Free Radical Scavenging Potential And LC-MS Based Identification Of Secondary Metabolites Of Deinococcus Radiodurans

Illiyas Maqbool, Ponniresan Veeramani Kandan, Ezhumalai Dhineshkumar, Nagarajan Rajendra Prasad

Abstract: Deinococcus radiodurans is an extremophilic organism that shows resistance towards radiation exposures and oxidative stress. The present study investigated the total phenolic and flavonoid content, antioxidant potential and presence of bioactive compounds in the crude secondary metabolite extract (CSME) of D. radiodurans by liquid chromatography-mass spectrometry (LC-MS) analysis. The total phenolic and flavonoid content in CSME of D. radiodurans was calculated to be 3.8 mg PGE equivalent/g CSME and 5.3 mg QE equivalent/g CSME, respectively. The results also show considerable scavenging activity of D. radiodurans CSME against DPPH (IC50 180 μg/ml), ABTS (IC50 210 μg/ml), hydroxyl (IC50 240 μg/ml) and superoxide (IC50 244 μg/ml) radicals. Further, a total of 286 metabolites were identified in the CSME of D. radiodurans. Among these, some major compounds present in the CSME of D. radiodurans are Mannosamine (m/z 149.21; environmental pollutant), Xanthone (m/z 195.15; insecticide and larvicide), Cytidine-5′-triphosphate disodium salt pyrimidine (m/z 165.19; pyrimidine biosynthesis and coenzyme in glycerophospholipid biosynthesis), Ethanolamine (m/z 441.4; pharmaceuticals, corrosion inhibitor, and chemical intermediate), Folic acid (m/z 61.08; pharmaceutical applications in end-stage renal disease, hyperhomocysteinemia), Octadecanoic acid (m/z 117.15; cosmetic applications), Butyrate (m/z 85.11; anti-inflammatory, used in ulcerative colitis and colorectal cancer treatment), O-Palmitoyl-L-Carnitine chloride (m/z 115.13; used for treating cardiomyocytes), pyrroloquinoline quinone (m/z 330.11; antioxidant) and Myxothiazol (m/z 487.32; mitochondrial cytochrome bc1 complex inhibitor). Therefore, this study proves that CSME of D. radiodurans could be a potential source of bioactive compounds that could be tested for various pharmacological properties.

Keywords: Extremophilic, secondary metabolites, Deinococcus radiodurans, oxidative stress, LC-MS analysis.

1. Introduction
Extremophiles survive in the most challenging environmental conditions. The extremophilic organisms require one or several extreme conditions to survive [1]. Secondary metabolites are small molecules that are not directly involved in the survival of an organism but rather play a key role in the long-term survival of productive organisms [2,3]. These small molecules are produced in response to growth retardation due to the depletion of nutrient sources such as carbon, nitrogen or phosphate [3]. It has been expected that the bioactive compounds isolated from extremophiles will provide useful drugs, especially antibiotics and effective anticancer drugs [4]. Recently, extremophilic organisms produced by secondary metabolites have been found to exhibit a wide range of pharmaceutical and therapeutic applications. These secondary metabolites are widely used as antibiotics, antitumor and antiviral agents [5,6]. Deinococcus radiodurans is a polyextremophile, a gram-positive and red-pigmented bacterium highly resistant to radiation exposures, oxidative stress, cold, dehydration, vacuum, and acid [7]. D. radiodurans has been listed in the "Guinness Book of World Records" as the toughest bacterium in the world [8].

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employed technique to find out secondary metabolites in a complex mixture. LC-MS is a qualitative and accurate quantitative analysis of bioactive components [18]. LC-MS technique enables real-time analysis of a variety of chemical species and therefore has emerged as an ideal tool for metabolomics studies [19]. LC-MS is a high-tech detection method based on high-resolution and highly sensitive chromatographic separation. When this technique is coupled to mass spectrometry (MS), additional information about each separated compound molecular mass, the elemental composition is achieved [20]. There is no sufficient information on metabolites synthesized by D. radiodurans. Therefore, in the present study, we identified secondary metabolites present in the crude extract of D. radiodurans using LC-MS analysis and explored the antioxidant potential of crude extract of D. radiodurans using in vitro free radical scavenging assays.

2. Materials and Methods

2.1. Reagents
Bacterial growth media and other reagents were purchased from Sigma Aldrich (Chennai, India). LC-MS grade ethyl acetate solvent was purchased from Himedia labs (Mumbai, India).

2.2. Growth media and culture conditions
D. radiodurans MTCC 4465 was obtained from a Pure Culture of Microbial Type Culture Collection (MTCC), Chandigarh, India. The cultures of D. radiodurans were maintained on TGY agar (0.5% tryptone, 0.1% glucose, 0.3% yeast extract supplemented with 1.5% agar) at 30 °C for 24-48 h. For the purpose of secondary metabolite production, the exponentially growing culture of D. radiodurans (OD = 0.5) was used to inoculate the Erlenmeyer flask with 500 ml of sterile autoclaved TGY broth. The broth culture containing the flask was then incubated at 30 °C in a shaker incubator for 5-7 days at 220 rpm for secondary metabolites.

2.3. Preparation of D. radiodurans CSME
Post incubation, the D. radiodurans broth culture was centrifuged at 10,000 rpm for 15 min. The cell-free supernatants were transferred to sterile polypropylene tubes. The pellet was stored at 4 °C for optional use. To the cell-free supernatant, an equal volume (1:1 v/v) of LC-MS grade ethyl acetate solvent was added and mixed well with secondary metabolites for complete extraction for vortexing. The solution is composed of an aqueous layer and a solvent layer. The upper solvent layer was taken out, stored into sterile vials and was further processed for LC-MS analysis.

2.4. Estimation of non-enzymatic antioxidant potential

2.4.1. Total phenolic content
The total phenolic content (TPC) of D. radiodurans CSME was determined according to the method of Lim et al. [21]. A total of 2.9 ml of distilled water and 0.5 ml of Folin-Ciocalteau’s reagent were mixed with the CSME. After 10 min of incubation, 1.5 ml of 20% sodium carbonate solution was added. The contents were mixed carefully and allowed to stand for 1 h in dark at 37 °C. The absorbance was measured at 725 nm. Phloroglucinol (PGE) was used as a standard. TPC was expressed as PGE equivalents / g of CSME.

2.4.2. Total flavonoid content
The total flavonoid (TFC) content of D. radiodurans CSME was measured according to Zhishen et al., 1999 [22]. Briefly, CSME of D. radiodurans was added to 3 ml of 5% (v/v) NaNO2 and incubated for 5 min at 37 °C. Next, 2 ml NaOH (1 M) and 0.3 ml AlCl3 (10%, v/v) were added. The absorbance was recorded at 510 nm. Quercetin (QE) was used as a standard. TFC was expressed as mg (QE) equivalents / g of CSME.

2.5. Determination of free radical scavenging activity

2.5.1. DPPH radical scavenging assay
The free radical scavenging activity of CSME was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [23] with slight modifications. A fresh methanolic solution of DPPH (0.2 mM) was prepared and incubated in the dark for 2 h prior to the analysis. After preparing different concentrations (0.031, 0.0621, 0.125, 0.25, 0.5 and 1 mg/ml) of CSME and standard ascorbic acid, 50 µl of each test sample and standard solutions were transferred to 96-well plate. DPPH methanolic solution (0.195 ml) was then added to each well and incubated for 1 h in the dark. The absorbance of the resulting mixture was measured at 540 nm by using a microplate reader. DPPH radical scavenging activity was expressed as percentage scavenging activity using the following formula: DPPH radical scavenging activity (%) = [(Abs blank – Abs sample)/ Abs blank] x 100

2.6.2. ABTS (2,2-Azino-Bis-(3-ethylbenothiazoline-6-Sulfonic Acid) assay
The D. radiodurans CSME was studied for its potential to scavenge the 2,2-Azino-bis-(3-ethylbenothiazoline-6-sulfonic acid) (ABTS) [24]. A freshly prepared solution of ABTS reagent (7 mM ABTS solution treated with 2.45 mM of potassium persulfate) was prepared. After preparing different concentrations (0.031, 0.0621, 0.125, 0.25, 0.5 and 1 mg/ml) of CSME and standard ascorbic acid, 100 µl of each test sample solution and standard ascorbic acid was mixed with 900 µl of ABTS stock solution. The absorbance was measured at 734 nm after 2 min of the incubation period. The ABTS radical cation scavenging activity has expressed a percentage of ABTS scavenged and was calculated using the following formula:

% ABTS scavenging = [(Abs control – Abs sample)/Abs control] x 100

2.6.3. Hydroxyl radical scavenging activity
The hydroxyl radical scavenging activity of CSME was measured by the method of Kunchandy and Rao, (1990) [25]. The reaction mixture (1 ml) containing 100 µl of 28 mM 2-deoxyribose in 20 mM KH2PO4-KOH buffer, pH 7.4, different concentration of the CSME and standard ascorbic acid (0.031, 0.0621, 0.125, 0.25, 0.5 and 1 mg/ml), 200 µl EDTA (1.04 mM), 200 µM FeCl3 (1:1 V/V), 100 µl of H2O2 (1 mM) and 100 µl ascorbic acid (1 mM), which was incubated at 37°C for 1 h. Next, 1 ml of thiobarbituric acid (1%) and 1 ml of trichloroacetic acid (2.8%) were added and incubated at 100 °C for 20 min in a water bath. After cooling, absorbance was measured at 523 nm. The CSME
was replaced with distilled water in the blank reaction mixture. The hydroxyl radical scavenging activity was expressed as percentage hydroxyl radical scavenging activity using the following formula:

\[
\text{% of hydroxyl radical scavenging} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100
\]

2.6.4. Superoxide radical scavenging assay
Each 3 ml reaction mixture sequentially contained 750 µl sodium phosphate buffer (50 mM, pH 7.8), 390 µL methionine (13 mM), 300 µl EDTA (100 nM), 1300 µL of each CSME and standard ascorbic acid concentrations (0.031, 0.0621, 0.125, 0.25, 0.5 and 1 mg/mL), 250 µL NBT (75 mM), and 6 µL riboflavin (2 mM). The reaction was started by turning on a fluorescent lamp (15 W), and illumination was run for 10 min. The production of violet color was monitored by measuring absorbance at 560 nm. Tubes without CSME were kept in the dark and served as blanks. The results were expressed as percent radical scavenging activity using the formula:

\[
\text{% Superoxide radical scavenging} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100
\]

2.7. Liquid chromatography-mass spectrometry analysis
LC-MS analysis was performed using an injection volume of 3 µl and samples were analyzed on Agilent 6540 B TOF/QTOF Mass Spectrometer Coupled with Dual AJS ESI ion Source. An ACQUITY UPLC HSS T3 (100×2.1 mm, 1.8 µm) column and pre-column (Phenomenex Security Guard™) was employed to separate sample. The column temperature was adjusted to 45°C and the flow rate was maintained at 0.5 ml/min. The acquisition range was 50 m/z to 1500 m/z and the scan rate was 1.00 spec/sec. The MS parameters were as follows: capillary voltage 3500V, nebulizer pressure 35 psi, drying gas 10L/min, gas temperature 325°C, vaporizer 200V, voltage charge 1000V; negative-ion mode capillary voltage 3500V, corona negative 15.0V, fragmentor 175V, skimmer 165.0V, octopole RF Peak 750V; positive ion mode capillary voltage 3500V, corona positive 4.0V, fragmentor 175V, skimmer1 65.0V and octopole RF Peak 750V. Metabolites were identified by an in-house database. Interpretation of the LC-MS mass spectrum of compounds identified in CSME of D. radiodurans was done using the SciFinder and Chemspider library with more than 62,000 patterns. The SciFinder and Chemspider library of the known components is stored in the spectrum of the mass spectrum. The name, molecular weight, and structure of the components of the test were confirmed.

2.8. Statistical analysis
All the values were expressed as means ± SD. The data were statistically analyzed by Duncan’s multiple range test (DMRT) using a statistical package program (SPSS). P-value (≤ 0.05) was considered statistically significant.

3. Results
3.1. Total phenol content
The total phenolic content present in CSME of D. radiodurans was found to be 3.8 mg phloroglucinol equivalents (PGE)/g of CSME (Figure 1A). Our results show that the CSME of D. radiodurans contain natural antioxidants which neutralize the ROS and free radicals. However, the total phenol content was found to be lower when compared to the phloroglucinol standard.

3.2. Total flavonoid content
The total flavonoid content in the CSME of D. radiodurans was found to be 5.3 mg quercetin equivalents (QE) per g of CSME (Figure 1B). Our results show that the CSME of D. radiodurans comprise of antioxidant molecules that scavenge ROS and other free radicals. Even though, the total flavonoid content was low when compared to the quercetin standard.

Figure 1. (A) Total phenolic content and (B) Total flavonoid content in CSME of D. radiodurans. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

3.3. DPPH scavenging activity of CSME
We observed that the CSME of D. radiodurans exerted significant DPPH scavenging activity. The CSME of D. radiodurans at a concentration of 0.5 mg/ml and 1 mg/ml exhibited 64 % and 83 % scavenging activity, respectively (Figure 2). The IC50 value of CSME was found to be 180 µg/ml while the standard ascorbic acid displayed an IC50 value of 367 µg/ml.

Figure 2. DPPH radical scavenging activity of D. radiodurans CSME. Different concentrations of D.
radiodurans CSME and ascorbic acid (standard) were prepared and their scavenging activity was determined. Values are given as means ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

3.4. ABTS scavenging activity of CSME
We observed a significant ABTS•+ radical scavenging activity of CSME of D. radiodurans in a dose-dependent manner. The CSME and ascorbic acid (standard) showed IC50 values of 210 µg/ml and 140 µg/ml, respectively (Figure 3). The results obtained display a strong radical scavenging activity.

![Figure 3. ABTS radical scavenging activity of D. radiodurans CSME.](image)

3.5. Hydroxyl radical scavenging activity of CSME
We observed that CSME of D. radiodurans exhibits potent hydroxyl radical scavenging activity in a dose-dependent manner. The IC50 values of CSME and ascorbic acid (standard) were found to be 240 µg/ml and 193 µg/ml, respectively (Figure 4). The result obtained emphasized a significant antioxidant capacity of CSME against hydroxyl radicals.

![Figure 4. Hydroxyl radical scavenging activity of D. radiodurans CSME.](image)

Values are given as means ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

3.6. Superoxide radical scavenging activities of CSME
The decrease of absorbance at 560 nm with CSME of D. radiodurans indicates the consumption of superoxide anion in the reaction mixture. From our results, superoxide radical scavenging by CSME followed a dose-dependent manner (Figure 5). The IC50 values of CSME and ascorbic acid (standard) were found to be 244 µg/ml and 221 µg/ml, respectively. The data proves that D. radiodurans CSME possesses strong superoxide radical scavenging activity.

![Figure 5. Superoxide radical scavenging activity of D. radiodurans CSME.](image)

3.7. LC-MS metabolite profiling of CSME
On comparison of the LC-MS mass spectra of each compound with the SciFinder and Chemspider library around 29 different probable metabolites were identified in ESI positive and negative ion modes (Table 1 and 2). The structural information of these identified compounds is also listed (Figures 6 and 7).
Table 1. Secondary metabolites identified in positive ion mode.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Formula</th>
<th>Name</th>
<th>Retention Time (RT)</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C3H7NO2</td>
<td>Alanine</td>
<td>6.431</td>
<td>131.17</td>
</tr>
<tr>
<td>2.</td>
<td>C4H8N2O3</td>
<td>Asparagine</td>
<td>10.15</td>
<td>131</td>
</tr>
<tr>
<td>3.</td>
<td>C2H7NO</td>
<td>Ethanolamine</td>
<td>12.078</td>
<td>62</td>
</tr>
<tr>
<td>4.</td>
<td>C4H4O4</td>
<td>Fumaric acid</td>
<td>12.777</td>
<td>105.09</td>
</tr>
<tr>
<td>5.</td>
<td>C3H7NO3</td>
<td>Serine</td>
<td>13.456</td>
<td>106</td>
</tr>
<tr>
<td>6.</td>
<td>C19H19N7O6</td>
<td>Folic acid</td>
<td>13.603</td>
<td>61.08</td>
</tr>
</tbody>
</table>

However, D. radiodurans CSME has no background signal with laser energy-adjusted from 10% to 90% (Figure 8). Under 90% of laser energy, we tested a clean target plate under control and confirmed that the little peaks arise from high laser energy under the target plate (Figure 9A). For comparison, several D. radiodurans matrices were examined and they exhibited abundant background peaks (Figure 9B).
Mannosamine, Phenylalanine, Cytidine-5'-triphosphate disodium salt, Ethanolamine, Fumaric acid, Serine, Folic acid, Tyrosine, Aspartic acid, Methionine, Glutamine, Succinic acid, Lactic acid, Threonine, Leucine, Octadecanoic acid, O-Palmitoyl-L-Carnitine chloride, Isopropyl alcohol, Pyrroloquinoline quinine, Xanthone, Butyrate and Myxothiazol.

We employed different metabolite standards to test the preliminary performance of D. radiodurans CSME as an LC-MS matrix. (Figure 10) shows the mass spectra of the detected amino acids, and they were all clearly detected with no background interference with [M-H] peaks.

The results show that these four peptides were detected as [M-H] ions with a clean background. The positive ion mode indicated the presence of Alanine (m/z 131.17), Asparagine (m/z 118.09), Ethanolamine (m/z 441.4), Fumaric acid (m/z 105.09), Serine (m/z 116.07), Folic acid (m/z 61.08), Tyrosine (m/z 527.12), Succinic acid (m/z 132.12), Threonine (m/z 131.17), Leucine (m/z 89.09), Octadecanoic acid (m/z 117.15), and O-Palmitoyl-L-Carnitine chloride (m/z 115.13), Isopropyl alcohol (m/z 60.09), Pyrroloquinoline quinine (m/z 330.11), Xanthone (m/z 195.15), Butyrate (m/z 85.11), Myxothiazol (m/z 487.32) (Figure 12 and 13).
Positive mode of LC-MS spectra of D. radiodurans CSME. (A), (B) Alanine, Asparagine, Ethanolamine, Fumaric acid, Serine, Folic acid, Tyrosine, Succinic acid, Threonine, Leucine, Octadecanoic acid and O−Palmitoyl−L−Carnitine chloride.

Small molecules like Mannosamine, Cytidine−5−triphosphate disodium salt, Ethanolamine, Folic acid, Octadecanoic acid and O−Palmitoyl−L−Carnitine chloride were detected with deprotonated peaks [M−H]. Abundant peaks were acquired, and the mass fingerprints of the two samples displayed a stark difference (Figure 14).

Positive mode of LC-MS spectra of D. radiodurans CSME. (A) Isopropyl alcohol, (B) Pyrroloquinoline quinine, (C) Xanthone, (D) Butyrate and (E) Myxothiazol.

The secondary metabolites were identified from positive as well as negative ion mode (Table 1 and 2) comprised of amino acids, polyphenols and flavonoids, quinones, vitamins, glycosyl and glycoside compounds and analogs, etc. These metabolites behave as excellent antioxidants. The identified polyphenols and flavonoids in this study i.e. xanthone and pyrroloquinoline quinine are potential free radical scavengers. The rest of the identified compounds comprised of saccharides and derivatives (mannosamine, maltose), alcohols (isopropyl alcohol, ethanolamine) esters (butyrate, octadecanoic acid), vitamins (folic acid) and other intermediate compounds (Myxothiazol, Cytidine−5−triphosphate disodium salt and O−Palmitoyl−L−Carnitine chloride). These entire compounds have a key role to play in the bio metabolism of diverse important molecules.

4. Discussion

Microbial species living in different extreme environments have proven to be a reliable source of novel therapeutic agents [27]. These organisms have been shown to support the production of certain metabolites that have led to harsh environmental conditions [28]. Extremophiles organisms produce novel bioactive compounds that have diverse therapeutic (antioxidant, antimicrobial, antiviral and anticancer) applications [29,30]. Among these polyextremophiles, radiation-resistant organisms grow and survive at extreme exposures as well as non-ionizing radiations [31]. Extensive studies and research have been conducted on the evaluation of extremophilic organisms in their bioactive secondary metabolites but very few studies have investigated the secretion of secondary metabolites by D. radiodurans. This organism is the toughest bacterium known until now [8]. This bacterium can survive lethal doses of radiation exposures (ionizing and non-ionizing) with minimal reduction in cell viability [7]. D. radiodurans has the potential to produce diverse bioactive compounds (secondary metabolites) having therapeutic potential against diverse health disorders. Antioxidants are important small molecules used as anti-aging agents in skin support because antioxidants help protect the skin from the toxic effects of free radicals that would otherwise impair and destroy healthy skin cells [32]. The synthesis of natural antioxidants is not restricted to only terrestrial sources. Radioresistant organisms have also proven to be reliable and rich sources of natural antioxidant compounds [33]. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [34]. Flavonoids and polyphenols are powerful antioxidants and are a necessary part of human health. Phenolics possess unique redox properties that contribute to neutralizing free radicals, decompose peroxides, and quenching of singlet and/or triplet oxygen [35]. The flavonoids act as excellent antioxidants by suppression of ROS formation, scavenging ROS and upregulation or protection of antioxidant defenses systems [36]. Different natural bioactive compounds including quinones, phenols xanthones have been reported in some extremophilic species of fungi. These bioactive compounds have been reported to exhibit radical scavenging activities, antimicrobial activities and anticancer activities [37]. The TPC and TFC in the CSME of D. radiodurans was calculated to be (3.8 mg PGE/ g of CSME) and (5.3 mg QE/ g of CSME), respectively (Figure 1A and 1B) which is the first report of total phenolic and flavonoid content in the bacterial strain employed in the present study. This shows that the presence of phenolic and flavonoid compounds plays a key role in neutralizing the harmful reactive molecular species and contributes towards the high antioxidant activity of CSME of D. radiodurans. Free radicals are highly reactive species derived either from...
metabolic reactions or as a response to external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. The free radicals damage the nucleus, cell membrane, DNA, proteins, carbohydrates, and lipids [38,39]. In a living system, hydroxyl radical and superoxide radical is being continuously formed in a process of reduction of oxygen to water. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage like a reduction of disulfide bonds in proteins, specifically fibrinogen, resulting in their unfolding and scrambled refolding into abnormal spatial configurations [40]. Consequences of this reaction are observed in many diseases such as atherosclerosis, cancer, and neurological disorders, and can be prevented by the action of non-reducing substances [41]. The ABTS and Superoxide anion radical is one of the strongest [42] and harmful ROS species to cellular components [43]. These radicals are reactive towards most antioxidants which include thiols, phenolics and ascorbic acid. The radical scavenging activities of CSME of D. radiodurans against DPPH, ABTS, hydroxyl and superoxide radicals were calculated as 84.7 %, 75.6 % and 85.1 %, respectively (Figure 2, 3, 4 and 5). Zheng et al., (2016) [37] reported that the exopolysaccharides in the crude extract of Bacillus cereus exhibited radical scavenging and antioxidant activity [44]. This suggests that the free radical scavenging potential of CSME of D. radiodurans may be due to the presence of different small molecule antioxidants that act as potential scavengers against the harmful effects of different free radicals. The LC-MS analysis in the present study focused primarily on non-volatile secondary metabolites, most of which cannot be analyzed with gas chromatography-mass spectrometry (GC-MS) (Figures 6 and 7). The LC-MS analysis of CSME of D. radiodurans displayed 29 major secondary metabolites in ESI positive and negative ion modes (Table 1 and 2). These metabolites comprised of amino acids, flavonoids, polyphenols, glycosyl and other analogs, etc. The major secondary metabolites in CSME of D. radiodurans are Mannosamine (m/z 149.21), is a hexosamine derivative of mannose which has been reported to possess biomedical applications of functionalized nanomaterials in different drug delivery strategies [45]. An organic compound, xanthone (m/z 195.15) which possesses anti-inflammatory, insecticidal and larvicidal activities were detected [46,47]. Cytidin-5′-triphosphate disodium salt (m/z 165.19) is a pyrimidine nucleoside triphosphate and has been reported to play a key role in pyrimidine biosynthesis and coenzyme in glycerocephospholipid biosynthesis was identified [48]. Ethanolamine (m/z 441.4) is the second-most-abundant head group for phospholipids and has been reported to possess pharmaceutical applications, as corrosion inhibitors, and as chemical intermediate was detected [49]. Folic acid (m/z 61.08) is a form of vitamin B9 and has been reported to reduce the risk of melanoma and breast cancer was detected in the present study [50]. Octadecanoic acid (m/z 117.15) is a C18 straight-chain saturated fatty acid component of many animal and vegetable lipids. This compound is known to exhibit potent anti-inflammatory activity [51]. Pyrroloquinoline quinine (m/z 330.11) is also known as redox cofactor was detected. Pyrroloquinoline quinine has been found to be one of the active scavengers of ROS during oxidative stress in D. radiodurans [14]. Butyrate (m/z 85.11) has been reported to exhibit anti-inflammatory, used in ulcerative colitis and colorectal cancer was also identified [52,53]. O−Palmitoyl−L−Carnitine chloride (m/z 115.13) is a protein kinase inhibitor and has been used for treating cardiomyocytes was detected [54]. Myxothiazol (m/z 487.32) has been used as an antibiotic which inhibits mitochondrial cytochrome b1 complex was also detected [55]. From the above results, D. radiodurans CSME consists of different secondary metabolites that possess diverse therapeutical and pharmacological properties. This proves that CSME of D. radiodurans is a promising source of different secondary metabolites of diverse therapeutical properties.

Conclusions
The present study investigated the presence of different known and unknown secondary metabolites in the D. radiodurans CSME. The results presented in this study prove that the bioactive compounds (secondary metabolites) present in the CSME of D. radiodurans exhibit diverse pharmaceutical activities. The present study provides primary evidence that CSME of D. radiodurans is a potential natural source of natural antioxidants bioactive compounds having therapeutic values. This study offers the first comprehensive scientific report of the different bioactive compounds in the CSME of D. radiodurans, highlighting its importance as a potential source of active therapeutical ingredients. However, further studies are required to identify the bioactive compounds present in the crude extract and to understand the underlying molecular mechanism(s) of these bioactive compound(s). Conflict of Interest The authors declare no conflict of interest.

References


[50] Μουρσελάς Σ. Πειραματικές μετρήσεις και μοντέλοποιηση ισορροπίας φάσεων σε μίγματα που περιέχουν ιοντικά υγρά τρίτης γενιάς.


