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IN VITRO ALFA-AMYLASE INHIBITORY EFFECTS AND ANTIOXIDANT ACTIVITY OF FLAVONOIDS ISOLATED FROM *Eugenia calycina*

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Abstract: Phenolic compounds isolated from natural sources show high antioxidant activities and significantly reduce the peak of postprandial glucose. Studies have shown that these compounds act as digestive enzyme inhibitor [1]. *Eugenia calycina* is species of Myrtaceae and native from Brazilian Savanna. This work describes the isolation and identification of flavonoids from *E. calycina* leaves and their antioxidant capacity and alfa-amilase inhibition. Leaves were collected in Uberlândia region and the ethanolic extract was submitted to liquid-liquid fractionation. Antioxidant activity of crude extract and their fractions were analyzed by DPPH (2,2-diphenyl-1-picrylhydrazyl) method and their alfa-amylase inhibition by GALG2CNP method. The oxidation potential was obtained by differential pulse voltammetry (DPV). Previous work showed high activity of crude extract and ethyl acetate fraction (EAF) [2]. In addition, EAF fraction showed high concentration of phenolic compounds. The EAF was submitted to chromatographic column using sephadex-LH20, eluted with ethyl acetate:methanol (8:2), and obtained seven fractions. F3, F4 and F5 showed higher antioxidant activity. Compounds 3 and 4 were isolated from F3, compound 2 from F4, and compound 1 from F5, by chromatographic column using silica gel 60G, eluted with ethyl acetate:methanol (9:1): 0.2% formic acid. These compounds were identified as rutin (1), isoquercitrin (2), quercitrin (3) and (-)-epicatechin (4) by ^{1}H and ^{13}C NMR analyses. Table 1 shows high antioxidant activity of all isolated flavonoids, which explains the excellent results observed in the extract and EAF. The glycosylated flavonols 1, 2 and 3 presented slightly lower activity than compound 4. This was also observed in DPV, compounds 1, 2 and 3 showed 402 mV as the oxidation potential while compound 4 showed 318 mV. The steric hindrance caused by saccharides of the glycosylation of the 3-hydroxyl group [3] can explain the decreased in the antioxidant activity in 1, 2 and 3. However, the compound 4 presented less alfa-amylase inhibitory effect. The C2=C3 double bond in conjugation with C4 carbonyl group of the compounds 1, 2 and 3 results in planar condensate ring structure that allows for formation of a highly conjugated π-system [4]. This promotes greater interaction between the compound and the enzyme, resulting in greater inhibition.

Table 1 - Analysis of isolated flavonoids, crude extract and its fraction from *E. calycina* leaves.

IC_{50} (μg mL^{-1})	1	2	3	4	Extract	EAF	BHT	Acarbose
DPPH	5.5±0.1	6.4±0.1	6.4±0.1	2.9±0.1	19.7±1.3	6.4±0.8	6.5±0.2	-
amylase	25.2±1.8	30.5±0.9	31.9±1.8	92.9±0.1	17.9±0.3	8.8±0.1	-	0.013±0.003

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