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Utilization of Chitin and Chitosan as Chromatography Supports for Separation of Alkaloids from Dichloromethane Extract of *Simaba ferruginea*

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SUMMARY. This work presents a comparative study of the efficacy of different chromatographic supports in the isolation of the canthin-6-one and 4-methoxycanthin-6-one, two bioactive alkaloids isolated from dichloromethane extract of *Simaba ferruginea*. When chitin was used as chromatography support, the yield of the compounds were higher than silica gel, a traditional chromatograpic support.

RESUMEN. "Utilización de la Quitina y Quitosano como Adsorbentes Cromatográficos para la Separación de Alcaloides del Extracto en Diclorometano de *Simaba ferruginea*". Este trabajo presenta un estudio comparativo de la eficacia de diversos adsorbentes cromatográficos en el aislamiento de cantin-6-one y 4 metoxicantin-6-one, dos alcaloides bioactivos aislados del extracto diclorometánico de *Simaba ferruginea*. Cuando la quitina fue utilizada como adsorbentes de la cromatografía, la producción de los compuestos era más alta que la correspondiente cuando se uso silica gel.

INTRODUCTION

Simaba ferruginea St. Hil. (Simaroubaceae), vulgarly known as "calunga" or "féo-da-terra", occurs frequently in Brazilian Cerrado being its rhizomes widely used in traditional medicine to treat gastric ulcers, fever, diarrhea, rheumatism, pain, and other disorders [1,2]. The literature reports just a few chemical and pharmacological studies regarding the genus Simaba [3-6]. Previous preliminary pharmacological studies carried out with the alcoholic crude extract from the rhizomes of *S. ferruginea* indicated promising antiulcerogenic effects against the ethanol and indomethacin gastric ulcer model [7]. Recently, we have demonstrated by bioassay-guided fractionation the presence of two alkaloids as the main active principles of this plant, identified as canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**) [7].

Chitin, poly-β-(1->4)-N-acetyl-D-glucosanine, is the second most abundant natural polysaccharide and exist largely in the shells of crustaceous. Chitin have been employed as a stationary phase in liquid chromatography for separation of a amino acids [8], flavonoisds [9], biflavonoids [10] and terpenes [11] where it was found that the separation was highly influenced by the hydrogen bond formed between the compounds and OH and -CONH- in polymer.

As an extension of our studies about utilization of chitin as chromatographic support, we have now evaluated the efficacy of this natural polysaccharide and its derivative chitosan for separation of alkaloids from *S. ferruginea.*

EXPERIMENTAL

Preparation of Stationary phase

Chitin flakes (85% N-acetylation) were obtained in NIQFAR laboratories according to the method of Rinaudo *et al.* [12]. The material was ground and sieved and fractions with sizes of

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between 43-100 µm were used for the chromatographic column. Chitosan (85% N-desacetylation) was obtained from Puripharma. The material was ground and sieved and fractions with sizes of between 43-100 µm were used for the preparation of the chromatographic column.

Plant Material

Rhizomes of *S. ferruginea* were collected in Cuiabá, in the State of Mato Grosso, Brazil, in October 2001. The material was identified by Dr. Germano Guarim Neto (Botanical Department, UFMT) and authenticated by Ms. Harri Lorenzi (Instituto Plantarum de Estudos da Flora de São Paulo). A voucher was deposited at the Central Herbarium (UFMT) under number 21.883.

Phytochemical Analysis

2 kg of dried powdered rhizomes were macerated with methanol at room temperature for seven days. The extract was separated by filtration and concentrated under reduced pressure to obtain 78 g of a brown residue. Part of this material (35 g) was suspended with methanol and water (7:3) and successively partitioned with solvents of increasing polarity, to give the following fractions and yields: *n*-hexane (4.16 g), dichloromethane (DCM) (1.53 g) and ethyl acetate (EA) (0.6 g).

Chromatographic separation

The dichloromethane fraction (250 mg) chromatographed on chitin and chitosan separately (7 g) packed into a glass column (1.0 x 30 cm) with the bed of 15 cm height. The elution was performed with a hexane:acetone gradient and fractions of 5 ml were collected. After being monitored by thin layer chromatography (TLC), the fractions eluted with hexane:acetone 60:40 v/v which showed a positive reaction with Dragendorgff were combined.

The purity of all isolated substances was examined by TLC precoated with a 0.25 mm layer of silica gel 60 HF_{254} from Merck and eluted with hexane:acetone 65:35 v/v. The compounds were detected by spraying with a Dragendorff solution or visualized under UV light (254 nm). The compounds were identified by direct comparison with authentic samples previously isolated from *S. ferruginea* [7].

RESULTS AND DISCUSSION

In this study, the separation of alkaloids canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**), from *S. ferruginea* (Fig. 1) was compared using different chromatographic supports, e.g. chitin, chitosan and silica gel. The results from chromatographic separation (amount and yield of compounds) are showed in Table 1.

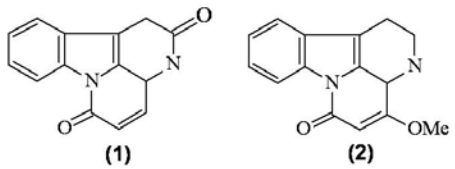

Figure 1. Structures of canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**).

The order of elution of alkaloids in chitin was the same used for silica gel, i.e., canthin-6-one (**1**) and 4-methoxycanthin-6-one (**2**). The compounds 1 and 2 were eluted from chitin column when hexane:acetona (60:40) was used as solvent. The yield of compounds **1** and **2**, separated with chitin, were 4 and 2 time higher respectively, when compared with yield using the silica gel as chromatography support.

Natural chitin, contain about 10-15% of deacetylated form (NH_2) free groups and 85-90% of the acetamide groups. The separation of alkaloids seems to occur by interaction of acetamide and OH-groups of chitin and alkaloids. The mechanims of the chromatographic separation of chitin is similar to the one of polyamide. Columns of polyamide are well suited for chromatographic separation of numerous organic compounds, especially polyphenols [13,14] alkaloids [15], phenylethanoid glycosides [16], carboxylic acids and aromatic amines [17]. The method is also useful for isolating naturally occurring compounds on a preparative scale.

Support	Canthin-6-one (1)		4-Methoxy-Canthin-6-one (2)	
	mg [1]	%	mg	%
Chitosan	-	-	-	-
Chitin	30.7 ± 2.9	8.1 ± 1.2	8.4 ± 0.4	2.2 ± 0.6
Silica gel [2]	3.0	1.2	2.8	1.1

Table 1. Efficiency of different support studied in the separation of alkaloids of *S. Ferruginea* (250) mg of dichloromethane extract. [1] Means for three experiments. [2] From reference 7.

On the other hand when the chitosan was used as sorbent, the alkaloids were not separated. The mixture of two alkaloids was eluted of the column when hexane:acetone 98:2 were used as eluent. These results show that the alkaloids have small interaction wit chitosan and this occur because the hydrogen bonding and acid-basic interactions between the chitosan and the organic compounds are the main driving force for chromatographic separation of these compounds.

Other interesting observation is the low yield of the pure compound (**1**) when silica gel was used as sorbent. This can be attributed to the strong interaction between the compound and free silanol groups from silica gel, resulting in the retention of large amount of compound in the column.

CONCLUSIONS

The results show that chitin can be used as an alternative chromatography support for separation of the alkaloids present in S. ferruginea because improving significantly the yield of the compounds.

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