PDO Rotonda’s Red Eggplant Extract: In Vitro Determination of Biological Properties and Minerals Bioaccessibility

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Abstract: Background: The Rotonda’s Red Eggplant belongs to the family of Solanum aethiopicum and it is cultivated in a specific area of Potenza (Basilicata, South of Italy) including villages of Rotonda, Viggianello, Castelluccio Superiore and Castelluccio Inferiore. The Red Eggplant cultivated in this area has gained the PDO, “Protected Designation of Origin”.

Objective: The aim of this research was to evaluate the use of PDO Rotonda’s Red Eggplant extract as a possible nutraceutical supplement. The antioxidant, antihypertensive, hypoglycemic, and hypolipidemic properties were in vitro evaluated.

Methods: The antioxidant activity was investigated by evaluating the scavenging properties against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals and by performing the Ammonium Molybdate and Folin-Ciocalteu assay. The hypoglycemic and antihypertensive activity was studied by evaluating the α-Amylase, α-Glucosidase and Angiotensin Converting Enzyme, respectively, inhibiting activity. In order to evaluate the hypolipidemic activity, the pancreatic lipase inhibiting property was determined and Oil Red O staining assay was performed. Finally, to evaluate the possible use of this extract as a minerals supplement, Selenium, Potassium and Chrome bioaccessibility was studied.

Results: The obtained results underline the good antioxidant, hypoglycemic, antihypertensive and hypolipidemic in vitro properties of the PDO Rotonda’s Red Eggplant extract. Moreover, the obtained data show a higher minerals bioaccessibility and this higher value could be ascribable to the natural phytocomplex of PDO Rotonda’s Red Eggplant, which increases the minerals bioaccessibility if compare it with a control sample.

Conclusion: The obtained results show that PDO Rotonda’s Red Eggplant extract, might be used as a possible nutraceutical supplement, along with traditional therapies, both for its biological properties and for its minerals bioaccessibility value.

Keywords: ABTS, ammonium molybdate, DPPH, minerals bioaccessibility, oil Red O, pancreatic lipase, Rotonda’s Red Eggplant extract, Solanum aethiopicum, α-amylase, α-glucosidase.

1. INTRODUCTION

Solanum aethiopicum is a vegetable crop belonging to the genus Solanum and it grows in Africa, where it is also known as Scarlet eggplant [1]. With the name “eggplant” other two cultivated species are also described that belong to subgenus Leptostemonum, such as S. melongena L., which is defined as brinjal eggplant and Solanum macrocarpon L., also defined gboma eggplant [2]. The scarlet eggplant (Solanum aethiopicum) is subdivided into four cultivar groups: Aculeatum which is commonly used as an ornamental, Gilo particularly used for its fruits while Kumba and Shum were used for their leaves [1].

Scarlet eggplant populations (an agro-ecotype locally named ‘melanzana rossa di Rotonda’) were found in a specific area of Potenza (Basilicata, South of Italy) including villages of Rotonda, Viggianello, Castelluccio Superiore and Castelluccio Inferiore [3]. The Red Eggplant cultivated in...
this area has gained the PDO, “Protected Designation of Origin” [4] and this area is characterized by a mild climate which is favorable for growing red eggplants [5].

In different studies, the chemical composition and the biological activity of Solanum aethiopicum were evaluated and the obtained data suggest that this scarlet eggplant is characterized by different biological properties probably due to its phenolic composition. Recent studies have shown that S. aethiopicum extract contains phenols, including chlorogenic acid [6] and that this phenolics-rich extract could be exploited for potential use in pharmaceutical formulations for preventive medicine in the management of type-2 diabetes and hypertension [7]. In a previous research, the antioxidant, hypoglycemic and anti-hypertensive activities of S. aethiopicum extract were evaluated [7] and the biological properties of the extract are probably correlated with its phenolic phytochemicals content [8]. Another research showed that Solanum Gilo and Solanum melongena are able to reduce serum total cholesterol, triglyceride and LDL cholesterol in hypercholesterolemic rabbits [9]. In addition, in another research, an extract obtained from S. aethiopicum showed an anti-inflammatory activity suppressing the egg albumin induced rat paw oedema both at the early and later phases of oedema [10], suggesting that this extract could also modulate different inflammatory conditions. According to these studies, the aim of the present research is to characterize, for the first time, the extract obtained from PDO Rotonda’s Red Eggplant, that belong to the other scarlet eggplant populations but has a PDO denomination which makes it different from the other eggplant. The purpose of this study is to evaluate the PDO RRE extract in terms of antioxidant, hypoglycemic, anti-hypertensive and hypolipidemic in vitro properties and to evaluate its healthy effects. Moreover, in order to characterize the extract in terms of mineral bioaccessibility the Selenium, Potassium and Chrome bioaccessibility was studied and the in vitro simulated gastrointestinal digestion protocol was used. These analyses allowed to evaluate the possible use of PDO Rotonda’s Red Eggplant (RRE) extract, along with traditional therapies, in the management of different metabolic disorders or of minerals deficiency.

2. MATERIALS AND METHODS

2.1. Sample Preparation

The whole fruit of PDO Rotonda’s Red Eggplant was crushed and mixed with a solution of 5% of hydrochloric acid in distilled water (in a proportion of 1:3) and was sonicated in a water bath for 1 hour. After that, the obtained solution was previously clarified by ultrafiltration and then by a nanofiltration. In order to obtain an extract highly concentrated in bioactive compounds, the obtained solution was frozen and freeze-dried. The fruit extraction with an acid solution allows to hydrolyze the glycoalkaloids present in S.aethiopicum [11].

2.2. Materials

All reagents and compounds used were obtained from Sigma Aldrich (Milan, Italy) and 3T3-L1 pre-adipocyte cells were obtained from the ATCC (ATCC® CL-173 ™).

PDO Rotonda’s Red Eggplant (RRE) dry extract was provided by Evra srl (Potenza, Italy).

2.3. Instrumentation

UV-Vis absorption spectra were obtained with a Jasco V-530 UV/Vis spectrometer (Jasco, Lecco, Italy).

2.4. Evaluation of Scavenging Activity on ABTS Radical

The RRE extract ability to scavenge ABTS (2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical was carried out according to literature with different concentrations of tested sample [12]. In order to produce ABTS+ radical cation, an aqueous ABTS solution (7mM) comes to react with a Potassium persulfate solution (2.45 mM) and then, the obtained mix solution, was left for 12-16 hours at room temperature and protected from light until used. The obtained reaction mixture was diluted with distilled water to an absorbance of 0.77 ± 0.030 at 734 nm. With the aim to evaluate the antioxidant properties of the RRE extract, different amounts of it were dissolved in distilled water and 1 ml of these were added to 2 ml of ABTS diluted solution. The obtained samples were allowed to stand at room temperature and protected from light for 5 minutes and, after that, their absorbance was measured at 734 nm. The IC_{50} value, and so the antioxidant activity of RRE extract, was expressed as percentage of scavenging activity and was calculated as follows:

\[
\text{Inhibition (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of the control in which RRE extract was replaced by 1 ml of distilled water; and \(A_1\) is the absorbance of different concentrations of the tested samples.

All determinations were assayed in triplicate and the obtained data were expressed as means (±SD).

2.5. DPPH Scavenging Activity

In the aim to verify the antioxidant activity of RRE extract, the DPPH (2,2’-diphenyl-1-picrylhydrazyl) radical assay was performed according to the literature [13]. The mix solutions were composed by 1 ml of a solution containing different amounts of RRE extract, 4 ml of a methanolic DPPH solution (200 µM) and 6 ml of Methanol. These reaction mixtures were incubated for 15 minutes in dark conditions and, after that, the absorbance of these samples, and so of residual DPPH, was evaluated at 517 nm. The radical scavenging ability of the tested RRE extract, expressed as IC_{50} value, was calculated according to the Eq. (1). All determinations were carried out in triplicate and data were expressed as means (± SD).

2.6. Evaluation of Total Antioxidant Activity

The total antioxidant activity of RRE extract was determined according to procedure reported in literature [14]. Briefly, 0.6 ml of an aqueous solution containing RRE extract (10 mg/ml) was mixed with 2.4 ml of reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 M Ammonium molybdate) and, after that, the reaction mixture was incubated at 90°C for 150 min. At the end of this time
and after cooling to room temperature, the absorbance of the sample was measured at 695 nm. The total antioxidant activity of the RRE extract was defined as mg of gallic acid equivalents per gram of extract. In order to obtain the calibration curve of gallic acid, six different standard solutions of it were performed with the same protocol. Each test was assayed in triplicate and data were expressed as means (± SD).

2.7. Folin-Ciocalteu Assay

In order to evaluate the total polyphenols content of RRE extract, the Folin-Ciocalteu assay was performed. 10 mg of the dried extract was mixed with 1 ml of Folin-Ciocalteu reagent, 1 ml of distilled water and 1 ml of a sodium carbonate solution (7.5% w/v). The mix of reaction was incubated for 2 hours at room temperature and in dark conditions [15]. At the end of this time, the absorbance of the solution was measured at 760 nm and the amount of total polyphenols was expressed as mg equivalent of Chlorogenic Acid per gram of extract (mgCAeq/g). In order to obtain the calibration curve of gallic acid, six different standard solutions of it were performed with the same protocol. Each test was assayed in triplicate and data were expressed as means (± SD).

2.8. Determination of α-Amylase Inhibition

The RRE extract ability to inhibit α-Amalyse enzyme was evaluated according to literature with slight modifications in the reaction time and temperature [7]. The amount of 500 µL of different concentrations of RRE extract dissolved in distilled water was incubated (for 15 min at 37°C) with 500 µL of 1% of Starch solution dispersed in PBS (Phosphate Buffer Solution), (1 x 10⁻³ mol L⁻¹, pH 7) and with 500 µL of porcine pancreatic α-Amylase enzyme (0.5 mg/ml in distilled water). To stop the reaction, 1 ml of Dinitrosalicylic acid colour reagent was added and the mixture was further incubated for 5 minutes in a boiling water bath. In these conditions, the reduction of 3,5-Dinitrosalicylic acid to 3-Amino-5-nitrosalicylic acid allows to quantify maltose which being detectable at 540 nm. The final concentration of maltose is related to the presence of α-Amylase inhibitor. The inhibition of α-Amylase was calculated as percentage of inhibition as follows:

\[
\text{Inhibition} (\%) = \left( \frac{Ac - (As - Ab)}{Ac} \right) \times 100
\]

where Ac is the absorbance of control without RRE extract, Ab is the absorbance of blank without α-Amylase and As is the absorbance of the sample. Each test was performed in triplicate and data were expressed as means (± SD).

2.9. Determination of α-Glucosidase Inhibition

Inhibition of α-Glucosidase enzyme was assessed according to the method present in literature [16]. The volume of 100 µL of porcine pancreatic α-Glucosidase solution (1.0 U/ml) prepared in PBS (0.1 M pH 6.9) was incubated with 50 µL of different concentrations of an aqueous solution of RRE extract for 15 min at 37°C. At the end of this time, 100 µL of p-Nitrophenyl-α-D-glucopyranoside (3mM) substrate was added and the obtained reaction mixture was incubated for 15 min at 37°C, again. Finally, 400 µL of Sodium Carbonate (Na₂CO₃) was added and, after stopping the reaction, the absorbance of released 4-Nitrophenol was read at 405 nm. The obtained results were expressed as inhibition percentage of the enzyme activity calculated according to Eq. (2). Each test was carried out in triplicate and data were expressed as means (± SD).

2.10. Determination of Angiotensin Converting Enzyme Inhibitory Activity

The RRE extract ability to inhibit ACE (Angiotensin Converting Enzyme) was evaluated by assessing the method reported in literature [17]. In a test tube the amount of 30 µL of different concentrations of RRE extract aqueous solution and of ACE solution (50 µL, 4 mU) prepared in PBS 0.2 M, pH 8.3 which contained Sodium chloride 0.3 M, were added and then incubated at 37°C for 10 min. In the aim to start the reaction, the volume of 220 µL of Hippuric acid-histidine-leucine (10 mM) substrate was added and the mixture was incubated for 30 min at 37°C, again. Finally, 220 µL of Hydrochloric acid (HCl 1.0 N) was added and the Hippuric acid, produced from the cleavage of substrate in the absence of an inhibitor, was extracted by adding 2 ml of Ethyl acetate. At the end of the extraction, the mixture was centrifuged and 1 ml of the upper Ethyl acetate layer was evaporated under vacuum. The absorbance of residue, which was redissolved in distilled water, was measured at 228 nm. The RRE extract ability to inhibit ACE was calculated according to the Eq. (2). Each test was performed in triplicate and data were expressed as means (± SD).

2.11. Measurement of Pancreatic Lipase Inhibition

The evaluation of porcine pancreatic lipase (type II) inhibition activity was assessed according to the method reported in literature [18] with modifications in the used wavelength. Briefly, 25 µL of different concentrations of RRE extract aqueous solution, 150 µL of Pancreatic lipase stock solution (3mg/ml), 150 µL (75 mM in Dimethyl Sulfoxide) of solution of P-nitrophenyl butyrate (p-NPB) substrate and 2 ml of PBS (0.01 M, pH 8) were incubated at 37°C for 30 min. In this test, the concentration of p-Nitrophenol was evaluated at 412 nm and the pancreatic lipase inhibition was expressed according to Eq. (2). All determinations were carried out in triplicate and data were expressed as means (± SD).

2.12. Cell Culture and Differentiation

The 3T3-L1 pre-adipocyte cells were cultured in DMEM supplemented according to the literature [19]. The medium was replaced every two days and NCS (Newborn Calf Serum) was used.

2.13. Determination of Cell Viability

In order to assess cell viability, MTT staining assay was used [20]. Different concentrations (10, 20, 30 µg/ml w/v) of RRE extract aqueous solution were incubated with 3T3-L1 pre-adipocyte cells (for 24 h at 37°C in humidified hair with 5% CO₂). At the end of treatment, each well was incubated
2.14. Oil Red O Staining Assay

At 570 nm in a microplate reader, the optical density was measured for 2 hours with fresh MTT and after that, 1 ml of Dimethyl Sulfoxide was added and the resultant chromogen formazan products were solubilized. The optical density was measured at 570 nm in a microplate reader.

2.15. In vitro Bioaccessibility Studies: Determination of Cr, K and Se after Simulated Gastrointestinal Digestion Protocol

For this purpose, the amount of 1g of RRE extract was digested in a way in which gastric and intestinal fluids were simulated. In order to simulate gastric digestion, 1 g of RRE extract was added to 50 ml of distilled water and this mixture was adjusted to pH 2 by adding HCl (5 M). Then, 3 ml of Pepsin solution (2% w/v of Pepsin dissolved in 0.1 M NaHCO₃) was added and to simulate the body temperature conditions, the reaction mixture was incubated for 1 hours at 37° under agitation. At the end of this time, the pH of this reaction mixture was adjusted to 6 by adding NaOH, 120 mM NaCl and 5 mM KCl, the pH value was adjusted to 7 and the mixture was incubated at 37°C for 1 h under agitation. In order to evaluate the minerals content by inductively coupled plasma optical emission spectrometry, the RRE extract was centrifuged for 10 minutes at 1000 g and then filtered.

An aqueous solution containing Sodium Selenite (Na₂SeO₃), KCl (Potassium Chloride) and Chromium Chloride was used as a control sample.

The measurement of the concentrations of Se, K and Cr minerals in the simulated GI solution after centrifugation and filtration (bioaccessible fraction), expressed as a percentage, is calculated using Eq. (3). The same equation was used to evaluate the minerals concentration in the control sample.

\[
\text{Minerals content (RRE extract after in vitro digestion)} \times 100
\]

\[\text{Minerals content (RRE extract before in vitro digestion)}
\]

2.16. Statistical Analysis

Data are reported as mean ± standard deviation values of independent experiments, which were done at least three times, each time with three or more independent observations. Statistical analysis was performed by Student’s t-test. A P value less than 0.05 was considered significant.

3. RESULTS AND DISCUSSIONS

3.1. Evaluation of Scavenging Activity on DPPH and ABTS Radicals

In order to evaluate the antioxidant activity of PDO Rotonda’s Red Eggplant extract, ABTS and DPPH assays were performed and the obtained results were expressed as IC₅₀ value. As shown in Fig. (1), RRE extract exhibits a very high DPPH inhibition activity, with an IC₅₀ value of 692 µg/ml and, as it is possible to note in Fig. (2), RRE extract inhibits ABTS radical with an IC₅₀ value of 324.5 µg/ml.

The antioxidant properties of PDO Rotonda’s Red Eggplant extract were investigated by evaluating its scavenging ability towards DPPH and ABTS radicals. The obtained results are in agreement with different studies in which the antioxidant activity and the total phenolic compounds of S. aethiopicum were studied. A previous study reported that S. aethiopicum species are a rich source of phenolic acids and, among these, chlorogenic acid is the one present in higher concentrations [6]. This phenolic acid, highly present in vegetables, has been shown to have several biological properties such as antioxidant properties, free-radical scavenging capacities and regulation of enzymatic activity [23]. For this reason, selecting vegetable varieties with high levels of phenolic compounds and of chlorogenic acid could be used as antioxidant sources and for the prevention of oxidative damage. Though there are different researches in which the phenolic composition and the antioxidant activity of S. aethiopi-
cum were discussed, in this study, the biological properties of an extract obtained from PDO Rotonda’s Red Eggplant were studied for the first time.

3.2. Evaluation of Total Antioxidant Activity

The total antioxidant activity of the RRE extract was expressed as mg equivalent of gallic acid per g of extract. By comparing the data with the gallic acid calibration curve, the amount of gallic acid equivalent (43.76 mg/g of extract) was determined. This test was evaluated by performing the molybdate assay. In this assay, the subsequent green phosphate/Mo (V) complex, formed by the reduction of Mo (VI) to Mo (V) was quantified at 695 nm. The obtained absorbance values indicate that RRE extract shows a significant antioxidant activity.

3.3. Folin-Ciocalteu Assay

The Folin-Ciocalteu assay allows to quantify the amount of total phenolic compounds present in RRE extract. The polyphenols content of the tested RRE extract was expressed as mg equivalent of Chlorogenic Acid per gram of dried extract (mg eqCA/g) and, in these experimental conditions, there are 77 mg eqCA/g of RRE extract. The obtained results underline the presence of antioxidant compounds in this kind of fruits and show that, in this extract, the amount of Chlorogenic Acid equivalents is higher if compared to the results obtained from another study in which an extract obtained from Solanum melongena was studied [24]. Indeed, the content of mg eqCA/g of extract is 7 mg/g and this result underline the differences between these two extracts that could be due both to the kind of fruits that to the type of the extraction that has been performed.

3.4. Determination of α-Amylase and α-Glucosidase Inhibition

In order to evaluate the RRE extract ability to manage metabolic disorders related to hyperglycemia, its ability to inhibit α-Amylase and α-Glucosidase enzymes was evaluated. The obtained results, expressed as IC₅₀ value, are shown in Table 1 and a considerable reduction of α-Amylase and α-Glucosidase activity was found. The RRE extract concentrations of 530 µg/ml and of 184 µg/ml inhibit 50% of the α-Amylase and α-Glucosidase activity, respectively. These enzymes are involved in the digestion and in the absorption of carbohydrates: α-Amylase hydrolyzes complex polysaccharides into oligosaccharides and α-Glucosidase breakdown them in glucose, which is ready to be absorbed. The subsequent intestinal absorption of glucose causes an increase of blood sugar levels [25]. The inhibition of these enzymes represents a valid alternative in the treatment of hyperglycemic conditions and, for this reason, the α-Amylase and α-Glucosidase inhibiting activity of RRE extract was evaluated. The obtained results show a stronger inhibition of α-Amylase and α-Glucosidase activity. According to another study, natural extracts characterized by a high concentration of phenolic compounds, could be considered good inhibitors of carbohydrate digestive enzymes and could be used to control hyperglycemic conditions [26]. These findings revealed that RRE extract could be used as a possible natural alternative in the treatment of hyperglycemic state.

3.5. Determination of Angiotensin Converting Enzyme Inhibitory Activity

In order to evaluate PDO Rotonda’s Red Eggplant extract as a possible remedy in the treatment of hypertension, its ACE inhibitory activity was study. The determination of ACE inhibitory activity is a measure of the ability of a sample to stop the development of angiotensin-2 and so the activation of aldosterone secretion that causes a subsequent elevation of blood pressure and then hypertension. The obtained results expressed as IC₅₀ value (20 µg/ml) in Table 1 clearly indicate the significant inhibitory activity of PDO Rotonda’s Red Eggplant extract. Inhibition of Angiotensin Converting Enzyme (ACE) activity is considered an important alternative approach in the treatment of hypertension. ACE is involved in the conversion of Angiotensin I in Angiotensin II, which, once released, causes the synthesis and release of aldosterone and the subsequent vasoconstriction and hypertension [27]. The obtained IC₅₀ value revealed that the concentration of 20 µg/ml is able to inhibit 50% of the enzyme activity. This activity is probably correlated with the phenolic compounds present in this
3.6. Measurement of Pancreatic Lipase Inhibition

In order to evaluate the RRE extract ability to inhibit pancreatic lipase enzyme, different amounts of it were dissolved in distilled water and the RRE extract concentration of 75 µg/ml (Table 1) inhibits 50% of the enzyme activity and by using increasing concentrations of the extract, the percentage of inhibiting activity increased also. Pancreatic lipase is a key enzyme that promotes the triglycerides hydrolyses and fat absorption as monoacylglycerols and fatty acids [30]. Because of its important role in the digestion of ingested fat, the pancreatic lipase inhibiting activity of several natural extracts was studied and so, the role of natural anti-obesity agents was defined [31]. In a previous study, the in vitro pancreatic lipase inhibition activity of different natural extracts was evaluated and, according to the obtained results, the authors concluded that these extracts, due to their pancreatic inhibition activity, could find a use as anti-obesity agents [32]. Our results revealed that RRE extract is able to inhibit pancreatic lipase enzyme in a dose dependent manner and, for this reason, also this extract could be used as a natural anti-obesity supplement.

3.7. Cell Viability Determination

Cell viability on 3T3-L1 cells was performed and the cytotoxicity of different concentrations (10, 20, 30 µg/ml) of RRE extract was evaluated (Fig. 3). As it is possible to note, every RRE extract concentration could be used, and it is possible to define this extract as non-cytotoxic to 3T3-L1 cells.

3.8. Oil Red O Staining

In the aim to evaluate the hypolipidemic activity of RRE extract, the inhibition activity on fat droplet formation in 3T3-L1 cells was evaluated. The obtained results show that RRE extract (20 µg/ml) decreases cellular lipid accumulation (Fig. 4). The reduction of lipid accumulation in the adipocytes was about 50% compared to MDI- treated control cells (Fig. 5). This performed protocol allowed to investigate the in vitro antiobesity properties of natural extract and so their ability to regulate adipocyte differentiation and lipid accumulation, which are related to the development of obesity. Different studies have evaluated the hypolipidemic effect of fruits belonging to the Solanaceae family such us Solanum melongena [33], but no study has researched these properties in the fruit of S. aethiopicum. Based on the obtained results, PDO Rotonda’s Red Eggplant extract could be used, along with traditional therapies, as a possible natural anti-obesity product.

3.9. In Vitro Bioaccessibility Studies: Determination of Cr, K and Se after Simulated Gastrointestinal Digestion Protocol

The minerals content in RRE extract before digestion was 0.115 µg/g for Se, 6200 µg/g for K and 0.317 µg/g for Cr, while after digestion their content was 0.0417 µg/g, 2500 µg/g and 0.152 µg/g, respectively. In the control sample, the same RRE extract minerals concentration was used and after in vitro digestion, the content of Se, K and Cr was 0.005

Table 1. α-Amylase, α-Glucosidase and angiotensin converting enzyme, pancreatic lipase inhibition activity of PDO Rotonda’s Red Eggplant extract. The results were expressed as IC50 value (means ± SD are presented).

<table>
<thead>
<tr>
<th>PDO Rotonda’s Red Eggplant Extract</th>
<th>IC50 of α-Amylase (µg/ml)</th>
<th>IC50 of α-Glucosidase (µg/ml)</th>
<th>IC50 of Angiotensin Converting Enzyme (µg/ml)</th>
<th>IC50 of Pancreatic Lipase (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>530 ± 0.3</td>
<td>184 ± 0.5</td>
<td>20 ± 0.4</td>
<td>75 ± 0.7</td>
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</table>
PDO Rotonda’s Red Eggplant Extract: In Vitro Determination of Biological Properties

**Fig. (4).** Inhibitory effect of RRE extract on lipid accumulation in 3T3-L1 adipocytes. (a): Untreated control cells. (b): Differentiated 3T3-L1 adipocytes were treated with RRE extract at concentration of 20 µg/ml and intracellular lipid accumulation was observed via microscope. The stained adipocytes were photographed at 100× magnification. The Oil Red O was eluted and quantified at 520 nm for quantification of lipid accumulation in the cells.

Fig. (5). The lipid accumulation was measured by Oil Red O staining. Results are presented as mean ±SD of at least three independent experiments. Significance was defined as *p <0.05.

### Table 2. In vitro bioaccessibility of Selenium, Potassium and Chrome mineral content in RRE extract and control sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Selenium (%)</th>
<th>Chrome (%)</th>
<th>Potassium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRE extract</td>
<td>36 ± 0.2</td>
<td>48 ± 0.3</td>
<td>40 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>5 ± 0.6</td>
<td>15 ± 0.5</td>
<td>12 ± 0.4</td>
</tr>
</tbody>
</table>

μg/g, 744 µg/g and 0.048 µg/g, respectively. The obtained data showed that the chemical composition of Rotonda’s Red Eggplant extract probably increases the minerals bioaccessibility of 7.2 times for Selenium, of 3.2 times for Chrome and of 3.4 times for Potassium if compared with a solution in which the same mineral content was dissolved (Table 2).

Selenium is an essential trace element and it is naturally present in many foods or used as a supplement in several Se enriched foods. It is an important component of enzymes for redox reactions as seleno-cysteine in thioredoxin reductase [34] and in glutathione peroxidase [35] and so protects from oxidative damage and infection. Selenium exists in two forms, which are good dietary sources of it: inorganic (selenate and selenite) and organic (selenomethionine and selenocysteine) forms [36]. Plant and vegetables accumulate the inorganic form and convert it into organic form, mostly selenocysteine, selenomethionine and their methylated derivatives. Intake recommendations for selenium and other nutrients are provided in the Dietary Reference Intakes and it is 55 µg/day for adults, 60 µg/day during pregnancy, and 70 µg/day during lactation with an upper limit of 400 µg/day with no adverse effects [37]. Selenium has a potential activity in health as well as in several disease conditions [38]; it is able to improve cardiovascular diseases and thyroid dysfunction. Selenoproteins help to prevent the oxidative modification of lipids, reducing inflammation and preventing platelets from aggregating [38], and for these reasons, different researches have suggested that selenium supplements could reduce the risk of cardiovascular diseases. Furthermore, Selenium concentration can help prevent or treat thyroid disease, in fact, it is higher in the thyroid gland than in any other organ in the body, and, like iodine, selenium has important functions in thyroid hormone synthesis and metabolism [39].
Trivalent chromium is essential for human nutrition [39] and play an important role in the regulation of bloods levels glucose [40] and in the modulation of carbohydrate and lipid metabolism. Intake recommendations of Chromium is 35 µg/day for men and 25 µg/day for women [41]. Several studies evaluated the role of Chromium in the glucose metabolism and the most widely accepted hypothesis is the involvement of an oligopeptide, named chromodulin [42]. This small oligopeptide is activated after binding four chromium atoms and only in this form is able to cause the insulin signal amplification [43]. After binding the insulin receptor, the activated chromodulin leads to the co-localization of GLUT4 protein (glucose transporter protein 4) to the cell membrane and so allows the glucose transport through the cell membrane [44]. Chromium supplementation could decrease glucose concentration in the blood, reduce the probability of atherosclerosis and heart attack [45].

Potassium is a mineral, which is able to manage both cellular and electrical functions, and to maintain normal blood pressure preventing several cardiovascular diseases [46]. This electrolyte is important also for its ability to modulate skeletal and smooth muscle contraction and to prevent osteoporosis, particularly in the older women [47]. Potassium is found in a wide range of foods such as leafy green vegetables, fruits, whole grain and fish (such as salmon) [47]. The consumption of this kind of foods allows to intake this electrolyte and so to ameliorate blood pressure and muscle contraction, and to prevent osteoporosis. Intake recommendations of Potassium are 3800 mg/die for men and 2800 mg/die for women [48]. The obtained results show a higher minerals bioaccessibility if compare it with a control sample. This higher value could be ascribable to the natural phytocomplex of PDO Rotonda’s Red Eggplant, which is able to increase the minerals bioaccessibility [49-51].

CONCLUSION

The objective of the present study was to characterize an extract obtained from PDO Rotonda’s Red Eggplant and to verify its biological properties and the probable use of it as nutraceutical supplement. The obtained in vitro data showed that extract intake could be a useful instrument, along with traditional therapies, in the management of different metabolic disorders such as hyperglycemic condition, hypertensive state and lipid accumulation and to ameliorate mineral deficiency. In the guidelines of Mediterranean diet, the daily consumption of vegetables is recommended because this kind of foods are good sources of natural antioxidants, such as vitamins, flavonoids, and other phenolics compounds, but often the recommended intake is not sufficient. In recent years, the attention has therefore been focused on nutraceutical supplements formulation, which could be used in diets characterized by a lower intake of fruits and vegetables. For all these reasons, the object of the present work was to study the biological properties and mineral bioaccessibility of PDO Rotonda’s Red Eggplant extract and to evaluate its possible use as nutraceutical supplement. The obtained results underline the good antioxidant, hypoglycemic, antihypertensive and hypolipidemic in vitro properties of the PDO Rotonda’s Red Eggplant extract. Moreover, the obtained data showed a higher minerals bioaccessibility and this higher value could be ascribable to the natural phytocomplex of PDO Rotonda’s Red Eggplant, which increases the minerals bioaccessibility if compare it with a control sample.

AUTHOR CONTRIBUTIONS

Francesco Puoci and Vincenzo Pezzi conceived and designed the experiments; Fabio Amone and Rocco Malivindi performed the experiments; Mariarosa Ruffo and Luca Scriverio analyzed the data; Domenico Gorgoglione and Filomena De Biasio provided the Red Eggplant dry extract; Ortenzia Ilaria Parisi wrote the paper.

ETHICS APPROVAL AND CONSENT TO PARTICI-PATE

Not applicable.

RESEARCH INVOLVING PLANTS

All the experimental research on plants was in accordance with guidelines of IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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