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Phenolic compounds in *Hypericum* species from the *Trigynobrathys* section

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ABSTRACT

Phenolic compounds of thirteen *Hypericum* species growing in South Brazil were investigated aiming to evaluate the usefulness of their distribution as a taxonomic character. The HPLC analysis of the methanolic fractions displayed similar chemical profile and significant contents variation among the investigated taxa, being chlorogenic acid the main metabolite quantified in most of the species (ranging from traces to 16.65 mg% of extract), followed by hyperoside (between 0.27 and 11.48 mg%), quercitrin (0.09 and 13.34 mg%), guaijaverin (0.14 and 2.91 mg%) and isoquercitrin (0.14 and 6.97 mg%), whereas rutin and the xanthone mangiferin were not detected.

1. Introduction

The genus *Hypericum* (Guttiferae) encompasses more than 400 species, which are accommodated in about 30 sections (Robson, 1990). The species growing in Rio Grande do Sul, South Brazil, belong to the sections *Brathys*, with two representatives, *Hypericum piriai* Arechav. and *Hypericum gentianoides* (L.) Britton, and *Trigynobrathys*, with a greater number of species. The section *Trigynobrathys* consists of 52 species of shrubs, subshrubs, shrublets, as well as perennial and annual herbs distributed over two subsections: *Connatum* and *Knifa*. The approximately 20 species growing in Southern Brazil belong to the former (Robson, 1990).

Species from the family Guttiferae have a strong tendency to accumulate phenolic compounds with the phloroglucinol substitution pattern. The phytochemical analysis of some native species resulted in the isolation of xanthones and phloroglucinol derivatives from the leaves and flowers of *Hypericum brasiliense* Choisy (Rocha et al., 1994, 1996), benzopyrans from the aerial parts of *Hypericum polyanthemum* Klotzsch ex Reichardt (Ferraz et al., 2001), phloroglucinol derivatives from *Hypericum myrianthum* Cham. & Schlecht. (Ferraz et al., 2002a), *Hypericum carinatum* Griseb., *H. polyanthemum*, *Hypericum caprifoliatum* Cham. & Schlecht. and *Hypericum connatum* Lam. (Nör et al., 2004), benzophenone derivatives from *H. carinatum* (Bernardi et al., 2005) and flavonoids from *Hypericum ternum* A. St.-Hil (Bernardi et al., 2007). In addition, tannins and essential oils were surveyed in some species (Dall'Agnol et al., 2003; Ferraz et al., 2005a) and the absence of hypericins was verified in eight investigated species (Ferraz et al., 2002b).

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The occurrence of these phenolic substances in the above mentioned species is linked to some biological activities such as monoamine oxidase inhibition (Rocha et al., 1995; Gnerre et al., 2001), antiviral (Schmitt et al., 2001; Fritz et al., 2007), analgesic (Viana et al., 2003), antimicrobial (Dall'Agnol et al., 2005; Fenner et al., 2005), antiproliferative (Ferraz et al., 2005b,c), acaricidal (Ribeiro et al., 2007) and antioxidant (Bernardi et al., 2007, 2008) exhibited for their extracts, fractions and isolated compounds.

Considering the medicinal properties already shown for some *Hypericum* species and the lack of information about other exemplars, the present study was conducted to quantitatively determine specific phenolic compounds occurrence among thirteen species belonging to the *Trigynobrathys* section, growing in a small geographic area of South Brazil. The presence of the flavonoids hyperoside, quercitrin, isoquercitrin, guaijaverin and rutin, as well as the phenolic acid chlorogenic acid and the xanthone mangiferin was investigated.

2. Material and methods

2.1. Plant material

Aerial parts in blossom of the plants were collected in various localities of the Rio Grande do Sul state (Table 1), south of Brazil, between September and December, 2008. Plants were identified by Sérgio Bordignon (UNILASALLE, Canoas, Brazil). Voucher specimens are deposited in the herbarium of Universidade Federal do Rio Grande do Sul (ICN).

2.2. Preparation of plant extracts

The extractions were performed using 0.5 g of powdered dry plant material, treated successively in an ultrasonic bath with dichloromethane, for elimination of lipophilic compounds, and afterwards with methanol for 15 min (until exhaustion). The methanolic fractions were combined, evaporated to dryness under reduced pressure, dissolved in 5 mL of HPLC grade methanol and filtered (0.22 μm pore size, Merck©).

2.3. HPLC determination of secondary metabolite contents

The metabolites were analyzed using a Waters HPLC system, comprising a Waters 2487 UV detector and a Waters 600 pump at flow rate of 1 mL min^{-1}. Reversed phase separations were carried out at room temperature using a Waters Nova Pack C_{18} column adjusted to a guard column Waters Nova Pack C_{18} 60 A.

Chlorogenic acid, guaijaverin, hyperoside, isoquercitrin and quercitrin were analyzed using isocratic elution with 14% CH_3CN, 86% H_2O, 0.05% TFA and detected at 254 nm. Chlorogenic acid (retention time of 3.52 min) was quantified by a calibration curve of pure standard (Sigma©) with excellent linearity ($r^2 = 0.9981$) between 3.91 μg mL^{-1} and 2000 μg mL^{-1}.

The flavonoids were expressed as hyperoside mass through a calibration curve with concentrations ranging from 36.25 μg mL^{-1} to 2320 μg mL^{-1} and ensured linearity ($r^2 = 0.9994$). Standards purified from *H. ternum* (Bernardi et al., 2007) were used to determine the concentrations of the compounds (expressed as mg% in the plant extract) and presented retention times of 17.80 min (hyperoside), 20.37 min (isoquercitrin), 26.65 min (guaijaverin) and 37.70 min (quercitrin). The presence of mangiferin and rutin (Merck©) (retention times of 4.2 and 15.83 min, respectively) was also investigated in the samples, using the same HPLC system.

3. Statistical analysis

One-way analysis of variance (ANOVA) was applied with a critical value of $p \leq 0.05$.

Table 1
Collection localities and habitat of the *Hypericum* spp. growing in South Brazil.

Species	Collection locality/habitat	Voucher number	Heigh[a]
H. campestre Cham. & Schlecht.	Caçapava do Sul, pasteurelands	Bordignon et al. 3119	460
H. caprifoliatum Cham. & Schlecht.	Porto Alegre, dry rocky roadsides	Bordignon et al. 2287	311
H. carinatum Griseb.	Glorinha, wet environmet	Bordignon & Ferraz 2309	50
H. connatum Lam.	Capão do Leão, stony slopes	Bordignon et al. 3076	21
H. cordatum Vell. Conc.	São Francisco de Paula, stony slops	Bordignon 3330	907
H. linoides A. St.-Hil.	São José dos Ausentes, stony lowland	Bordignon et al. 3317	1100
H. lorentzianum Gilg. ex R. Keller	Porto Alegre, pasteurelands	Bordignon et al., 3401	90
H. megapotamicum	Capão do Leão, wet pasteures	Bordignon & Salazar 1532	21
H. myrianthum Cham. & Schlecht.	Paraíso do Sul, pasteurelands	Bordignon et al. 3059	108
H. polyanthemum Klotzsch ex Reichardt	Caçapava do Sul, stony lowland	Bordignon et al., 3118	250
H. rigidum A. St.-Hil.	Jaquirana, damp grassland	Bordignon et al. 3063	900
H. salvadorense Robinson	Glorinha, stony lowland	Bordignon & von Poser 3452	50
H. ternum A. St.-Hil.	São Francisco de Paula, dry rocky roadsides	Bordignon & Ferraz 3073	907

[a] Meters above the sea level.