Determination of Total Phenol Content (TPC) by the Folin-Ciocalteu Method

The TPC of each extract was determined with the Folin-Ciocalteu (FC) method as described in [1,2] with only few modifications. The FC reagent was diluted 10-fold with water. 0.1 Milliliters of the methanolic extract (obtained as described in section 2.2.1) were added to 0.75 mL of the diluted FC reagent. The solution was left standing in the dark for 10 min and then 0.75 mL of a Na_2CO_3 aqueous solution (2% w/v) were added. The resulting solutions were left standing in the dark for 3 h. The absorbance (λ at 765 nm) was red in disposable optical Corning® 96 well plates from Merck Life Science (Merck KGaA, Darmstadt, Germany) using a Sunrise microplate reader (Tecan Italia Srl, Milan, Italy). All the measurements made with 0.2 mL solutions were performed in triplicate for each extract and the results were expressed as mg of gallic acid equivalents (GAE)/g dry extract (d.e.).

Determination of the Total Antioxidant Capacity (TAC) by the FRAP Method

The reducing power was determined according to the method described in [1,2], with only few modifications. The FRAP reagent was prepared from 2.5 mL of a TPTZ solution (10 mM) in HCl (40 mM, aqueous) and 2.5 mL of a $FeCl_3$ aqueous solution (20 mM), mixed with 25 mL of NaOAc (300 mM, pH 3.6). For the determination of the antioxidant activity, 1.5 mL of obtained FRAP reagent were mixed with 0.1 mL of the methanolic extract solution (previously obtained as described in section 2.2.1). The reaction mixture was kept in the dark for 4 min at room temperature before measuring the spectrophotometric assay. The absorbance (λ at 593 nm) was red in disposable optical Corning® 96 well plates from Merck Life Science (Merck KGaA, Darmstadt, Germany) using a Sunrise microplate reader (Tecan Italia Srl, Milan, Italy). All the measurements made with 0.2 mL solutions were performed in triplicate for each extract and the results were expressed as mg of Trolox equivalents (TE)/g dry extract (d.e.).

Determination of the Radical Scavenging Capacity by the DPPH Method

The radical scavenging capacity was measured by using the DPPH method as described in [1,2], with only few modifications. DPPH was progressively solubilized in HPLC-grade EtOH until a concentration producing an absorbance of 0.65 (± 0.02) at 517 nm was reached. A time frame of approximately 2 h was necessary to stabilize the above absorbance value, keeping the solution a temperature of 4 °C. A volume of 0.05 mL of methanolic extract (obtained as described in section 2.2.1) was added to 2.95 mL of the stabilized DPPH solution. The absorbance was determined at 517 nm after 30 min of incubation in the dark at room temperature. The absorbance was red in disposable optical Corning® 96 well plates from Merck Life Science (Merck KGaA, Darmstadt, Germany) using a Sunrise microplate reader (Tecan Italia Srl, Milan, Italy). All the measurements made with 0.2 mL solutions were performed in triplicate for each extract and the results were expressed as mg of Trolox equivalents (TE)/g dry extract (d.e.).

Determination of the Radical Scavenging Capacity by the ABTS Method

The radical scavenging capacity was measured by using the ABTS method as described in [1,2], with only few modifications. A solution of ABTS+ was prepared at a concentration of 0.36% (w/v) in water and a solution of $K_2S_2O_8$ was prepared at a concentration 0.2% (w/v) in water. Then, two volumes of the ABTS+ solution and one volume of the $K_2S_2O_8$ solution were combined. The flask was covered with aluminium foil and allowed standing overnight at room temperature in the dark. The obtained ABTS•+ solution was diluted with EtOH until getting an absorbance of 0.70 (± 0.05) at 734 nm. An aliquot of the ABTS+•/EtOH solution (4 mL) was added to the extract (0.06 mL) and the mixture was left standing in the dark for 6 min. The absorbance was red in disposable optical Corning® 96 well plates from Merck Life Science (Merck KGaA, Darmstadt, Germany) using a Sunrise microplate reader (Tecan Italia Srl, Milan, Italy). All the measurements

made with 0.2 mL solutions were performed in triplicate for each extract and the results were expressed as mg of Trolox equivalents (TE)/g dry extract (d.e.).

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