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Article

New Extraction Technique for Alkaloids

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Um método de extração de produtos naturais foi desenvolvido. Comparado aos outros métodos, a nova técnica é rápida, mais eficiente e consome menos solvente. A extração de alcalóides de produtos naturais, como *Hyoscyamus muticus, Datura stramonium* e *Ruta graveolens,* consiste no uso de uma solução sonicada, contendo um surfactante como agente extrator. Os alcalóides são precipitados pelo reagente de Mayer, dissolvidos em uma solução alcalina, e então, extraídos com clorofórmio. Este artigo compara os resultados obtidos com outros métodos, mostrando claramente as vantagens do novo método.

A method of extraction of natural products has been developed. Compared with existing methods, the new technique is rapid, more efficient and consumes less solvent. Extraction of alkaloids from natural products such as *Hyoscyamus muticus, Datura stramonium* and *Ruta graveolens* consists of the use of a sonicated solution containing a surfactant as extracting agent. The alkaloids are precipitated by Mayer reagent, dissolved in an alkaline solution, and then extracted with chloroform. This article compares the results obtained with other methods showing clearly the advantages of the new method.

Keywords: alkaloids, extraction, sonication, surfactants, Mayer reagent

Introduction

The growing interest in secondary metabolites of plants has directed attention to methods for their extraction. Natural products are extracted by conventional methods such as Soxhlet and room temperature solvent extraction,[1-7] or by ultrasound,[8-14] microwaves,[15-18] supercritical solvents[19-30] or other methods.[31-34]

In this paper a new method is developed to extract alkaloids, which are among the most important groups of secondary metabolites.[35] We propose here a method combining ultrasound with surfactants, where properties of wetting, dispersion, solubilisation and emulsification[36-37] reduce the solvent and time necessary for the extraction of alkaloids from natural products. The selected plants for this study are *Hyoscyamus muticus, Datura stramonium* and *Ruta graveolens*.

Experimental

Plant material

Hyoscyamus muticus was collected in the far south of Algeria in spring 2001, *Datura stramonium* and *Ruta graveolens* were harvested in the north east of Algeria (summer 2002). Aerial parts of the plants were air dried in the shade for several days at room temperature, ground and stored in glass flasks to protect them from humidity and light.

Solvent extraction

Powdered plant material (10 g) was wetted with 15 mL of NH_4OH (25%, m/m) and room temperature solvent extraction was performed with 300 mL of ethyl acetate for 72 h. The extract was filtered and the solvent was evaporated in a rotary evaporator under reduced pressure at 40 °C. The residue, dissolved in H_2O and acidified with H_2SO_4 to pH 3-4, was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH 9-10 with NH_4OH (25%, m/m), it was extracted with chloroform, the extract washed with distilled water to neutral pH, dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids.

Soxhlet method

The extraction was undertaken with 10 g of powdered plant material and 300 mL of ethyl acetate in a Soxhlet

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apparatus for 18 h. The final extract was filtered and the filtrate was concentrated under vacuum. The residue was treated in the same manner as described for the maceration method (see above).

Principle of the new method

A sample of 10 g of powdered plant material was suspended in 400 mL of surfactant solution in a glass beaker and sonicated for 2.5 h in an ultrasonic bath at a constant temperature of 25 °C. The extract was separated by simple filtration and the residual material washed with 20 mL of pure water. The solution of combined filtrates was acidified with sulfuric acid solution (2%, m/m) to pH 3-4 and the alkaloids were precipitated with 15 ml of Mayer reagent.[6] The precipitate was dissolved in an alkaline solution of sodium carbonate (5%, m/m) and extracted with $CHCl_3$. The organic layer was washed with water to neutral pH, dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain alkaloids.

Thin layer chromatography, IR and UV were used to compare crude alkaloids obtained by the different procedures. All samples presented the same qualitative profile.

Results and Discussion

Preliminary experiments set were performed with sonication of powdered leaves of *Datura stramonium* in surfactant solution of sodium dodecylsulfate 0.1% (m/v) at room temperature to determine the best extraction time. The collected results (Figure 1) showed that the highest yield was achieved by this method after 2.5 h with no significant variation. All experiments were duplicated and this time was selected, as standard.

The following experiment was made to choose between an anionic (SDS: Sodium dodecyldsulfate, Aldrich product) and a non-ionic surfactant (Emulgen: Polyethyleneglycoldodecyl ether, Aldrich product) and to determine the effects of surfactant concentration on the extraction. The results (Figures 2 and 3) showed that the presence of surfactant enhances the amount of total alkaloids extracted even at low concentration (0.01%, m/v). Moreover, no significant difference was observed between the two types of surfactant.

It appeared that more the total alkaloid contained in the plant material, the higher the concentration of surfactant required to extract it in high yield.

A concentration of 0.2% (m/v) of surfactant was selected to compare this new method and the classical one.

The collected results (Figure 4) concerning the comparison between conventional extractions (soxhlet and

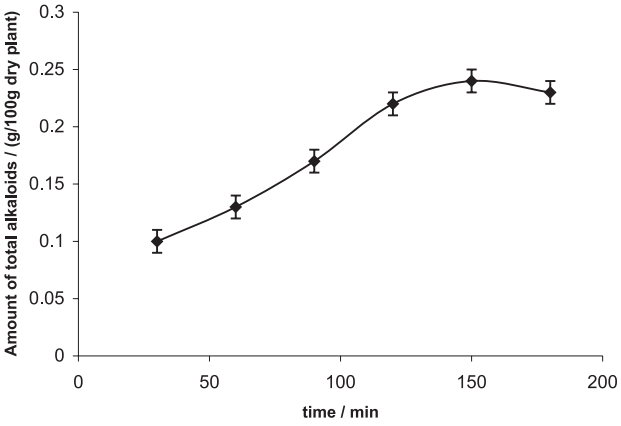

Figure 1. Total alkaloids extracted (g) according to time (min).

room temperature solvent extraction) and the extraction of the three plants under study in the presence of surfactant, showed the following points: In all cases, SDS or Emulgen lead to the same results; in the case of *Datura stramonium*, there is no significant variation between the different extraction methods; in the cases of *Ruta graveolens* and *Hyoscyamus muticus*, the use of surfactant results in a better extraction of total alkaloids.

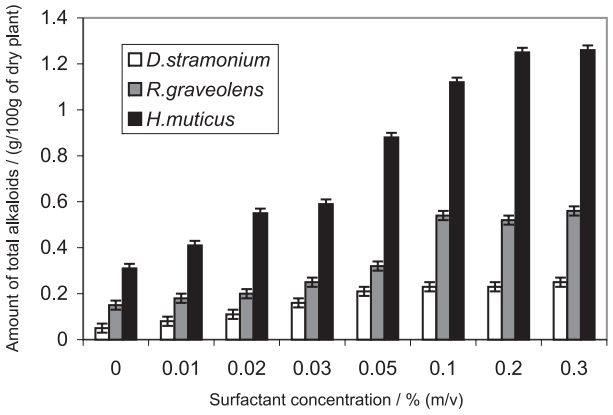

Figure 2. Variation of total alkaloids amount with concentration of SDS.

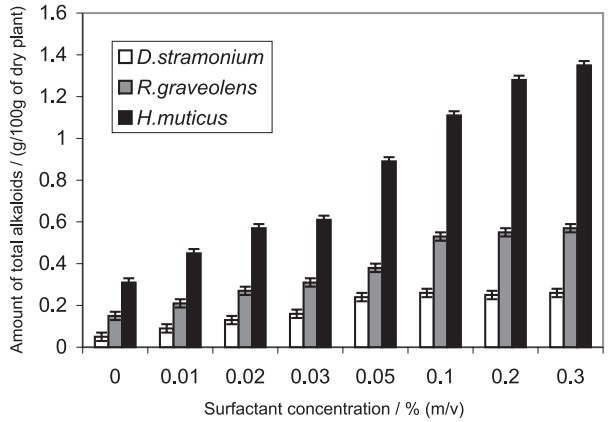

Figure 3. Variation of total alkaloids amount with concentration of Emulgen.

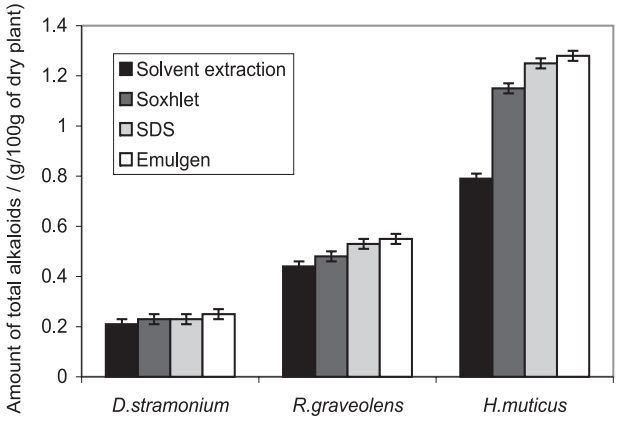

Figure 4. Total alkaloids (g/100g of dry plant) obtained with different methods.

Conclusions

The study carried out, confirms that the method using surfactants in the extraction of alkaloids is more efficient, the alkaloids recovered having similar qualitative characteristics of those obtained by conventional extraction methods. The method is a useful alternative technique and can probably be expanded to other secondary metabolites (essential oils and polyphenols are in the course of study). To make this method more universal will require optimisation of parameters such as temperature, nature of surfactants, volume and concentration of surfactant solution.

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