Flavonoids isolated from leaves of *Eugenia calycina* (Myrtaceae)

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Abstract

Flavonoids were isolated from *Eugenia calycina* leaves that presented antioxidant activity and α-amylase inhibition.

Introduction

Eugenia calycina is a species of the Myrtaceae family and endemic of the Brazilian Savanna. Previous works[1] showed that extracts of the leaves presented high antioxidant activity and α-amylase inhibition. This enzyme is responsible for digesting carbohydrates, thus its control is relevant for the treatment of diabetic patients. Studies have shown correlation between flavonoids and its inhibitory action on digestive enzymes[2]. In this work, flavonoids were isolated from leaf extracts.

Results and Discussion

Leaves were collected in Uberlândia (Minas Gerais, Brazil) and ethanol extract was prepared by maceration, which was submitted to a liquid-liquid fractionation (Hexane, CH_2Cl_2, AcOEt, *n*-BuOH). Antioxidant activity of the extract and its fractions were analyzed by DPPH free radical method[3] and α-amylase inhibition by GALG2CNP method[4]. Previous studies showed high activity of the crude extract and ethyl acetate fraction, and strong correlation with phenolic compounds[2]. This fraction was subjected to column chromatography using Sephadex LH-20 as stationary phase and ethyl acetate:methanol (8:2) as eluent. Seven fractions were obtained and F3, F4 and F5 showed high activity. Quercitrin and (-)-epicatechin were isolated from F3. Isoquercitrin was isolated from F4. Rutin was isolated from F5. In all cases, a chromatographic column with silica gel 60G as stationary phase and ethyl acetate:methanol:formic acid (9:1:0.02) as mobile phase was used. NMR spectrum revealed the same substitution pattern in the aromatic rings to flavonoids (C5, C7, C3' and C4' substituted by OH). The disaccharide of rutin present in C3 position was identified by HSQC, which revealed the two doublet of doublets of the diastereotopic hydrogens of the C6'' (glucose). The correlation between H1''' (rhamnose) and C6'' (glucose) was possible to identify by HSBC. The difference between quercitrin and isoquercitrin is related to the type of monosaccharide substituted in the C3 position. The isoquercitrin has a glucose group and therefore it was observed diastereotopic hydrogens of the C6''. Quercitrin has a rhamnose group and it was observed doublet for C6''-hydrogen. (-)-Epicatechin presented characteristic hydrogens such as singlet from C2 and two double doublet for the diastereotopic hydrogens of the C4[5]. The absolute configuration of (-)-epicatechin was determined by optical rotation obtained in a digital polarimeter and compared with the literature data[6].

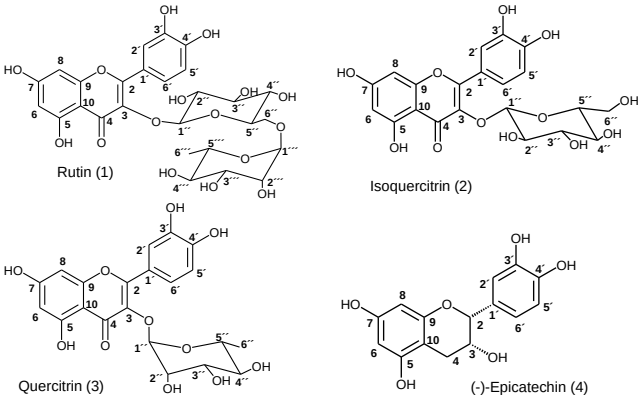

Figure 1. Isolated molecules from *E. calycina* leaves.

As shown in Table 1, the isolated compounds presented good results for the performed activities (IC_{50}<50: high activity; 100<IC_{50}>50: moderate activity).

Table 1. Analysis of leaf extract and isolated flavonoids.

IC_{50} (μg mL^{-1})	1	2	3	4	Extract[2]
DPPH*	5.5±0.1	6.4±0.1	6.4±0.1	2.9±0.1	19.7±1.3
Amylase**	25.2±1.8	30.5±0.9	31.9±1.8	92.9±0.1	17.9±0.3

Positive control: *BHT: 6.5±0.2 μg mL^{-1}; **Acarbose: 0.013±0.003 μg mL^{-1}

Conclusions

It was possible to isolate flavonoids and verify the antioxidant activity and α-amylase inhibition. These compounds justify the activity observed in the leaf extracts. Mass spectrometric dereplication will be performed in future work to identify these flavonoids in the flowers and branches extracts.

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