

Separation of Bioactive Biflavonoids from *Rheedia gardneriana* Using Chitosan Modified with Benzaldehyde

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This paper shows the influence of benzenic groups on the chitosan surface for the separation of bioactive biflavonoids from *Rheedia gardneriana* leaves. The yield of the biflavonoids using chitin modified with benzaldehyde (CH-Bz) as adsorbent in column chromatography was higher than that achieved with silica gel and chitosan. The presence of benzenic groups decreases the polarity of chitosan and consequently the interaction of hydrogen bonding between phenolic hydroxyl (OH) of biflavonoids and amine groups of the adsorbent. Therefore, the separation of these compounds appears to be the result of hydrophobicity and π - π interaction among electrons from the aromatic ring in sorbent and biflavonoid molecules.

Key words: Chitosan-benzaldehyde, Biflavonoids, *Rheedia gardneriana*

Introduction

Rheedia gardneriana Pl. Tr. (Guttiferae), commonly known as “bacopari”, is frequently used in folk medicine in Brazil for treatment of various pathologies such as inflammations, infections and pain (Balmé, 1982). Biological studies have demonstrated that the ethyl acetate fraction causes an analgesic effect in mice (Luzzi *et al.*, 1997) and has antimicrobial properties against pathogenic microorganisms (Verdi *et al.*, 2004). Recent chemical studies with this fraction indicated the presence of biflavonoids such as fukugeside, fukugetin, volkensiflavone and I3-naringenin-II8-eriodictyol (GB2a), see Fig. 1 (Luzzi *et al.*, 1997), I3-naringenin-II8-4'-OMe-eriodictyol (Gb-2a-II-4'-OMe) (Cechinel-Filho *et al.*, 2000) and epicatechin (Verdi *et al.*, 2004).

Biflavonoids isolated from *R. gardneriana* have similar chemical structures and a high polarity due to the presence of several hydroxyl groups. These characteristics result in a low yield of these compounds when silica gel is used as adsorbent in column chromatography (CC). Recently we have conducted studies in order to improve the separation process of these compounds using chitin and chitin-Fe as stationary phases in CC (Rodrigues *et al.*, 2000a, b). More recently, we have shown that

chitosan modified with benzaldehyde (CH-Bz) can be used as chromatographic support for separations of phenolic compounds, such as flavonoids from *Aleurites moluccana* (Girardi *et al.*, 2003).

In the present work we have extended our investigations on this field and compared the efficiency of CH-Bz in the separation of the biflavonoids present in the ethyl acetate fraction of *R. gardneriana* with some adsorbents reported in the literature.

Material and Methods

Preparation of the ethyl acetate fraction

R. gardneriana was collected in Blumenau, in the south of Brazil, in September 1997 and voucher specimen were deposited in Dr. Roberto Miguel Klein Herbarium (Blumenau) under numbers 534 to 540.

An hydroalcoholic extract was obtained after maceration with 50% ethanol at room temperature (150 g dried leaves in 1.2 l) for 15 d. The extract was concentrated under reduced pressure, and successively partitioned with hexane, dichloromethane, ethyl acetate and butanol. The ethyl acetate fraction, rich in biflavonoids, was selected for chromatography studies.

Table I. Efficiency of different supports in the separation (300 mg of ethyl acetate fraction) of biflavonoids from *R. gardneriana*.

Support	Volkensiflavone		Fukugeside		GB2a		Fukugetin	
	[mg]	(%)	[mg]	(%)	[mg]	(%)	[mg]	(%)
CH-Bz ^a	13	4.3	105	35.0	9	3.0	21	7.0
Chitin-Fe ^b	10	3.4	94	31.0	10	3.4	36	12.0
Chitin ^c	15	5.0	126	42.0	20	6.6	14	4.6
Silica gel ^d	4.5	1.5	8.1	2.7	11.2	3.7	63	21.6

^a Mean of two experiments.

^b Rodrigues *et al.*, 2000a.

^c Rodrigues *et al.*, 2000b.

^d Luzzi *et al.*, 1997.

Chromatography

The adsorbent CH-Bz (with substitution degree of 19%) was prepared through Schiff's reaction and characterized as previously described (Girardi *et al.*, 2003).

300 mg of the ethyl acetate fraction, containing the biflavonoids (fukugeside, GB-2a, volkensiflavone and fukugetin, Fig. 1), were chromatographed by CC (2.0 × 30 cm) using 6 g of CH-Bz eluted with a CHCl₃/MeOH gradient. Fractions of 5 ml were collected, and monitored by thin layer chromatography (TLC). By elution with CHCl₃/MeOH 70:30 (v/v) the fractions which showed a positive reaction with FeCl₃ were combined. The compounds were detected by spraying with FeCl₃ (2% in ethanol) or by visualization under UV light (254 nm). The compounds were identified by direct comparison with authentic samples (Luzzi *et al.*, 1997).

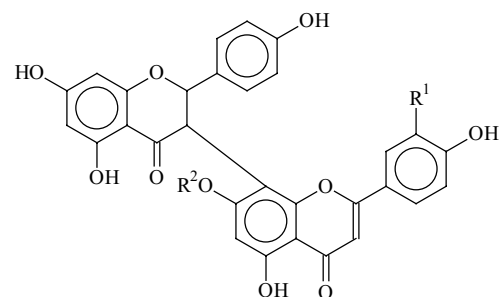
Results and Discussion

We have previously reported that CH-Bz is effective as an adsorbent for separation of flavonoids, suggesting that the separation process is influenced by the π - π electron interaction between benzenic groups present in flavonoids and on the chitosan surface (Girardi *et al.*, 2003).

Table I shows the biflavonoids yields with different adsorbents. The yields for volkensiflavone (Fig. 1) using CH-Bz are higher when compared with that of silica gel and chitin-Fe. On the other hand, yields of fukugetin and GB2a are lower when compared with that of chitin-Fe and silica gel.

The retention of biflavonoids on the chitin surface seems to occur by the interaction of hydrogen

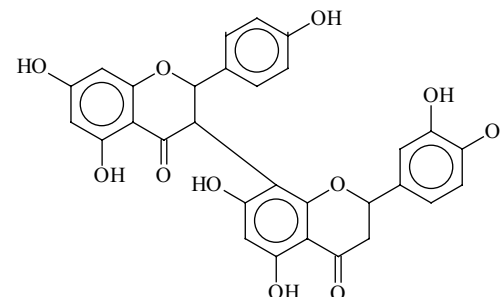
bonding between phenolic OH and NH₂ of the polymer surface (Rodrigues *et al.*, 2000a). The presence of benzenic groups on the chitosan surface decreased the polarity of the adsorbent and consequently the contribution of the interaction by hydrogen bonding between phenolic OH and NH₂ groups on the chitosan surface, responsible



R¹, R² = H: Volkensiflavone

R¹ = OH, R² = H: Fukugetin

R¹ = OH, R² = β -D-gluc: Fukugeside



13-Naringenin-II8-criodietylol (GB2a)

Fig. 1. Molecular structure of biflavonoids from *Rheedia gardneriana*.

for the fukugeside retention on chitosan and chitin (Rodrigues *et al.*, 2000a).

In chitin-Fe, the interaction of phenolic OH and iron adsorbed onto chitin is the main factor responsible for separation of biflavonoids (Rodrigues *et al.*, 2000b). The presence of OH neighbor groups in the aromatic ring (GB2a and fukugetin) favors the interactions of the type metal:ligand in IMAC chromatography (Holmes and Schiller, 1997; Garbec-Porekar and Menart, 2001). The presence of aromatic rings in the polymer increases the hydrophobicity of the adsorbent and reduces the importance of the metal:OH phenolic interactions.

In addition, the separation of biflavonoids by the CH-Bz sorbent can be attributed to π - π interaction, but the hydrophobic interaction may also play an important role in the separation of these compounds on the sorbent (Rodriguez *et al.*, 2000).

Another important observation relates of the relationship between the amount of fukugeside and fukugetin separated on silica gel and CH-Bz,

respectively. The small amount of fukugeside obtained by Luzzi *et al.* (1997) can be attributed to the strong interaction of the glucosidic residue with OH-silanol groups from silica gel, and this interaction results in retention of compounds on the stationary phase during chromatographic procedures.

On the other hand, the amount of fukugeside obtained in this work is larger when compared with the amount isolated by Luzzi *et al.* (1997), showing the importance of choice of the stationary phase in the separation of these compounds.

Therefore, the hypothesis that the small amount of fukugeside present in the ethyl acetate extract could be related to its transformation in fukugetin during the stage of removal of the solvent is not substantiated.

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