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Study of antiinflammatory activity of metabolites isolated from *Tripodanthus acutifolius*

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ABSTRACT

A novel glycosylated phenylbutanoid (tripodantoside) and four flavonoids isolated from *Tripodanthus acutifolius* leaves were assayed on their abilities to inhihibit cyclooxygenase-2 (COX-2) and hyaluronidase catalyzed reactions in order to assess their antiiflammatory activities. Quercetin glycosides (rutin, hyperoside and isoquercitrin) showed higher hyaluronidase inhibitory activity than nicotiflorin (a kaempferol glycoside) and tripodantoside. Tripodantoside exerted higher COX-2 inhibitory activity than flavonoids, though that inhibition was lower than acetyl salicylic acid. Tripodantoside aglycone was significant more active against COX-2 than the glycoside. As folk medicine indicates that infusion from *T. acutifolius* leaves is employed as anti-inflammatory, the inhibitory effects showed by the assayed substances may partially contribute to that activity.
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Keywords: Antiinflammatory activity , *T. acutifolius*, phenols

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Introduction

Tripodanthus acutifolius (Ruiz & Pavón) Van Tieghem (Loranthaceae) is an endemic shrub that grows in the arid and semiarid region of northwestern Argentina. It is used in traditional medicine as anti-inflammatory. The infusion prepared from leaves of this species possesses phenolic compounds with recognised free radical scavenging activities (Soberon *et al*. 2009a). As free radical scavenging substances are ascribed to possess anti-inflammatory activities (Schemppa *et al*. 2006), the purified compounds were studied on their abilities to inhibit enzymes related to inflammatory process in order to assess the anti-inflammatory activities. The results were compared with those of reference substances.

Experimental

Plant material:

T. acutifolius leaves were collected from the province of Tucumán, in northwestern Argentina. Plant was taxonomically identified by Dr A.R. Sampietro (Facultad de Bioquímica, Química y Farmacia-UNT-Argentina). Voucher specimens were deposited in the herbarium of the Instituto de Estudios Vegetales for future references.

Extract preparation:

Infusion was prepared from *T. acutifolius* leaves and the extraction yield was calculated as described elsewhere (Soberón *et al*. 2009a). The dried material obtained represented the extracted material (EM).

Isolation and identification of phenolics from *T. acutifolius* infusion:

T.acutifolius infusion active compounds were purified and structurally elucidated as described elsewhere (Soberon *et al*. 2009a).

Anti-inflammatory activity:

Anti-inflammatory activity was assayed by measuring the inhibitory effect of substances on cyclooxygenase-2 (COX-2) and hyaluronidase catalyzed reactions. The inhibitory effect on COX-2 reaction was assayed as described elsewhere (Sud'ina *et al*. 2008). Malondialdehyde generated from COX-2 reaction products is proportional to the COX-2 activity (Sharma *et al.* 2001), and could be detected at 532 nm as a 2-thiobarbituric acid reactive substance. The inhibitory effect on hyaluronidase catalyzed reaction was evaluated as described by Takahashi *et al.* (2003), and the fragments containing N-acetylglucosamine were quantified. Acetyl salicylic acid (AAS) was employed as reference. The concentrations inhibiting the enzymatic activity by 50% (IC_{50}s) were calculated by graphic interpolation of the concentration-enzyme activity curves (Soberón *et al.* 2009b).

Statistic análisis:

Data were analyzed by either Student's t test or one way ANOVA, considering a probability level lower than 0.05 ($p < 0.05$) as statistically significant.

Results and discussion

The extraction yield of *T. acutifolius* leaves obtained from hot water (infusion) was 47.6 ± 4.0 g of EM per 100 g of dry leaves. Four glycoflavonoids were purified and identified as: rutin, nicotiflorin, hyperoside and isoquercitrin, along with an unusual glycosilated phenylbutanoid: tripodantoside. Quercetin glycosides (rutin, hyperoside and isoquercitrin) showed higher hyaluronidase inhibitory activity than nicotiflorin (a kaempferol glycoside) and tripodantoside. There were significant differences between the IC_{50} values of the isolated compounds and AAS ($p < 0.05$) (Table 1).

Table 1:Tripodantoside aglycone; Nt: nicotiflorin; Hp: hyperoside; Iq: isoquercitrin

Substance	Hyaluronidase assay		COX-2 assay	
	mM	mg . mL^{-1}	µM	µg . mL^{-1}
AAS	0.47	0.08	12.5	2.2
Tp	27.90	9.60	50	17.2
Ag-Tp	43.91	8.00	42	7.6
Rt	1.70	0.95	110	73.1
Nt	3.70	2.20	>160	>95.1
Hp	1.70	0.79	46	21.4
Iq	1.67	0.78	52	24.1

The assayed substances exerted low inhibition on hyaluronidase activity, because the IC_{50} values are in the millimolar order (1,67-43,91 mM).

Tripodantoside COX-2 inhibitory activity was significant higher than flavonoids activities ($p< 0.05$), though that inhibition was almost four-fold lower than acetyl salicylic acid (Table 1). Tripodantoside aglycone was significant more active against COX-2 than the glycoside (p<

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0.05). Flavonoids with a bulky sugar moiety, such as nicotiflorin and rutin, showed lower COX-2 inhibitory activity than those with only one sugar bounded to the aglycone (hyperoside and isoquercitrin).

The COX-2 inhibitory effects showed by the assayed substances, specially tripodantoside and its aglycone, sets them as novel compounds for studies involving COX-2 activity.

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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