# An Improved Extraction of Marrubiim from *Marrubium vulgare*

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## Key Words

Column liquid chromatography
Chitin stationary phase
Marrubiin
Analgesic activity

## Summary

Marrubiin is the main active compound isolated from *M. vulgare*, a medicinal plant used in folk medicine to cure several diseases. The present study shows that chitin, an abundant natural polymer, may be successfully in chromatography column to separate marrubiin from complex mixtures. The experimental procedure described here represents an efficient and rapid method to obtain such compound in high yield.

## Introduction

*Marrubium vulgare* (Labitae) is a medicinal plant used in folk medicine in Brazil and many countries for the treatment of a variety of ailments, especially gastroenterical and respiratory disorders [1, 2]. We have previously demonstrated that hydroalcoholic extract obtained from the aerial parts and roots of this plant exibits marked antispasmodic and analgesic effects when analysed in several pharmacological models [3, 4]. One of the active compounds responsible for these activities was identified as the diterpenoid marrubiin (**1**), see Figure 1 [5]. This compound also is stated to stimulate secretions of the bronchial mucosa and to possess anti-arrhythmic properties [2].

Several authors have reported the isolation of (**1**) from different plants, including *M. vulgare* , and some of them have proved that this compound is an artefact produced during the extraction process [6–8]. However, all experimental procedures involved expensive solvents and time-consuming methods. Since (**1**) exerts different pharmacological properties [2, 5] and could represent a potential therapeutic tool, we propose here an improved and alternative method for isolation of this compound from *M. vulgare* using chitin, a natural biopolymer, as a chromatographic support.

## Experimental

### Preparation of Extract

*Marrubium vulgare* L. was collected in Bom Retiro, Santa Catarina, south of Brazil, in September 1996, and classified by Prof. Leila da Graça Amaral (Department of Botany, Federal University of Santa Catarina (UFSC), Florianópolis, SC). Voucher was deposited in FLOR herbarium (UFSC) under number 26699.

Air-dried aerial parts and roots of *M. vulgare* (1.3 kg) were powdered and macerated with 50 % ethanol-water (3.5 L) for approximately 10 days at room temperature. After solvent removal under reduced pressure at 90 °C, the extract was then cooled over for two days and the precipitate (3 g) was filtered for further chromatographic procedures. Similar procedures were performed only with the roots of this plant.

### Preparation of Chromatographic Support

Chitin flakes (85 % N-acetylation) were obtained in NIQFAR/FAQFAR laboratories according to the literature method from shrimp shells captured Atlantic south cost [9, 10]. The material was ground, sieved and fractions with size between 43–65 μm were used for preparation of the chromatographic column.

### Chromatography

3 g of the precipitate described above was chromatographed on a column chromatography (CC) (3.0 × 30 cm) using 35 g of chitin eluted with hexane and fractions of 5 mL were collected. After monitoring by thin layer chromatography (TLC) eluted with hexane:ethyl acetate 2:1, the similar fractions which exhibited only one spot by spraying with anisaldehyde-sulfuric reagent, were combined to give pure marrubiin (1.54 g; yield = 0.12 %). It was identified on basis of its spectroscopic



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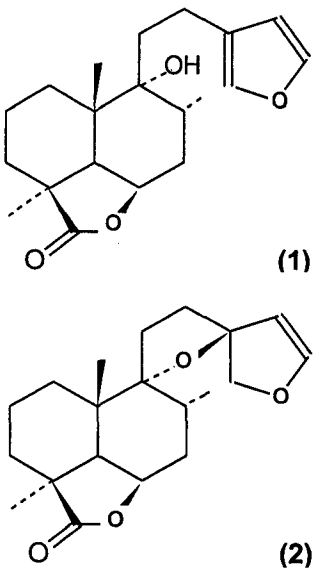


**Figure 1**

data ($^1$H and $^{13}$C-NMR, IR, UV) [6, 7], melting point determination and co-TLC with an authentic sample.

The same experimental procedure was repeated by using silica gel as stationary phase in CC, given 0.07 % of marrubiin (**1**).

An aliquot of the hydroalcoholic extract obtained from roots of *M. vulgare* was analysed by TLC in comparison with an authentic sample of **1**.

## Results and Discussion

In the last years, our research group has increased efforts to obtain analgesic compounds isolated from Brazilian medicinal plants, using the active constituents as a model for the synthesis of more potent derivatives. The analgesic effects of *M. vulgare* and consequently marrubiin [3–5], which presents several reactive centers, led us to investigate a rapid and inexpensive method for separation of this compound. Since marrubiin is an artefact generate from premarrubiin (**2**), see Figure 1, we prepared different extracts from the whole plant, such as methanolic, ethanolic, chloroformic and hydroalcoholic and analysed them by TLC. The last extract showed to possess more concentration of (**1**), being then selected for phytochemical separation. However, TLC of the extract obtained from the roots indicated the presence of small quantity of marrubiin. Solvent removal at 90 °C proved to be the best experimental conditions to convert premarrubiin into marrubiin. The purification of this compound was carried out by using open chromatography column over chitin, which showed excellent results as solid support, quickly affording a high yield of pure marrubiin. This simple process eliminates the need of repeated chromatography column (CC) obtained with other solid supports, i.e., silica gel, alumina and cellulose. Our results confirmed that the use of chitin as stationary phase in CC gave best yield of marrubiin than silica gel (about 1.7-fold more). Other advantages to use chitin as support in CC consist in the facility to obtain the raw material (the major waste by-product of the shellfish processing industry), once Itajaí city has an important fishing port. Although chitin is widely employed in different fields of chemistry, chromatographic applications of this polymer are limited and further studies are required. However, studies using chitin as a support in TLC indicated its ability to separate mixtures of phenols, amino acids or inorganic ions, being more effective than cellulose, silica gel and polyamides [11, 12].

Other studies conducted with marrubiin (**1**) revealed that it is produced in poor yield by cell suspension and callus cultures obtained from seedlings of *M. vulgare*[13]. More recently have been shown that this compound seems be biosynthesized in *M. vulgare* via a non-mevalonate pathway [14].

In summary, the experimental procedure described in the present investigation represents a rapid, inexpensive and efficient method for isolation of marrubiim from *M. vulgare*. In addition, this method will may be useful for the isolation of other active compounds from medicinal plants. Presently, the studies are in progress to determine the retention mechanism on chitin.

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