Alternative extraction of alkaloid anti-carcinogens from Brazilian "vinca rosea" using Ion exchange chromatography

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Abstract

Extracts in ethanol and ethanol-ammonia of dried leaves from *Catharanthus roseus*, gathered at Rio de Janeiro state, were adsorbed in a strongly acidic cation exchange resin with sulfonic acid group, using the finite bath method, resulting in an alkaloid retained fraction and an acidic and neutral unretained fraction. High Performance Liquid Chromatography showed the isolation of the alkaloid fraction to be highly selective and with good performance, with an absence of alkaloids in the unretained fraction, while the retained fraction presented 1,54-6,35 mg/g of vindoline and 0,12-0,91 mg/g of vinblastine, common for an alkaloid-rich concentrate, usually obtained by classic extraction with several steps using solvents.

The isolation of the alkaloid fraction in phytochemical methodology involves column chromatography and successive liquid-liquid extractions, using acidic media and solvents with different polarities[1]. In production scale, extraction processes with solvents and acids generate inconvenient side-effects, like the recuperation of large volume of solvents which are generally hazardous, the formation of emulsions that are difficult to separate, the difficulty of solubilizing substances with a high molecular weight and the diluted solutions of the extracts[2]. Ion Exchange Chromatography in a non-aqueous medium, which is much used to separate acidic, basic and neutral fractions from petroleum derivatives[3], is a simple and alternative method, free from the inconveniences of classic extraction. Macroporous cation exchange resin type styrene-divinylbenzene copolymer containing sulfonic acid group showed high fixation capacities (1,66 milliequivalent-grams per gram of resin) and high recovery levels (85-96%) of standards organic bases[4]. The application of this chromatographic technique has been shown to be promising in obtaining the alkaloid fraction from *Peschiera affinis*, using a strong cation exchange resin[4].

In the same way, this method can be applied to isolate the alkaloid fraction that contains vinblastine and vincristine, dimeric alkaloids of commercial interest due to their antineoplastic pharmacological properties. These alkaloids are extracted from *Catharanthus roseus* leaves, a plant known as Vinca rosea or periwinkle, which is cultivated as a decorative plant in many countries, including Brazil[5]. The plant is the only natural source of these alkaloids, as there is no synthesis for their commercial production, except deriving from two other monomeric alkaloids, vindoline and catharanthine, which are both extracted from the same plant[6].

The design of alternative techniques for isolating the fraction of these alkaloids is economically important, taking advantage of this plant in Brazil, since the *Catharanthus roseus* leaves collected in the city of Magé, state of Rio de Janeiro, and analyzed by high performance liquid chromatography (HPLC), showed vinblastine levels comparable to those of plants in other regions of the world[7].

In the present work, we determine the recuperation and selectivity in retaining the alkaloid fraction containing vindoline and vinblastine from *Catharanthus roseus* in a cation exchange resin using the finite bath method, searching to value the advantages of extraction via ion exchange chromatography.

As can be seen in Table 1, identical levels were obtained for ethanolic and ethanolic-ammoniac extracts, around 60 mg per gram of dried leaf. The quantities of fixed alkaloids per

Table 1. Average data for extraction levels and of retained masses in adsorption of the *Catharanthus roseus* alkaloids in one gram of Lewatit SP112 resin

Condition	I	II	III	IV
dried leaves (g)		5		10
extraction solvent	EtOH	NH_3/EtOH[1]	EtOH	NH_3/EtOH[1]
extract (g)	0.40	0.29	0.59	0.59
retained fraction (mg/g resin)	34.5	60.3	84.0	209.2
retained vindoline[2] (mg/g resin)	1.54	2.16	5.03	6.35
(mg/g retained fraction)	44.6	35. 8	59.9	30.4
retained vinblastine[3] (mg/g resin)	0.12	0.32	0.91	0.84
(mg/g retained fraction)	3.4	5.3	10.9	4.0

[1] 3.5 %p/v ammonia in ethanol; [2] quantified as hydrochloride; [3] quantified as sulfate.

gram of resin, determined via external normalization in HPLC, are presented in Table 1, for different extraction solvents and different quantities of extract to mass of resin.

The unretained fraction was defined as the non-alkaloid fraction, given the absence of alkaloids, as shown in the chromatogram, even in more concentrated solutions (10 mg/mL in methanol) (Figure 1a), while the chromatographic result for the retained fraction (Figure 1b), was shown the presence of vindoline and vinblastine from the retention times of the standards injected under the same analysis conditions. The retained fraction was characterized as the alkaloid fraction, due to presence of vindoline and vinblastine at contents (Table 1) according to a concentrate rich in alkaloids, as reported in the literature from classic extraction[9,10] of up to 20 mg/g and 0,50-8,30 mg/g, respectively. These results demonstrate the selectivity of the compounds retained on the resin.

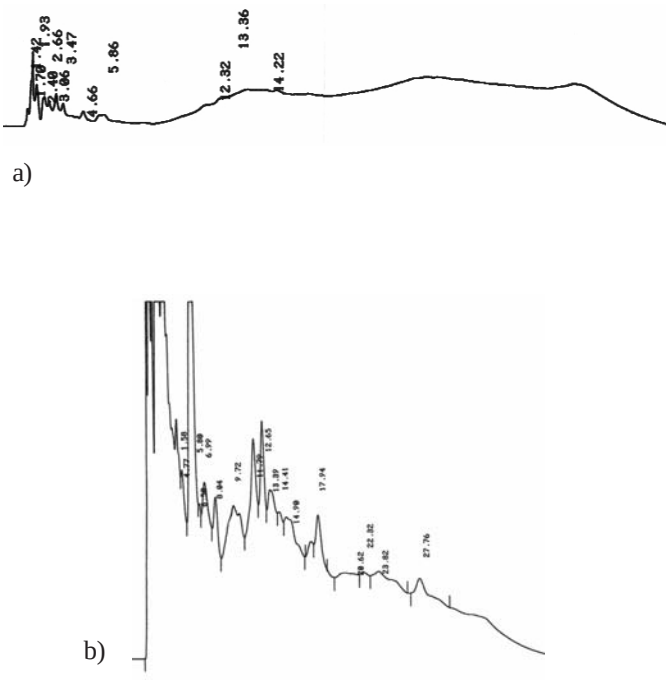

Figure 1. Analysis by HPLC of the fractions obtained from alcoholic extract of *Catharanthus roseus* in adsorption on resin Lewatit SP 112 by finite bath method: a. unretained fraction (to 10 mg/ml); b. retained fraction (to 5 mg/ml). Signals: vindoline at 8.0 min and vinblastine at 17.9 min.

The high fixation capacity of the resin was shown when the raising of the mass from 5 to 10 g of leaves were used (Table 1).

The good selectivity and high retention levels for the isolation of alkaloid fractions from the Vinca by cation exchange resin point to the technique as a feasible alternative to the consecutive extraction stages using solvents. Besides, the ionic exchange chromatography process allows large-scale automation.

Material and Methods

Catharanthus roseus plants were collected in the Magé city of Rio de Janeiro State, and the leaves, which had been dried at room temperature, were ground and extracted, by maceration in 40 ml of ethanol P.A. grade ("ethanolic extracts") or a solution of ammonia 3.5 %w/v in ethanol ("ethanolic-ammoniac extracts") for each 10 g of dried leaf, in a light-proof environment for 48 h. The extracts were filtered and evaporated until they were dry, and after being weighed were diluted in ethanol. Portions of these solutions, the equivalent of extracts containing 5 and 10 g of leaf, were added to 1 g of highly acidic cation exchange resin, Lewatit SP112 (Bayer), composed of styrene-divinylbenzene copolymer with sulfonic acid groups. The mixture was stirred at room temperature for 2 h to retain the alkaloids.

The supernatant was filtered and the resin rinsed five times, with 7.0 ml of a 1:1 v/v solution of toluene/ethanol, to remove the unretained components. The organic solutions used to rinse the substance were added to the supernatant after filtration, thereby making the unretained fraction. Ammonium hydroxide concentrate (15 ml) was added to the resin, stirred for one hour to bring about the total desorption of the retained alkaloids. The final solution was filtered and the resin rinsed five times with 10 ml of ethanol. The alcoholic solutions were added to the concentrated alkaline solution, thereby forming the retained fraction.

The retained and unretained fractions were evaporated until they were dry, then they were weighed and re-diluted at a concentration of 5 mg/ml of methanol and analyzed (20 ml) by HPLC, using a similar technique to that presented in the literature[11] with a m-Bondapak C18 column 300 mm x 3.9mm, 10 µm, with the gradient 20:30:50:0.02 to 29:36:35:0.02 V/V of methanol-acetonitrile-ammonium acetate buffer-triethylamine, at room temperature, with a flow rate of 1.5 ml/min and detection at 280 nm.

Under the same conditions, standard solutions were injected with 40 µg/ml of vinblastine sulfate and vindoline hydrochloride in methanol, to determine their retention time, quantification and characterization in the samples. The linearity of the chromatographic method to alkaloids has been proven in previous work[8]. The levels of vindoline and vinblastine in the samples were measured by external normalization, relating the integration areas of each substance to the concentration in the standard solutions.

Acknowledgments

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References

[1] Cechinel VF, Yunes RA. Estratégias para obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceito sobre modificação estrutural para otimização da atividade. Química Nova. 1998; 21(1): 99-105

[2] Zief M Kiser R. An overview of Solid Phase Extraction for sample preparation. American Laboratory. 1990; 70-83

[3] Green JB, Hoff RJ, Woodward PW, Stevens LL. Separation of liquid fossil fuels into acid, base and neutral concentrates. An improved nonaqueous ion exchange method. Fuel 1984; 63: 1290-1301

[4] Soares KCC, Saraiva E, Taddei LEM, Carvalhaes, SF. Caracterização da capacidade de fixação e rendimento de recuperação de bases orgânicas em resinas catiônicas macroporosas com aplicação em *Peschiera affinis*. 23º Reunião da Sociedade Brasileira de Química (Annual Meeting of the Brazilian Society of Chemistry). 2000

[5] Potier P. Rhône-Poulenc lecture. Search and Discovery of New Antitumor Compounds. Chemical Society Reviews. 1992; 21:113-119

[6] Huhtikangas A, Pennanen S, Lousnamaa M. Process for the preparation of dimeric *Catharanthus* alkaloids. WO 89/12056 (1989), FI 89/00104 (1989), Appl. FI 882755 (1988), Class. Int. C07D 519/04

[7] d'Avila LA, Mazzei JL, Carvalhaes GK, Martins HM, Anastácio CS, Carvalhaes SF, Furtado EG. Extração alternativa de alcalóides anti-cancerígenos da Vinca Rosa Brasileira. XV Simpósio de Plantas Medicinais do Brasil (Symposium on Medicinal Plants of Brazil). 1998

[8] Mazzei JL, Martins HM, Carvalhaes GK. Análise de alcalóides da Vinca brasileira por CLAE. XXXV Congresso Brasileiro de Química (Brazilian Congress of Chemistry). 1995

[9] Atta-ur-Rahman, Bashir M. A rapid procedure for the isolation of catharanthine, vindoline and vinblastine. Journal of Medicinal Plant. 1983; 47:246-256

[10] Gunasekera SP. Method of isolation vinblastine. WO 88 03135 (1998), Appl US 922514 (1996), Class. Int. C07D 519/04

[11] Naaranlahti T, Nordström M, Huhtikangas A. Determination of *Catharanthus* alkaloids by reversed-phase high-performance liquid chromatography. Journal of Chromatography. 1987; 410(2):488-493

Constituintes químicos do extrato acetato de etila das partes aéreas de *Solanum paludosum* Moric.

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Abstract

Phytochemical investigation of the aerial parts of S. paludosum afforded 3,4,7,8-tetramethyl gossypetinr, 3,7-dimethyl kaempferol, 3-methyl kaempferol, 3-methyl apigenin, and 3-methyl quercetin ethers, besides N-p-trans-coumaroyltiramine, and protocatecuid acid. The structures were established from spectral data of the natural substances and the permethyl and acetyl derivatives of tetramethyl gossypetin.

Solanum paludosum Moric. é uma espécie incluída no subgênero *Leptostemonum* (Solanaceae) que se apresenta sob forma de arbusto. Estudo com espécies de *Solanum* da Paraíba[6] revelou a presença de 0,67 % do alcalóide solasodina nos frutos verdes de *Solanum paludosum*[1]. Este alcalóide é um importante precursor para formação de hormônios esteroidais.

Em comunicação anterior foram descritos o isolamento e a identificação de duas substâncias das partes aéreas de *S. jabrense*, o alcalóide Nβ-metiltetraidro-β-carbolina[7], e o sesquiterpeno solavetivona[8], que apresentou efeito espasmolítico inespecífico em íleo isolado de cobaia[2,3].

Este trabalho relata o resultado obtido na investigação do extrato acetato de etila da parte aérea de *Solanum paludosum*.

Material e Métodos

Material vegetal: a parte aérea de *Solanum paludosum* foi coletada em janeiro de 1999 no Campus Universitário, João Pessoa, Paraíba. Uma exsicata encontra-se depositada no Herbário Prof. Lauro Pires Xavier (M. F. Agra 3224 JPB), Universidade Federal da Paraíba, João Pessoa, Paraíba.

Extração e isolamento: o material coletado foi seco, pulverizado (1,1 kg) e percolado em etanol. O resíduo obtido do extrato