F and G (p 0.000). On day 17, fasting blood glucose mean of Group C was significantly different compared to Group E (p 0.005).

**Table (6): Effects of algal extracts on the fasting blood glucose level of normal and alloxan-diabetic albino mice.**

<table>
<thead>
<tr>
<th>Groups treatment</th>
<th>Fasting blood glucose level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>A Normal (Non Diabetic)</td>
<td>98± 17.6</td>
</tr>
<tr>
<td>B Diabetic (Alloxan only)</td>
<td>109± 2.1</td>
</tr>
<tr>
<td>C Glibenclamide</td>
<td>115± 7.4</td>
</tr>
<tr>
<td>D (C. trinodis) (Pet. Ether)</td>
<td>99± 11.4</td>
</tr>
<tr>
<td>E (C. trinodis) (Chloroform)</td>
<td>113± 10.7</td>
</tr>
<tr>
<td>F (M. aeruginosa) (Pet. Ether)</td>
<td>126± 7.2</td>
</tr>
<tr>
<td>G (M. aeruginosa) (Chloroform)</td>
<td>106± 3.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM: *p<0.05 = significant; **p<0.001; NS= Non significant: Group A and B is compared: Group B and C are compared: Group C is compared with Groups D, E, F and G.

**Body weight**

As shown in Table (7), on day 1, all groups had no significant difference in body weight (p 0.789). Groups B, C and G exhibited loss of body weight with significant (p<0.05) compared to Group A on day 3, while, the other groups (D and E) exhibited a gradient increase of body weight. On day 10, Groups D and E showed a significant difference (p<0.05) in body weight but all the other groups had no significance.

On day 17, groups E, F and G were not significantly different compared to group C. On the other hand, groups D and E had non-significant difference compared to group C on day 24. On day 31, the results showed that groups B and C had a significant increase on body weight.

**Body temperature**

Body temperature was ranged between 35.5°C to 37.8°C. On days 1, 3, 17, 24 and 31, the body temperature means had no significant intra-group variation (p>0.05). It gives the highest degree in group B, but group A, C, D and E showed few differences in its temperature degree. For groups D and E, a significant difference (p<0.05) was observed compared to group C on days 3 and 10 while groups F and G were non-significant (Table 8).
Table (7): Effect of algal extracts on the body weight (g) in normal and alloxan diabetic albino mice

<table>
<thead>
<tr>
<th>Groups treatment</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 17</th>
<th>Day 24</th>
<th>Day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Normal (Non Diabetic)</td>
<td>23±0.4</td>
<td>24±0.5</td>
<td>24±0.5</td>
<td>23±0.5</td>
<td>23±0.3</td>
<td>24±0.3</td>
</tr>
<tr>
<td>B Diabetic (Alloxan only)</td>
<td>25±1 NS</td>
<td>19±0.4 *</td>
<td>23±1.4 NS</td>
<td>22±0.3 NS</td>
<td>23±0.3 NS</td>
<td>20±0.3 ''</td>
</tr>
<tr>
<td>C Glibenclamide</td>
<td>23±1 NS</td>
<td>22±0.6 *</td>
<td>21±0.4 NS</td>
<td>22±1.4 NS</td>
<td>22±0.4 *</td>
<td>22±0.1 *</td>
</tr>
<tr>
<td>D (C. trinodis) (Pet. Ether)</td>
<td>26±2.6 NS</td>
<td>25±0.3</td>
<td>19±0.3</td>
<td>20±0.5</td>
<td>21±0.3 NS</td>
<td>22±0.2 NS</td>
</tr>
<tr>
<td>E (C. trinodis) (Chloroform)</td>
<td>24±2 NS</td>
<td>26±0.4 *</td>
<td>26±0.5 **</td>
<td>21±0.5 NS</td>
<td>22±0.5 NS</td>
<td>23±1 NS</td>
</tr>
<tr>
<td>F (M. aeruginosa) (Pet. Ether)</td>
<td>24±1 NS</td>
<td>22±1 NS</td>
<td>21±0.8 NS</td>
<td>die</td>
<td>die</td>
<td>die</td>
</tr>
<tr>
<td>G (M. aeruginosa) (Chloroform)</td>
<td>20±2.4 NS</td>
<td>21±0.4</td>
<td>22±0.6 NS</td>
<td>die</td>
<td>die</td>
<td>die</td>
</tr>
</tbody>
</table>

Values are mean ± SEM: *p<0.05 = significant; **p<0.001; NS= Non significant: Group A and B is compared: Group B and C are compared: Group C is compared with Groups D, E, F and G.

Table (8): Effect of algal extracts on the body temperature (°C) in normal and alloxan diabetic albino mice

<table>
<thead>
<tr>
<th>Groups treatment</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 10</th>
<th>Day 17</th>
<th>Day 24</th>
<th>Day 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Normal (Non Diabetic)</td>
<td>35.7±0.3</td>
<td>36±0.1</td>
<td>36.2±0.1</td>
<td>36.1±0.1</td>
<td>36.5±0.1</td>
<td>36.3±0.1</td>
</tr>
<tr>
<td>B Diabetic (Alloxan only)</td>
<td>35.8±0.4 NS</td>
<td>36.6±0.1</td>
<td>36.4±0.1 NS</td>
<td>35.9±0.1</td>
<td>36.7±0.1 NS</td>
<td>37.8±0.1 ''</td>
</tr>
<tr>
<td>C Glibenclamide</td>
<td>35.8±0.4 NS</td>
<td>36.3±0.1 NS</td>
<td>36.1±0.1 NS</td>
<td>36.2±0.2 *</td>
<td>36.3±0.1</td>
<td>36.7±0.1 ''</td>
</tr>
<tr>
<td>D (C. trinodis) (Pet. Ether)</td>
<td>36.2±0.2 NS</td>
<td>36±0.0 NS</td>
<td>36.3±0.1 NS</td>
<td>36.1±0.1 NS</td>
<td>36.4±0.1 NS</td>
<td>36.6±0.1 NS</td>
</tr>
<tr>
<td>E (C. trinodis) (Chloroform)</td>
<td>36.2±0.1 NS</td>
<td>35.9±0.2 *</td>
<td>35.7±0.3 NS</td>
<td>35.9±0.1</td>
<td>36.4±0.2 NS</td>
<td>36.7±0.1 NS</td>
</tr>
<tr>
<td>F (M. aeruginosa) (Pet. Ether)</td>
<td>36.4±0.2 NS</td>
<td>36.1±0.1 NS</td>
<td>35.8±0.4 NS</td>
<td>die</td>
<td>die</td>
<td>die</td>
</tr>
<tr>
<td>G (M. aeruginosa) (Chloroform)</td>
<td>36.4±0.2 NS</td>
<td>36.4±0.1 NS</td>
<td>35.5±0.3 NS</td>
<td>die</td>
<td>die</td>
<td>die</td>
</tr>
</tbody>
</table>

Values are mean ± SEM: *p<0.05 = significant; ''p<0.001; NS= Non significant: Group A and B is compared: Group B and C are compared: Group C is compared with Groups D, E, F and G.

Oral glucose tolerance test (OGTT)

Figure (2) showed that, at zero time, the fasting blood glucose were 77, 182, 172, 180 and 171 mg/dl for groups A (non-diabetic control group), B (-ve control), C (+ve control), D and E (treated with C. trinodis extracts), respectively. After 30 min., groups A, C, D and E have been recorded the maximum rate of fasting blood glucose then decreased after 2h to reach 80, 171, 179 and 175 mg/dl, respectively. On the other hand, by increasing of time, the fasting blood glucose increased.

Figure (2): Fasting blood glucose (mg/dl) during oGTT of all groups.
Cytotoxicity test
Treatment with various concentrations of C. trinodis extract caused a dose-dependent cytotoxicity in HepG2 cells. Maximum viability rate was observed at 3.1 mg/ml concentration while 50% viability was observed at 112 mg/ml concentration in ether extract. Also, Chloroform extract showed the maximum viability rate at 3.1 mg/ml concentration but 50% viability was observed at 77 mg/ml concentration (Figure 3). On the other hand, the two fractions of M. aeruginosa are deadly affect HepG2 cells.

DISCUSSION
Alloxan itself is nontoxic. It is rapidly taken up by the pancreatic β-cells. Alloxan has a direct effect on islet cell permeability and acts at the site of hexose transport as it inhibits glucose-stimulated insulin release (Tomita et al., 1994). It also interferes with the generation of glucose derived energy by inhibiting glycolytic flux and pyruvate oxidation (Borg et al., 1979). This destruction was attributed with high blood glucose level and was recorded in diabetic mice (Group B) as compared to normal control mice (Group A) (Table 6). Alloxan-induced model of hyperglycemia in mice was used to investigate the effect of two solvent fractions of C. trinodis and M. aeruginosa on lowering of blood glucose level. Unfortunately, we couldn’t complete groups F and G (M. aeruginosa extracts) due to death of mice (Table 6). It is well known, however, that M. aeruginosa produce the microcystin-LR toxin that may cause a haemorrhagic shock (Hermansky et al., 1990). After intraperitoneal injection of doses of M. aeruginosa, microcystin appears to be transported by bile acids transporter in both the intestine and the liver. About 70% of the toxin was rapidly localized in the liver (Runnegar et al., 1991) via a carrier-mediated transport system followed by the inhibition of serine protein phosphatases 1 and 2A. The protein phosphorylation imbalance causes disruption of the liver cytoskeleton which leads to massive hepatic haemorrhage that can cause death (Romanowska et al., 2002). Thus, the two fractions of Microcystis aeruginosa had no cytotoxic effect on HepG2 cells (Figure 3). Moreover, all extracts of M. aeruginosa reveals the presence of palmitic acid which were associated with higher diabetes risk (Wenjie et al., 2015) with the highest percentage (Table 1, 3, 4 and 5). C. trinodis fractions were effective in lowering the blood glucose level in alloxan-induced diabetic mice (Table 6, Figures 1 and 2) and had a cytotoxic effect against HepG2 (Figure 3). These effects may be attributed to the presence of secondary metabolites which identified by the GC-MS analysis on various extracts of C. trinodis and enumerated along with molecular formula, retention time, molecular weight and peak area. GC-MS analysis of petroleum ether extract of C. trinodis revealed the presence of hydrocarbons, unsaturated fatty acids, steroid, terpenoids and vitamin E (Table 1). While, the chloroform extract reveals the presence unsaturated fatty acids (Table 2). Fucosterol is the primary sterol found in brown algae like C. trinodis. Recently, considerable interest has been generated regarding fucosterol due to its potential antioxidant; anti-inflammatory, anti-diabetic effects that made it as an effective hepatoprotective agent that could be useful for preventive therapies against oxidative stress-related hepatotoxicity (Choi et al., 2015). Poly unsaturated fatty acids reduce insulin resistance and thus, decrease the risk of type 2 diabetes (Das, 2002). This is accordance with Das and Suresh (2001) who suggest that polyunsaturated fatty acids can prevent alloxan-induced diabetes mellitus in experimental animals and may be useful to prevent diabetes mellitus in the high-risk population. The compounds identified by GC-MS in all extracts are medicinally valuable and possess various pharmaceutical applications even in minimal dose. For instance, hexadecanoic acid which is a palmitic acid compound found to be anti-oxidant, anti-hypcholesterolemic, nematicide, pesticide, turbinant activities and hemolytic 5-alpha is a reductase inhibitors. These results are strengthened by the findings of Sermakkani and Thangapandian (2012) who observed the presence of this compound in methanol extract of Cassia italica leaves. Unfortunately, palmitic acid lowers insulin resistance and incident diabetes (Mozaffarian et al., 2010). On the other hand, the percentage of arachidonic acid (5, 8, 11, 14-tetraecicosatetraenoic acid) shown to positively correlated with insulin sensitivity (Das, 2002). Oleic acid (9-octadecenoic acid) reported to have an anti-inflammatory, nematicide, insecticufge, anticancer and antiadibiotic properties. The monounsaturated oleic acid did not affect DNA fragmentation and induced β-cell proliferation. Moreover it prevented the deleterious effects of both palmitic acid and high glucose concentration (Maedler et al., 2003). The combinations of essential fatty acids and vitamin E have general utility in the treatment of a variety of diseases such as.
disorders of inflammation, in alcoholism and in schizophrenia complications (Horrobin, 1992). Di-terpenoid compounds (4, 8, 12, 16-tetramethyl heptadecane and neophytiadiene) were reported to possess antimicrobial, anticancer and anti-inflammatory activity (Praveen et al., 2010) and exhibited a significant decrease in blood glucose level (Lu et al., 2010). The steroid component of the petroleum ether fraction of C. trinodis could support the fasting blood glucose level lowering mechanism and assist indirectly the health of the diabetic mice since these metabolites had been reported in ethanolic extract of Musa fruits to have lipid lowering effect (Kaimal et al., 2009).

CONCLUSION

In the present study, 20 and 19 chemical compounds have been identified by GC-MS analysis from the petroleum ether, while 10 and 19 chemical compounds have been identified from the chloroform extract of C. trinodis and M. aeruginosa, respectively. M. aeruginosa had no anti-diabetic and deadly cytotoxic effect on HepG2 cells. On the other hand, C. trinodis showed hypoglycemic and antioxidant properties. Microcystis aeruginosa was found to contain high percentage of palmitic acid. Meanwhile, Cystoseira trinodis contains terpenoids, steroids, unsaturated fatty acids (arachidonic and oleic acid) and vitamin E. These compounds may mediate the beneficial effect of our extracts. Further studies should be performed including isolation and purification of the bioactive chemical compounds present in the extract in order to prove their potential in clinical studies.

REFERENCES


KAIMAL, S., K. S. SUJANATHA, AND S. GEORGE.


الملخص العربي
تهدف هذه الدراسة إلى تعيين المكونات الفيتو كيميائية لكل من مستخلصي البترويل إيثر والكلوروفرم لطبلي السيستوسيرينات والميكروسيستس أريونوزا وذلك باستخدام أجهزة الـ $^1$H NMR وGC-MS و$^1$H NMR. هذا الابحاث على وجود العديد من الأحماض الدهنية، الاستيرادات، التربيعات الفيتوولومات التي ينتمون إلى فئة سكر الدم. وجد أنه مستوي الجلوكوز في الدم ينخفض مع تأثير الصوديوم في المحلول. إثر كل المجموعات المعالجة بمستخلصي البترويل إيثر و الكلوروفرم لطبلي السيستوسيرينات إيران و الكولوريومسات للمكونات الغير معالجة، وجد أيضا أن مستخلصي طبلم السيستوسيرينات إيران. جذب تأثير على تقليل حيوية خلايا أكي المريضة، وللوحي الأ pazان المستقل عن مقاطع 50% من الخلايا لمستخلصي البترويل إيثر والكلوروفرم. أريونوزا على فخفض سكر الدم ولا على تقليل حيوية خلايا أكي المريضة.