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Plumbagin quantification in roots of *Plumbago scandens* L. obtained by different extraction techniques

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ABSTRACT

The *Plumbago* genus belongs to the Plumbaginaceae family and it is known due to its variety of biological uses, most of them attributed to the presence of naphthoquinones. Plumbagin is a naturally occurring naphthoquinone that can be obtained from roots of *Plumbago scandens* L. In order to find out the better technique for plumbagin extraction, were applied: static maceration, dynamic maceration, with assistance of ultrasonic waves and in Soxhlet apparatus. Four compounds were qualitatively detected in all extracts: the naphthoquinones plumbagin and *epi*-isoshinanolone, palmitic acid and sitosterol. Plumbagin was always the major component in all analyzed extracts and it was quantitatively determined by gas chromatograph coupled with mass spectrometer. Soxhlet was the most efficient extraction technique however, prolonged heating time promoted plumbagin degradation.

Key words: Plumbaginaceae, *Plumbago scandens* L., plumbagin, extraction techniques.

INTRODUCTION

The plant kingdom represents an extraordinary resource of organic compounds. However, only few plants have been investigated chemically. The rapid disappearance of tropical forests has meant that it is essential to have access to methods which lead to the rapid isolation and identification of bioactive natural products and also minimize the amount of plant material used in a phytochemical study (Hostettmann et al. 1997).

Plumbago scandens L. (Plumbaginaceae) is a scandent subshrub distributed in America, native from Brazil. The chemical profile of the genus is marked by the presence of naphthoquinones, flavonoids and terpenoids (Paiva et al. 1995).

Quinones structurally encompass pigments, antibiotics, vitamin K and coenzymes. The last ones function in metabolism as one-electron transfer agents by virtue of their property to form reversible rather stable semiquinone radicals on reduction. Quinones biosynthesis is diversified, some types come from polyketide pathway, while others derived from shikimic acid route (Torssel 1983). These compounds represent a chemical defense for plants and have many known biological properties.

Plumbagin (2-methyl-5-hydroxy-1,4-naphthoquinone) (Figure 1) is a naturally occurring yellow

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pigment, produced by members of Plumbaginaceae, accumulated mostly in roots (Van der Vijver 1972).

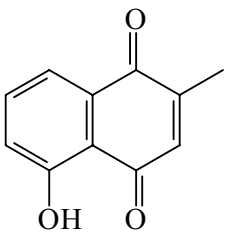

Fig. 1 – Plumbagin structure.

Biological activities of crude plant extracts from different *Plumbago* species as well as of plumbagin have been studied exhaustively. Plumbagin showed anticancer (Melo et al. 1974), leishmanicidal (Chan-Bacab and Peña-Rodriguez 2001) and bactericidal (Durga et al. 1990) activities, being also effective against insects (Kubo et al. 1980, Ghosh et al. 1994).

Secondary metabolites concentration in plant organs can vary along the year and different extraction techniques may interfere quantitatively and qualitatively in the extract composition.

Conventional extraction methods with organic solvents, as maceration or hot extraction in Soxhlet apparatus, are widely used to obtain plant extracts. The amount of dried plant extract depends on the analytical technique that will be employed (Sargenti and Vichnewski 2000). Other parameters should be considered such as: type of solvent, the organic solvent volume, the temperature and the extractive process duration.

The objective of the present work was to find out the better extraction technique of the naphthoquinone plumbagin, in terms of efficiency.

MATERIALS AND METHODS

Solvents

The solvents used (chloroform and ethyl acetate) were PA grade.

Plant Material

Roots of *Plumbago scandens* L. were collected at Fundação Oswaldo Cruz *campus*, Rio de Janeiro State, Brazil. A voucher of this plant was deposited at Rio de Janeiro Botanical Garden Herbarium (RB) under the number 340.340.

Extracts Preparation

Roots of *P. scandens* were oven dried at 40°C and powdered (5 mm of particle diameter). The plant (9.5 g) was extracted with 300 ml of chloroform by different extraction techniques: static maceration (24h), dynamic maceration (10h + 14h of static maceration), extraction with aid of ultrasonic waves (2h) and extraction in the Soxhlet apparatus at different process duration (2h, 5h, 10h). Chloroform was employed as solvent for these extractions, once it was reported in literature for nafthoquinones extraction (Sidhu and Sankaram 1971, Zhong et al. 1984, Pakulski and Budzianowski 1996). All the solutions were evaporated to dryness under reduced pressure. The extraction efficiency was defined as follows:

$$\underset{\text{(w/w)}}{\text{Percentage extraction}} = \frac{\text{mass of extracts}}{\text{mass of dried material (roots)}} \times 100$$

Instruments

Instrumentation consisted of an Agillent Technologies gas chromatograph model 6890N equipped with a mass selective detector, model 5973 and an automatic injector model 5683. Capilar column HP-5MS (5% phenyl, 95% methyl syloxan), 30 m $\times$ 0.25 mm $\times$ 0.25 μm. The data acquisition was performed by HP Chemistation Data Acquisition Software.

Sample Preparation

A portion of the crude chloroform extracts (2 g each) was dissolved in ethyl acetate (1 ml), and injected into a gas chromatograph coupled with a mass spectrometer.

Chromatographic Conditions

The following conditions were used: helium as carrier gas, mass detector, detector temperature = 280°C, injector temperature = 270°C, flow rate 1.0 ml/min, split of 1:20, injection volume = 1.0 μl, initial temperature = 120°C and oven program from 5°C/min to 290°C followed by an isoterm period of 20 min.

Standard Solutions

For system calibration, individual stock solution of plumbagin (previously isolated from roots of *P. scandens* and identified by spectrometrical techniques) was prepared with ethyl acetate in volumetric flask. Known concentration aliquots of plumbagin (0.10; 0.50; 1.00; 1.50; 2.00; to 2.50 mg/ml) were used in order to have a standard curve (Figure 2).

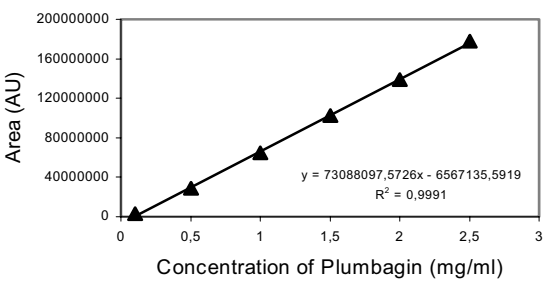

Fig. 2 – Standard curve with known concentration of plumbagin.

RESULTS AND DISCUSSION

The efficiencies of plumbagin extraction by different techniques were compared. As expected, Soxhlet extraction was the most efficient technique (Figure 3). Its efficiency is due to the employment of a small volume of solvent which is continuously renewed in contact with the plant material, promoting more interactions between them.

Extractions in Soxhlet apparatus for 5 hours demonstrated a high efficiency, yielding almost 50% over the extract obtained in 2 hours of extraction. Comparatively 10 hours of extraction yielded approximately 8.5% more than the obtained in 5 hours.

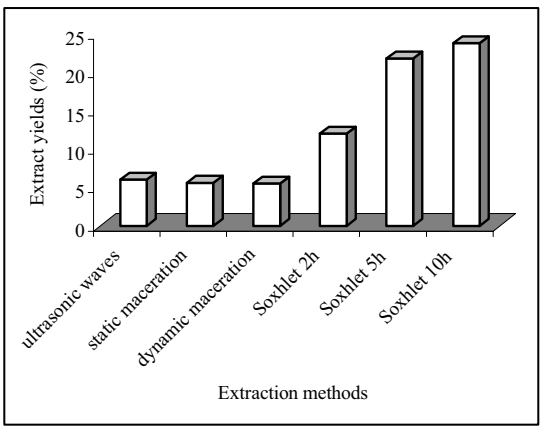

Fig. 3 – Extract yields (%) of the crude chloroform extracts from roots of *Plumbago scandens* L. obtained by different extraction techniques.

A sample of each extract solution in ethyl acetate (2 mg/ml) was submitted to a gas chromatograph coupled with a mass spectrometer. The samples were submitted to a chromatographic fractionation twice in order to guarantee the fidelity of the experiment. None of the used extraction techniques showed qualitative change of the chemical composition, since the same compounds were identified in all extracts by GC/MS retention time and molecular fragmentation. The chromatograms and the spectral analysis demonstrated the presence of four main compounds identified as the naphthoquinone plumbagin, the naphthoquinone *epi*-isoshinanolone, palmitic acid and sitosterol (Figure 4). Plumbagin was always the major component, independent of the extraction technique. The variation profile of these compounds in different extraction techniques can be observed in Figure 5.

Plumbagin was isolated from roots of *P. scandens* and identified by comparison of its spectral data to those reported in the literature (Carvalho 1986). This compound can be dissolved in solvents with low and medium polarity and its melting point is at 74°C. Its quantitative determination was carried out in all extracts. The results are described in Table I. The lowest level of this substance was observed in the extract obtained by static maceration

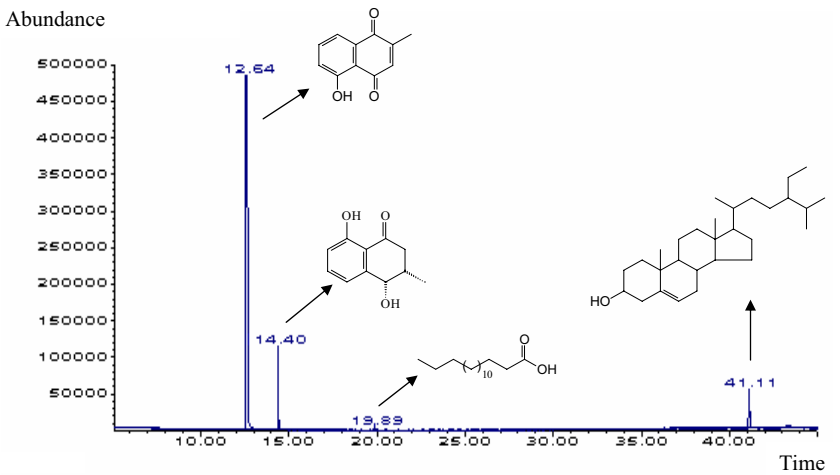

Fig. 4 – Chromatogram of the crude chloroform extract from roots of *Plumbago scandens* obtained by dynamic maceration.

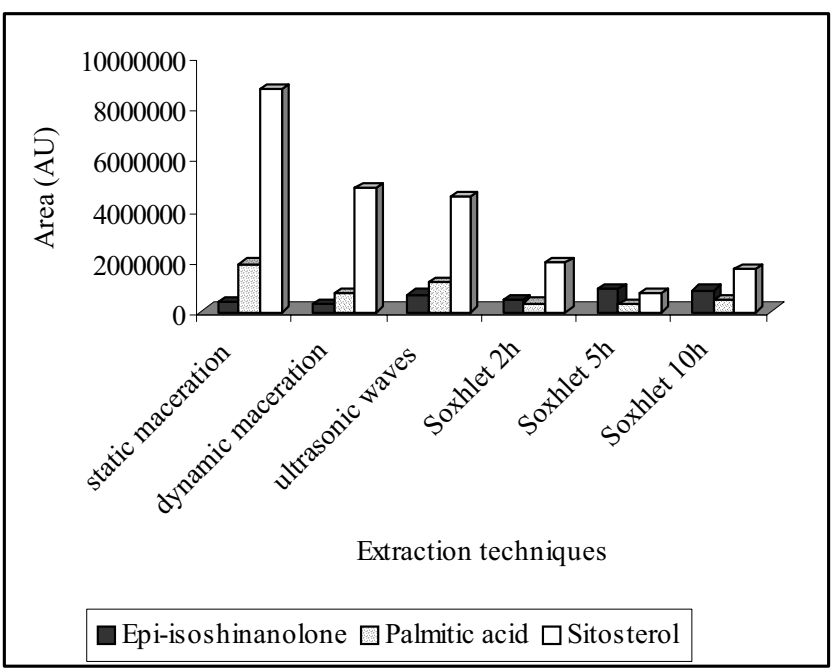

Fig. 5 – Profile variation of the main constituents of the *Plumbago scandens* root extracts obtained by different extraction techniques.

(6.47%), while the highest content was verified in the Soxhlet extraction for 2, 5 and 10 hours (35.78; 57.65 and 50.27, respectively). However, the process duration of 10 hours demonstrated a decrease of 12.8% in the plumbagin content, suggesting its degradation under long exposition time to high temperature. It is interesting to point out the 30h time hot extraction reported by former researchers study-

TABLE I

Plumbagin content (mg/ml) and its percentage in roots of *Plumbago scandens* according to different extraction techniques.

Extraction technique	mg/ml	% of plumbagin
Static maceration	0.18	6.47
Dynamic maceration	0.33	15.51
Assistance of ultrasonic waves	0.44	21.87
Soxhlet – 2 hours	0.93	35.78
Soxhlet – 5 hours	1.56	57.65
Soxhlet – 10 hours	1.06	50.27

ing *Plumbago* species (Sankaram et al. 1976).

The naphthoquinone plumbagin is a compound of great interest due to its biological properties. This work aimed to investigate the better extraction technique for this compound from plant material. Plumbagin can be isolated in large amounts, especially from roots of *Plumbago* species and represents an important target for new pharmacological tests. It could be concluded that the extraction in Soxhlet apparatus was optimal to reach high percentage extraction of plumbagin, however the process duration should not be so long over 5h, in order to maintain the better yield of plumbagin.

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RESUMO

O gênero *Plumbago* pertence à família Plumbaginaceae e apresenta uma variedade de atividades biológicas, a maioria atribuída à presença de naftoquinonas. Plumbagina é uma naftoquinona natural que pode ser obtida a partir de raízes de *Plumbago scandens* L. Na tentativa de descobrir a melhor técnica de extração dessa substância foram usadas, maceração estática, maceração dinâmica, extração com auxílio de ultrassom e extração em Soxhlet. Quatro substâncias foram detectadas qualitativamente: as naftoquinonas plumbagina e *epi*-isoshinanolona, o ácido palmítico e o sitosterol. Plumbagina foi sempre o componente majoritário em todos os extratos analisados e sua determinação quantitativa foi realizada através de cromatografia com fase gasosa acoplada à espectrometria de massas. A extração em Soxhlet foi a técnica mais eficiente para obtenção de plumbagina, entretanto a longa exposição a temperaturas elevadas favoreceu a degradação da plumbagina.

Palavras-chave: Plumbaginaceae, *Plumbago scandens* L., plumbagina, técnicas de extração.

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