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Proceeding Paper

Blackcurrant (*Ribes nigrum* L.) and Raspberry (*Rubus idaeus* L.) Ethanolic Extracts: Inhibitory Effects on Pancreatic Lipase and Antioxidant Activity †

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Abstract: A single paragraph of about 100 words to give a brief introduction to your work.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article yet reasonably common within the subject discipline.)

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1. Introduction

In the last years, several studies analysing the composition, the bioactivity of red fruits and a relationship between fruits intake and reduced risk of several chronic diseases, including obesity, have been published. Fruits are sources not only of vitamins, minerals, dietary fiber but also of several healthy phytochemicals. Blackcurrant (*Ribes nigrum* L.) and raspberry (*Rubus idaeus* L.) fruits are a rich source of polyphenols that have showed to possess antioxidant, anti-inflammatory, antimicrobial, and hypoglycaemic properties [1,2]. In this study, in order to investigate as the environmental factors may influence types and contents of active substances and to prospect a potential use as new functional foods and/or nutraceuticals, we investigated the radical scavenging effects and lipase inhibitory activity of the ethanolic extract of blackcurrant *(Ribes nigrum* L.) and raspberry (*Rubus idaeus* L.) collected in Southern Italy. Several previous studies have in fact demonstrated that plants that grow in various environments produce different active substance contents because of their wide distribution in different geological zones. This will result in variations in their internal qualities also in the same species from different growing regions [3].

2. Materials and Methods

Plant materials and extraction procedure. Ripe fruits were collected in southern Italy (WGS84: 39° 87′ 13″ N, 16° 06′ 53″ E). Fresh fruits (350 g) were exhaustively extracted by maceration by using ethanol as solvent (4 × 1.2 L). Dry extracts were stored in brown glass bottles, and kept at 4 °C before analyses.

Total polyphenols content (TPC) and total flavonoids content (TFC). TPC was determined by using the Folin-Ciocalteu method in which extracts (concentration of 1.5 mg/mL) were mixed with water, sodium carbonate 15% (*w*/*v*), and Folin-Ciocalteu reagent [4]. After an incubation of 2 h at room temperature, the absorbance was read at 765 nm by using a UV-vis Jenway 6003 spectrophotometer (Milan, Italy). For TFC determination, extracts (concentration of 1.5 mg/mL) were added to water and sodium nitrite 5% (*w*/*v*). After 5 min, aluminium chloride 10% (*w*/*v*) was added. After other 6 min, sodium hydroxide 1 M and water were added. Then, the absorbance was read at 510 nm [4].

Radical scavenging activity. The radical scavenging activity was investigated by using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests [4]. In the ABTS test, an ABTS radical cation solution was prepared and mixed with a solution of potassium persulfate and left before the use for 12 h in the dark. The ABTS solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. After the addition of the lavender extract to the ABTS solution, the absorbance was read after 6 min at 734 nm. In the DPPH test, a mixture of DPPH ethanol solution (1.0×10^{-4} M) and extracts at different concentrations were prepared and kept for 30 min in the dark. The bleaching of DPPH was determined by reading the absorbance at 517 nm. Ascorbic acid was used as positive control in both assays.

Pancreatic lipase inhibitory activity test. To investigate the lipase inhibitory activity, a previously reported protocol was adopted [5]. Concisely, extracts at different concentrations were mixed with the enzyme, Tris-HCl buffer (pH 8.5), and 4-nitrophenyl octanoate, and the mixture was incubated for 30 min at 37 °C. Then, the absorbance was measured at 405 nm. Orlistat was used as a positive control.

Statistical analysis. Experiments were performed in triplicate. Prism GraphPad Prism Software (San Diego, CA, USA) was used to calculate the concentration causing 50% inhibition (IC_{50}). Data were analysed by One-way analysis of variance (ANOVA) and significant differences were calculated according to Tukey's test.

3. Results and Discussion

The fresh fruits of *R. nigrum* and *R. idaeus* from Calabria (southern Italy) have been subjected to maceration by using ethanol as solvent obtaining extraction. Yields of 11.8 and 11.2% for blackcurrant and raspberry, respectively. A TPC value of 501.1 and 483.7 mg chlorogenic acid equivalents/100 g plant materials for raspberry and blackcurrant, respectively, was found. Blackcurrant extract was characterized by the highest TFC with a value of 35.1 mg quercetin equivalents/100 g plant materials in comparison to raspberry (26.3 mg quercetin equivalents/100 g plant materials).

Raspberry extract was the most active in ABTS test with an IC_{50} of 1.6 μg/mL, value comparable to that of the positive control.

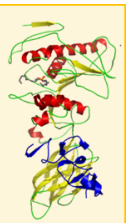
In recent years, inhibitors of pancreatic lipase have received great attention from researchers, and inhibitors from natural source have still attracted much attention due to their wide range of sources, structural diversity, and low toxicity and side effects. The results of our study showed that the ethanolic extracts of both blackcurrant and raspberry have significant inhibitory activity against this enzyme, better than the positive control orlistat. In fact, *R. Idaeus* exhibited an IC_{50} value of 5.1 μg/mL. An IC_{50} of 30.2 mg/mL was found for blackcurrant.

Both extracts were more active than orlistat (IC_{50} of 37.1 μg/mL).

Sample	ABTS test (IC_{50}, μg/mL)	DPPH test (IC_{50}, μg/mL)
R. nigrum	3.3 ± 0.5 [b]	4.7 ± 0.6 [a]
R. idaeus	1.6 ± 0.1[a]	8.9 ± 0.8 [b]
Sign.	**	**
Positive control		
Ascorbic acid	1.1 ± 0.4	5.2 ± 0.2

Data are expressed as mean ± standard deviation (n= 3). Differences were assessed by a One-way analysis of variance (ANOVA) test completed with a multiple comparison Tukey's test. Results followed by different letters in a same column are significantly different at **p< 0.01.

Sample	Pancreatic lipase IC_{50} μg/mL
R. nigrum	30.2 ± 1.8[b]
R. idaeus	5.1 ± 0.9[a]
Sign.	**
Positive control	
Orlistat	37.1 ± 1.1



Data are expressed as mean ± standard deviation (n= 3). Differences were assessed by a One-way analysis of variance (ANOVA) test completed with a multiple comparison Tukey's test. Results followed by different letters in a same column are significantly different at **p< 0.01.

4. Conclusions

The present research points to the potential value of blackcurrant and raspberry extracts in inhibiting lipase and exerting antioxidant effects. The most promising results were obtained with raspberry extract and will contribute towards the development of new functional foods with anti-obesity effects.

This report provides some basic evidence for the effects of ethanolic extracts of these fruits and suggests future in vivo studies for the identification of the molecules that exhibit anti-oxidative and anti-lipase effects and for the development of new products with beneficial health properties for the prevention and/or treatment of metabolic disorders.

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