5th BCNP Brazilian Conference on Natural Products
XXXI RESEM
Atibaia – SP – Brazil
ISBN: 978-85-66836-10-3
Oct. 26–29th 2015

Biological activity of the ethanol extract and fractions of the leaves from *Inga laurina* (Sw.) Willd.

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Abstract: Many studies about phenolic compounds, especially flavonoids, demonstrate the ability of these compounds to capture free radicals (antioxidant activity) and to protect the human body from various diseases, such as Alzheimer's disease, diabetes, Parkinson's disease, among others [1,2]. Studies with plants extracts from genus *Inga* have been demonstrated antioxidant, antimicrobial and antitumor activities [3-5]. The aim of the present study was to identify the bioactive compounds of the leaves from *Inga laurina* (Sw.) Willd. The ethanolic extract was prepared by maceration and the crude extract (42.0 g) was dissolved in MeOH:H_2O (9:1) and submitted to liquid-liquid extraction by using hexane, chloroform, ethyl acetate and n-butanol, successively. The antioxidant activity of the samples was evaluated by DPPH free radical scavenging assay [6] and *α*-amylase inhibitory activity was made by the kinetic method with the substrate *α*-(2-chloro-4-nitrophenyl)-*β*-1,4-galactopiranosilmaltoside (Gal-*α*-G2-CNP), using microplates and spectrophotometric detection (Table 1) [7]. The ethyl acetate fraction was submitted to a bioguided fractionation using Sephadex LH-20 as stationary phase and eluted with a gradient of increasing methanol in ethyl acetate/methanol (9:1). Compounds of the bioactive fraction were identified by high resolution and tandem mass spectrometry (HRMS and MS/MS).

Table 1: Antioxidant and *α*-amylase inhibitory activities of ethanol extract and fractions from *I. laurina*.

Samples	Antioxidant activity	*α*-Amylase inhibitory activity
	EC_{50} (µg/mL)	EC_{50} (µg/mL)
Ethanol extract	10.4 ± 1.1	7.9 ± 0.1
Hexane	44.4 ± 0.8	11.8 ± 0.5
Chloroform	31.1 ± 1.6	-
Ethyl acetate	5.4 ± 0.8	8.1 ± 0.01
Butanol	15.3 ± 1.9	6.2 ± 0.2
BHT	7.3 ± 0.3	-
Acarbose	-	0.013 ± 0.003

The ethanol extract and fractions showed EC_{50} (effective concentration) values below 50 mg L^{-1}, indicating good antioxidant and *α*-amylase inhibitory activities, except for the chloroform fraction, that had no inhibition of *α*-amylase at the tested concentrations. After fractionation of ethyl acetate extract, the collected fractions were analyzed by TLC and was possible to identify the flavonoid myricetin-3-*O*-rhamnoside by ^{1}H and ^{13}C NMR. The flavonoids: quercetin-3-*O*-rhamnoside, myricetin, myricetin-*O*-(*O*-galloyl)-deoxyhexose, myricetin-3-*O*-*β*-D-galactopyranoside and myricetin-(*O*-galloyl)-hexose were identified by HRMS and MS/MS. Furthermore, the fragmentation pattern of these compounds was compared with those published to confirm the chemical structures. According to several studies that report flavonoids as evidenced bioactive compounds [1-3], we can conclude that the good result of the EC_{50} of ethyl acetate fraction in the antioxidant and *α*-amylase inhibitory activities can be assigned to these compounds.

Acknowledgments: IQUFU, FAPEMIG, CNPq, RQMG and IF GOIANO for the financial supports.

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