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Phytochemical Analysis and in vitro Biological Activities of an Ethyl Acetate Extract from the Peel of Punica granatum L. var. Dente di Cavallo

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Abstract : Hyperglycemia represents the main pathogenic factor in the development of diabetes complications and has been found associated with mitochondrial dysfunction and oxidative stress, which in turn increase cell dysfunction. Therefore, counteract oxidative species appears to be a suitable strategy for preventing the hyperglycemia-induce cell damage and support the pharmacotherapy of diabetes and metabolic diseases. Antidiabetic potential of many food sources has been linked to the presence of polyphenolic metabolites, particularly flavonoids such as quercetin and its glycosylated form rutin. In line with this evidence, in the present study, we assayed the potential anti-hyperglycemic activity of an ethyl acetate extract from the peel of Punica granatum L. var. Dente di Cavallo (PGE), a fruit well known to traditional medicine for the beneficial properties of its edible juice. The effect of the extract on the glucidic metabolism has been evaluated by assessing its ability to inhibit α-amylase and α-glucosidase, two digestive enzymes responsible for the hydrolysis of dietary carbohydrates: their inhibition can delay the carbohydrate digestion and reduce glucose absorption, thus representing an important strategy for the management of hyperglycemia. Also, the PGE ability to block the release of advanced glycated end-products (AGEs), whose accumulation is known to be responsible for diabetic vascular complications, was studied. The iron-reducing and chelating activities, which are the primary mechanisms by which AGE inhibitors stop their metal-catalyzed formation, were evaluated as possible antioxidant mechanisms. At last, the phenolic content of PGE was characterized by chromatographic and spectrophotometric methods. Our results displayed the ability of PGE to inhibit α-amylase enzyme with a similar potency to the positive control: the IC_{50} values were 52.2 (CL 27.7 - 101.2) µg/ml and 35.6 (CL 22.8 - 55.5) µg/ml for acarbose and PGE, respectively. PGE also inhibited the α-glucosidase enzyme with about a 25 higher potency than the positive controls of acarbose and quercetin. Furthermore, the extract exhibited ferrous and ferric ion chelating ability, with a maximum effect of 82.1% and 80.6% at a concentration of 250 µg/ml respectively, and reducing properties, reaching the maximum effect of 80.5% at a concentration of 10 µg/ml. At last, PGE was found able to inhibit the AGE production (maximum inhibition of 82.2% at the concentration of 1000 µg/ml), although with lower potency with respect to the positive control rutin. The phytochemical analysis of PGE displayed the presence of high levels of total polyphenols, tannins, and flavonoids, among which ellagic acid, gallic acid and catechin were identified. Altogether these data highlight the ability of PGE to control the carbohydrate metabolism at different levels, both by inhibiting the metabolic enzymes and by affecting the AGE formation likely by chelating mechanisms. It is also noteworthy that peel from pomegranate, although being a waste of juice production, can be reviewed as a nutraceutical source. In conclusion, present results suggest the possible role of PGE as a remedy for preventing hyperglycemia complications and encourage further in vivo studies.

Keywords : anti-hyperglycemic activity, antioxidant properties, nutraceuticals, polyphenols, pomegranate
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