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Isolation of flavonoids from *Aleurites moluccana* using chitosan modified with benzaldehyde (CH-Bz) as chromatographic support

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This paper describes the preparation, characterization and use of a derivative of chitosan as a chromatographic sorbent. Chitosan modified with benzenic ring (CH-Bz) was used to separate two flavonoids, swertisin and 2″-O-rhamnosylswertisin, from ethyl acetate fraction of *Aleurites moluccana*. The results showed that CH-Bz can be used as a sorbent for the separation of flavonoid compounds. The studies showed that CH-Bz in column chromatography produces goods results, separation of the flavonoid compounds.

1. Introduction

Aleurites moluccana L. (Willd) (Euphorbiaceae), known in Brazil as "Nogueira-da-Índia" is frequently used in folk medicine to treat fever, headache, tumours, diarrhoea and asthma [1].

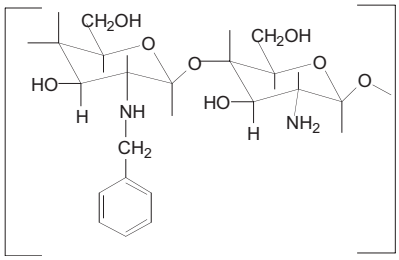
Meyre-Silva et al. showed that butanolic and ethyl acetate extracts of this plant contain the flavonoid 2″-O-rhamnosylswertisin which exhibits antinociceptive effects in mice [2]. However when silica gel is used as a sorbent in column chromatographic separation, the yield of this flavonoid is very low [3].

On the other hand we recently used chitin and fully N-acetylated chitin to separate of flavonoids from *A. moluccana*. The results showed that the reduction in the free NH_2^- group improved the yield of phenolic compounds [4]. We could increase the hydrophobic interactions of chitosan, incluing the π-π interaction of a stationary phase for flavonoids, by a simple modification of chitosan with benzaldehyde. The presence of π-π interactions between the analyte and a sorbent can be very useful for separating compounds with similar retention behaviors in traditional sorbents. This type of interaction can be defined as an interaction between π-electrons of the stationary phase and those of the compounds [5].

In this study, we prepared a chitosan-based stationary phase with aromatic moieties and tested the separation of flavonoid compounds present in an ethyl acetate fraction of *A. mollucana*.

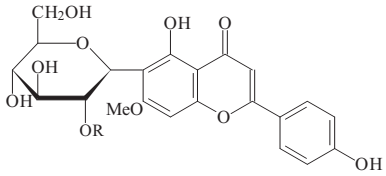
2. Investigations, results and discussion

The Schiff reaction between chitosan and aldehyde gives the respective aldimine, which can be hydrogenated to products less susceptible to hydrolysis. The degree of chitosan substitution was determined by potentiometric titration. The reaction of chitosan with benzaldehyde produced a 0.19 substitution in NH_2 groups of chitosan.



Chemical structure of CH-Bz

The IR spectrum of chitosan modified with benzaldehyde (CH-BZ) exhibits a band at 1641 cm^{-1} attributed to −C=C− stretching and bands at 650–750 cm^{-1} attributed to C−H out-of-plane deformation of the aromatic ring. This band is absent in chitosan [6]. The NMR spectrum of the CH-Bz shows a peak near 2.0 ppm attributed to the protons of $-CH_2$, peaks between 2.5–3.5 ppm attributed to the protons of the glycosidic ring and peaks at 7.5–8.5 ppm attributed to the protons of the benzenic ring [6]. The results of chromatographic separation of swetisin (**1**) and 2″-O-rhamnosylswertisin (**2**), on CH-Bz sorbent are compared in the Table.



1- R = H
2- R = Rhamnosyl
1-Swertisin, 2-2″-O-Rhamnosylswertisin

Table: Efficiency of different support studied in the separation of flavonoids of *A. moluccana* (150) mg of ethyl acetate extract

Support	Swertisin		2″-O-Rhamnosylswertisin	
	mg	%	mg	%
Chitin[1]	11.2	8.0	10.9	7.2
Chitin-100[1]	12.8	13.5	15.8	10.5
Silica gel[1]	3.0	2.3	9.2	6.1
CH-BZ	31.5	21.0	36.0	24.0

[1] From Morsch et al. 2002 [4]

It has recently been reported that some aminopropyl modified silyl silica gel derivatives interact with π-electron-rich compounds, such as polyaromatic hydrocarbons, through a π-π electron interaction (π-π interaction) [7]. As mentioned above, π-π interactions are assumed to exist in the CH-Bz separation mode. Thus, the sorbent was tested for the separation of flavonoids from the ethyl acetate extract of *A. mollucana*.

The presence of the amino group favors the formation of a strong hydrogen bond between phenolic OH and NH_2 present in chitin. On the other hand, full N-acetylation of the chitosan significantly reduces the amount of the free NH_2 groups from the sorbent. The interaction among the OH groups of swertisin and 2″-O-rhamnosylswertisin with the acetamide groups is less intense than for the NH_2 groups, therefore the yield of the compounds is larger when chitin is used as a sorbent [4].

In the case of CH-Bz, the addition of benzenic groups in the sorbent increases its hydrophobic character. These aromatic groups lead to interactions of the π-π type with the flavonoids that are weaker than the interactions for the hydrogen bond. The presence of theses groups at the chitosan surface decreases the polarity of the sorbent. The interactions between phenolic OH with the NH_2 groups of the chitosan is less than that of the interactions among the π-electrons of the aromatic groups of the sorbent and the flavonoids. Thus, we estimated that the interaction is also attributed to the π-π interactions between π-electron clouds of CH-Bz and flavonoids.

The results indicate that CH-Bz sorbent has a particular ability to separate the flavonoids from *A. mollucana* extract. The results should be informative for improving the yield of other phenolic compounds present in medicinal plant extracts.

3. Experimental

3.1. Plant material

A. moluccana was collected in Itajaí, in the state of Santa Catarina, Brazil, in February 1998, and identified by Dr. Ademir Reis. A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí) under number VC Filho 001.

A methanolic extract was obtained after maceration with methanol at room temperature for 10 days (812 g dried leaves). This extract was concentrated and then successively partitioned with hexane, dichloromethane, and ethyl acetate, respectively. The ethyl acetate, rich in flavonoids, was dried at room temperature (1.6 g).

3.2. Preparation of the stationary phase

Chitosan (76% N-desacetylation) was obtained by basic hydrolysis of chitins [8]. The material was ground and sieved and fractions of 43–80 μm were used for the preparation of the chromatographic column.

The chitosan derivative was synthesized through Schiff's reaction using a modified conventional method. An methanolic dispersion of chitosan was continuously stirred and refluxed with benzaldehyde (Aldrich) for 48 h. The polymer were purified in a Soxhlet with ethanol and acetone for removal of excess benzaldehyde. Afterwards, the imine groups were reduced with cyanoborohydride (Aldrich) for 12 h. The solid was washed with water to remove the excess cyanoborohydride, filtered and dried at 60 °C. The material obtained was characterized by IR, NMR and potentiometric titration [9]. The IR spectra were obtained in KBr disk on a IR-FT, Bomem MB-100 spectrophotometer. The polymer was solubilized in CD_3COOD/D_2O (3% w/w) and a ^1H NMR Spectrum was obtained in 200 MHz on a Bomem 200 spectrophotometer. The potentiometric titration was carried out using an ORION- pHmeter model A920.

3.3. Chromatography

150 mg of the ethyl acetate fraction, containing the biflavonoids, was chromatographed on a column (2.0 × 30 cm) using 3 g of CH-BZ eluted with $CHCl_3$: MeOH. Fractions of 5 ml were collected. After monitoring by thin layer chromatography (TLC) (mobile phase: $CHCl_3$: MeOH 70 : 30 v/v), the fractions which showed a positive reaction with $FeCl_3$ were combined.

The purity of all the isolated substances was examined by TLC precoated with a 0.25 mm layer of Merck silica gel 60 HF_{254} and eluted with $CHCl_3$: MeOH 85 : 15 v/v. The compounds were detected by spraying with $FeCl_3$ (2% in ethanol) solution or visualization under UV light (254 nm). The compounds were identified by direct comparison with authentic samples.

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References

1 Duke, J.: A Handbook of Medicinal Herbs, p. 29, CRS Press, New York 1991
2 Meyre-Silva, Yunes, R. A.; Soares Santos, A.; Dal Magro, J.; Delle-Monache, F.; Cechinel-Filho, V.: Planta Med. **65**, 293 (1999)
3 Meyre-Silva, C.; Mora, T. C.; Soares Santos, A.; Dal magro, J.; Yunes, R. A.; Biavatti, M. W.; Cechinel-Filho, V.: Phytomedicine **5**, 109 (1998)
4 Morsch, M.; Girardi, L. G. J.; Cechinel-Filho, V.; Meyre-Silva, C.; Rodrigues, C. A.: Z. Naturforsch. **55**C, 857 (2002)
5 Reubsaet, J. L.; Vieskar, R.: J. Chromatogr. A **841**, 147 (1999)
6 Silverstein, R. M.; Bssler, G. C.; Morrill, T. C.: Identificação Espectrofotométrica de Compostos Organicos, p. 45, 5. ed, Guanabara Kogan, Rio de Janeiro 1994
7 Makashima, K.; Fuchigami, Y.; Kuroda, N.; Kinoshita, T.; Akiyama, S.: J. Liq. Chrom. Rel. Technol. **23**, 2533 (2000)
8 Rinaudo, M.; Milas, M.; Dung, P. L.: Int. J. Biol. Macromol. **15**, 281 (1993)
9 Brugnerotto, J.; Lizardi, J.; Goycoolea, F. M.; Argüelles-Modal, W.; Desbriéres, J.; Rinaudo, M.: Polymer **42**, 3359 (2001)