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Antioxidant flavonol glycosides from *Nectandra grandiflora* (Lauraceae)

Flavonóis glicosilados antioxidantes de *Nectandra grandiflora* (Lauraceae)

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ABSTRACT

The bioassay-guided fractionation of the ethanol extract from *Nectandra grandiflora* leaves led to the isolation of two flavonol glycosides which inhibited the bleaching of b -carotene on the TLC assay. Both compounds had their molecular structures elucidated by means of extensive use of uni- and bidimensional NMR techniques and were identified as 3-*O*-b -rhamnosylkaempferol and 3-*O*-b -rhamnosylquercetine.

Keywords: *Nectandra grandiflora*, antioxidant, flavonoid glycoside

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RESUMO

O fracionamento cromatográfico do extrato etanólico das folhas de *Nectandra grandiflora* resultou no isolamento de dois flavonóides glicosilados que apresentaram atividade antioxidante inibindo a oxidação do b -caroteno em CCDC. As substâncias isoladas tiveram suas estruturas elucidadas através de técnicas espectrométricas de RMN uni- e bidimensional e foram identificadas como 3-O-b -ramnosy kaempferol and 3-O-b -ramnosylquercetina.

Palavras-chave: *Nectandra grandiflora,* antioxidante, flavonóide glicosilado

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Introduction

Nectandra grandiflora is an arboreous species belonging to Lauraceae and is mainly found in Asia southeast and Brazil[10]. It is popularly known as "canela amarela" and "caneleira"[11] in Brazil, which are references to the cinammon smell of its bark. The infusion of its leaves is used as a folk medicine for its antirheumatic, digestive and diuretic properties[5], and previous chemical and pharmacological studies on this species reported the occurrence of alkaloids and terpenes in its bark ethanolic extract[12]. Flavonoids are also found in many species of Lauraceae as glycosides or aglycones and exhibit a number of biological activities including antibacterial, antiviral, antiinflammatory and antioxidant[7,8]. Antioxidants play a role in the maintenance of the pro/antioxidant balance by neutralizing the radicalar oxygen and nitrogen species (ROS and RNS), which are responsible for deleterious processes in biological systems. ROS/RNS initiate radicalar reactions with membrane lipids, for instance, leading to oxidative stress conditions, which may include descompartmentalization of cellular fluids, desfunctionalization and cell death ultimately[6].

The number of hydroxyl groups and the pattern of the aromatic rings hydroxylation of antioxidant molecules can be correlated to the antioxidant efficiency of phenolic compounds[2]. The acidic phenolic hydrogens can be easily released in the radicalar form to neutralize ROS/RNS, generating phenoxyl radicals which are well stabilized through resonance[13]. The ease of donation of hydrogen radicals is related to the radical scavenging ability of antioxidants, which may be assessed by a number of methods, including the spectrophotometric assay with the stable free radical diphenyl pycrylhydrazyl (DPPH)[4].

This paper deals with the detection of antioxidant compounds in the ethanolic extract of *Nectandra grandiflora* leaves, which inhibited the bleaching of b -carotene on TLC plates. The fractionation of the extract through chromatography in order to isolate and elucidate the molecular structures of the compounds responsible for the antioxidant activity and the evaluation of the scavenging activity of the isolates were subsequently undertaken.

Materials and methods

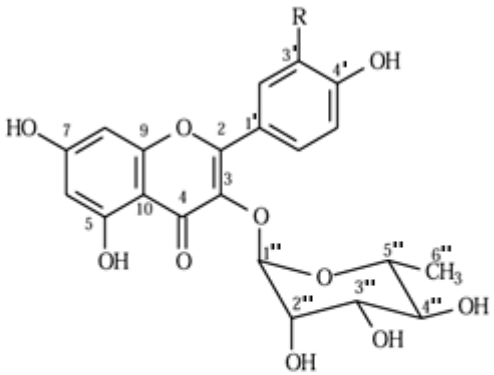
- PLANT: Leaves from *Nectandra grandiflora* were collected in Estação Ecológica Juréia-Itatins, Guaraú, in Peruíbe, in January 1999 and were identified by botanist Dr. Inês Cordeiro. A voucher specimen was deposited at Botanic Garden, São Paulo.
- ANTIOXIDANT TESTS - *Bleaching of* b *-carotene on TLC*: The test was carried out on TLC plates. After developing and drying, plates were sprayed with a 0.02% solution of b -carotene (Aldrich) in CH_2Cl_2. Plates were placed under natural light until discoloration of background. The yellow spots remaining indicated the presence of antioxidant substances. Spectrophotometric assay on the reduction of DPPH: The test of reduction of 2,2-diphenyl-1-pycrylhydrazyl was carried out with 0,004% DPPH solution in MeOH, which was added

to the compounds. Absorbance at 517 nm was determined after 30 min and the percentage of activity was calculated [4].

- ISOLATION: Dried and ground leaves (5.0 kg) were submitted to extraction with ethanol at room temperature. The ethanolic solution was evaporated under vacuum and afforded the ethanolic extract (13.2 g). After dissolution in MeOH/H_2O (9:1), this extract (5.0 g) was submitted to liquid-liquid partition with organic solvents: hexane, chloroform and ethyl acetate, subsequently. These fractions were analysed by TLC revealed with b -carotene solution, disclosing the presence of antioxidant compounds in the ethyl acetate fraction. The chromatographic separation of this fraction was performed on a Sephadex LH-20 column eluted with methanol and afforded 42 subfractions, which were pooled after comparison of their chromatographic profile through TLC analysis. Fractions (25-31) and (32-36) were subsequently purified by preparative TLC ($CHCl_3$/MeOH/H_2O/HOAc 79/18/2/1) and afforded compounds **1** (7.1 mg) and **2** (6.0 mg), respectively.

Results and discussion

The antioxidant test disclosed the presence of one pale-yellow spot at Rf 0.50 and one orange spot at Rf 0.30 due to the presence of compounds **1** and **2**, respectively, in the ethyl acetate fraction. The phytochemical work on this fraction led to the isolation of 3-*O*-b -rhamnosylkaempferol (**1**) and 3-*O*-b -rhamnosylquercetine (**2**) (Figure 1) which have been identified through analysis of their spectrometric data and comparison with literature[3,9].



R
1 H
2 OH

FIGURE 1 - Flavonol glycosides **1** and **2**.

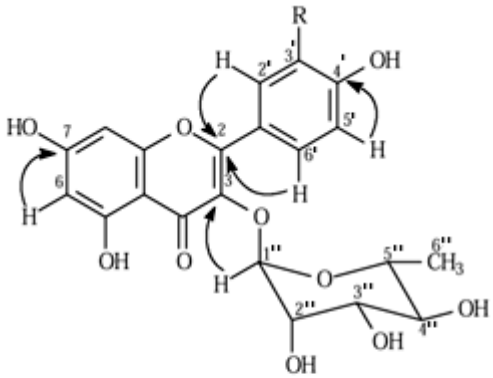
The ^{1}H NMR spectrum of compound **1** disclosed the presence of two doublets at d 6.86 (8.5 Hz) and 7.66 (8.5 Hz) evidencing a *p*-hydroxylated B ring and two broad singlets at d 5.88 and d 6.04 for the 5,7,9-trioxygenated A ring of a flavonoid (Table 1). One doublet at d 0.77 (6.0 Hz) of a methyl group, one dublet at d 5.27 (1.0 Hz), in addition to signals at d 3-4 suggested disclosed the presence of a rhamnosyl moiety linked to the aglycone.

Table 1 – ^{1}H and ^{13}C NMR data for compound **1** (DMSO-d_6, 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR).

Position	δ_C, mult.	δ_H, mult, *J* (Hz)	HOMOCOSY	HMBC
2	155.5 *s*	-	-	H2'/H6'
3	134.0 *s*	-	-	H1"
4	176.0 *s*	-	-	-
5	162.0 *s*	-	-	H6
6	100.6 *d*	5.88 *br s*	H8	-
7	162.0 *s*	-	-	H6
8	95.0 *d*	6.04 *br s*	H6	-
9	157.1 *s*	-	-	-
10	101.0 *s*	-	-	H6
1'	120.0 *s*	-	-	H3'/H5'
2'	130.2 *d*	7.66 *d* (8.5 Hz)	H3'	-
3'	115.4 *d*	6.86 *d* (8.5 Hz)	H2'	-
4'	160.2 *s*	-	-	H2'/H6'
5'	115.4 *d*	6.86 *d* (8.5 Hz)	H6'	-
6'	130.2 *d*	7.66 *d* (8.5 Hz)	H5'	-
Rhamnose: 1"	101.6 *d*	5.27 *d* (1.0 Hz)	H2"	-
2"	70.1 *d*	3.96 *m*	H1"	-
3"	70.3 *d*	3.48 *m*	-	-
4"	70.4 *d*	3.09 *m*	-	-
5"	71.2 *d*	3.09 *m*	-	-
6"	17.5 *q*	0.77 *d* (6.0 Hz)	-	-

The ^{13}C NMR spectrum showed signals at d 70.1, 70.3, 70.4 and 71.2 for hydroxymethine carbons, one signal at d 17.5 for one methyl carbon and at d 101.6 for the anomeric carbon, which confirmed the presence of a rhamnosyl unit linked to the aglycone. This spectrum also showed signals at d 176.0, d 155.5 and d 134.0 suggesting the presence of an a ,b -unsaturated ketone moiety of a flavonol. Comparison with literature data for flavonol aglycones evidenced downfield shifts for C-2 (Dd ~9.5 ppm) and C-4 (Dd ~ 2.0 ppm) and upfield shift for C-3 (D d~2.0 ppm) for compound **1** suggesting the location of the sugar moiety at C-3[1]. The remaining signals of this spectrum confirmed the presence and oxygenation pattern of the A and B rings.

Correlations observed in the HOMOCOSY, TOCSY, HMQC and HMBC spectra allowed the complete assignment of ^{1}H and ^{13}C NMR signals to the hydrogens and carbons of compound **1**. The HMBC spectrum showed correlation of the anomeric hydrogen H-1" (d 5.27) with C-3 which confirmed the position of the rhamnosyl moiety at C-3 (Figure 2). These remarks led to the establishment of the structure of compound **1** as 3-*O*-b -rhamnosyl kaempferol.



R
1 H
2 OH

FIGURE 2 – Selected HMBC (H⟶ C) correlations for compounds **1** and **2**.

The ^{1}H NMR spectrum of compound **2** showed two doublets at d 6.78 (8.0 Hz) and d 7.24 (2.0 Hz) and one double-doublet at d 7.20 (2.0 and 8.0 Hz) which were assigned to H-5', H-2' and H-6', respectively, suggesting the presence of a 3,4-dioxygenated B ring on flavonoid **2** structure (Table 2).

Table 2 – ^{1}H and ^{13}C NMR data for compound **2** (DMSO-d_6, 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR).

Position	δ_C, mult.	δ_H, mult., *J* (Hz)	HOMOCOSY	HMBC
2	156.0 *s*	-	-	H2'/H6'
3	133.5 *s*	-	-	H1"
4	177.0 *s*	-	-	-
5	162.0 *s*	-	-	H6'
6	100.0 *d*	5.97 *br s*	H8	-
7	162.0 *s*	-	-	H6
8	94.1 *d*	6.14 *br s*	H6	-
9	157.0 *S*	-	-	-
10	101.0 *s*	-	-	H6
1'	120.0 *s*	-	-	H5'
2'	115.0 *d*	7.24 *d* (2.0 Hz)	-	-
3'	149.5 *d*	-	-	-
4'	145.5 *s*	-	-	H2'/H5'
5'	115.4 *d*	6.78 *d* (8.0 Hz)	H6'	-
6'	120.8 *d*	7.20 *dd* (2.0 e 8.0 Hz)	H2'/H5'	H2'
Rhamnose: 1"	101.8 *d*	5.24 *d* (1.0 Hz)	H2"	-
2"	70.1 *d*	3.96 *m*	H1"/H3"	-
3"	70.3 *d*	3.52 *m*	H2"/H4"	-
4"	71.3 *d*	3.13 *m*	H3"/H5"	-
5"	70.5 *d*	3.25 *m*	H4"/H6"	-
6"	17.5 *q*	0.81 *d* (6.0 Hz)	H5"	H4"

The ^{13}C NMR spectrum showed signals at d 149.5 and d 145.5, assigned to quarternary aromatic carbons C-3' and C-4' and signals at d 115.0, d 115.4 and d 120.8, which were assigned to the methine aromatic carbons C-2', C-5' and C-6' and confirmed the 3,4-dioxygenation pattern of B

ring. The remaining ^{1}H and ^{13}C NMR signals had chemical shifts similar to those of compound **1**, including the HMBC correlation of C-3 (d 133.5) and the anomeric hydrogen H-1" (d 5.24), which confirmed the position of the rhamnosyl unit at C-3 (Figure 2) and allowed us to establish the structure of compound **2** as 3-*O*-b -rhamnosylquercetine, also known as quercitrin.

The antioxidant activity observed for pure compounds **1** and **2**, evidenced by inhibition of b -carotene bleaching on TLC and by reduction of DPPH, was due to structural features of flavonoids as phenolic hydroxyl groups, a ,b -insaturated ketone moiety and a - or b -hydroxyketone units. These sub-structures causes enhancement of the antioxidant activity by improving the stabilization ability of the flavonoid radicalar species after release of a phenolic hydrogen radical of its molecular structure.

The brighter yellow color observed on the TLC for compound **2**, meaning a higher antioxidant activity, was due to the additional hydroxyl on B ring, composing a catechol (*ortho*-dihydroxy) group, which confers greater stabilization ability for the flavonoid radical through hydrogen bonding (Scheme 1). The evaluation of the radical scavenging activity of compounds **1**, **2** and rutin using the spectrophotometric assay with DPPH gave the percentual absorbance variation (%A) of the DPPH solution for each compound in the several concentrations used. The original purple solution with unreduced DPPH turns into yellow color after addition of the antioxidant and completion of the radicalar reaction, in which the extended conjugation of DPPH is interrupted. The catechol moiety was also responsible for the stronger radical scavenging ability of compound 2 and standard compound rutin, when compared to the kaempferol derivative 1 (Figure 3).

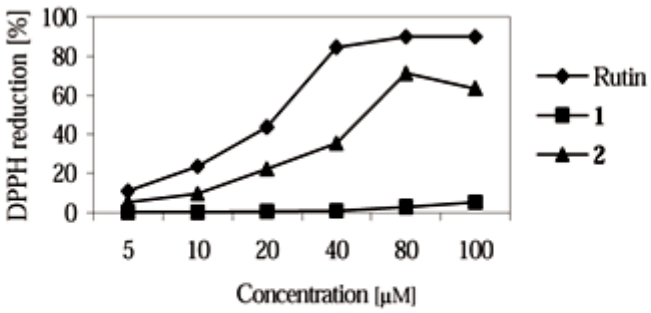


FIGURE 3 – Scavenginig activities of compounds **1** and **2** on DPPH radical compared to those of rutin. Measurements at 517 nm, determination after 30 min.

Conclusions

The pursue for antioxidant compounds from the ethanol extract of *Nectandra grandiflora* leaves led to the isolation and identification of two flavonol glycosides. Flavonoids are secondary metabolites widespread in plant kingdom and their occurrence in leaves has been related to the need of protection from the oxidative processes. Leaves are the primary site for photosynthetic reactions due to their high content in chlorophylls. They are thus continuously exposed to UV/Vis light which is not only necessary for photosynthesis, but also responsible for the generation of radicalar species that might damage subcellular structures if the leaves had not an efficient system for quenching ROS/RNS. The glycosilated flavonols isolated in this work might thus play a role in the protecting

system of *Nectandra grandiflora* leaves against oxidative stress.

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