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COMMUNICATION

Development of a Profitable Procedure for the Extraction of 2-H-1-Benzopyran-2-one (Coumarin) from *Mikania glomerata*

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ABSTRACT

*This report deals with a new procedure suitable for the extraction of coumarin **1** from* Mikania glomerata*. The aim of this investigation is to obtain this compound in an economically profitable way, taking into account the yield of its extraction, the cost, and the time of the overall process. Fresh and dried plants collected in several areas of the State of Rio de Janeiro were used, and seasonal effects on coumarin content were studied. Obtained results indicated that extraction with a 1% (w/v) NaOH solution, under appropriate conditions, allows a simple and complete recovery of the desired product and that the best yields were obtained with the fresh aerial parts of the plant. Season and area of harvesting effects have also been studied*

KEY WORDS: Mikania glomerata; Extraction procedure; Coumarin; Phytopharmaceutical technology.

INTRODUCTION

The genus *Mikania* consists of approximately 300 identified species, spread throughout all tropical America. Despite this large number of species, only 20 of these have been actually studied from a chemical point of view and some of their constituents isolated and identified (1). Within this *Mikania* genus, the species *M. glomerata* is one of the most used in Brazilian etnopharmacology, mainly because of its anti-inflammatory, bronchodilator, anti-asthmatic, and spasmolytic activity. A more systematic and scientific approach to a deeper investigation on this plant started with the studies by Oliveira (2) that were recently continued by Cabral et al. (3). Coumarin **1**,

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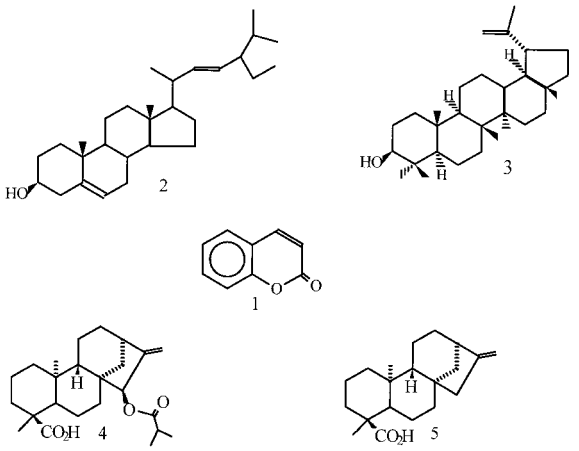

Figure 1. Compounds extracted from *Mikania glomerata*.

stigmast-22-en-3-ol **2**, lupeol **3**, isobutyryloxy-kaurenoic acid **4**, and kaurenoic acid **5** were isolated and identified (Fig. 1), and laboratory tests indicated coumarin **1** as responsible for the pharmacological effects reported above (4). This substance is widely used in pharmaceutical and fine chemistry industries, thus it also has a significant commercial value. Consequently, considering that coumarin is currently produced only by synthetic procedures, whereas extractive processes are used successfully by the Merck Brazil to obtain pharmaceutical products such as pilocarpine **6** and rutin **7** (Fig. 2), the possibility of developing a new extractive process with the purpose of obtaining coumarin at low cost appears to be very interesting and stimulating, both from both economical and ecological points of view. In this work we describe the development of a new extraction procedure that is suitable for the production of coumarin from *M. glomerata* in a selective and economically feasible way.

EXPERIMENTAL PROCEDURES

Materials and Methods

Plant Material

The plant material was collected in 1996 and 1997 at Teresópolis, Vassouras, Campo Grande and Jacarepagu, Rio de Janeiro, Brazil.

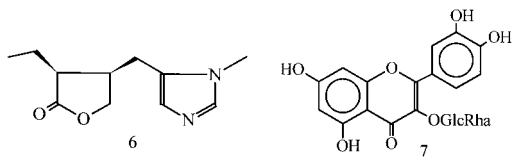

Figure 2. Chemical structure of pilocarpine 6 and rutin 7.

General Experimental Procedures

The HPLC analysis was carried out using a Shimadzu Liquid chromatograph with an UV detector and a model 7010 sample injection valve (Rheodyne, CA) with a 20-μL loop. All separations were performed on a C_{18} Chromosorb column (10 mm, 250×4.6 mm i.d.). The chromatograms were integrated by means of an IBM microcomputer. The solvents used for the extraction and analysis (MeOH, EtOH, hexane, and H_2O) were HPLC grade. All reagents used were analytical grade.

Extraction Procedure

The aerial parts of the *M. glomerata* Sprengel were cut in pieces of approximately 0.5 cm by means of a knife mill, then 10 g of this material was extracted three times with 40 mL of solvent, refluxing each time for 30 min. Constant magnetic stirring was maintained throughout the extraction process (dynamic remaceration). For an appropriate comparison, the same procedure was carried out, in parallel, at room temperature. Coumarin concentration was then determined in the obtained solutions. The solvents tested for these extraction processes were hexane, ethanol, water, and a 1.0% w/v aqueous solution of NaOH.

HPLC Analysis

Coumarin content in the various extracts was determined by HPLC using a 1:1 water : methanol mixture as mobile phase at a flow rate of 0,5 mL/min. The UV detector was set at the wavelength of 220 nm. The chromatograms of samples obtained from the NaOH aqueous solution and from the EtOH extraction are shown in Figure 3 as examples.

RESULTS AND DISCUSSION

For a preliminar evaluation of the coumarin **1** content that is usually present in the species *M. glomerata*, this work was initiated with an investigation of the ideal solvents that would be capable of exhaustively extracting the raw substance from the plant material. For this purpose, the solvents initially tested were ethanol and hexane because of the high solubility in such solvents of the product that had to be extracted (5). The plant material was refluxed three times for 30 min with the solvent; each time the solvent was removed and replaced. In most cases, this method allowed a complete recovery of coumarin, as confirmed by a further extraction in the same experimental conditions that showed how no coumarin could be detected in a fourth portion of solvent. As noted above, all quantitative

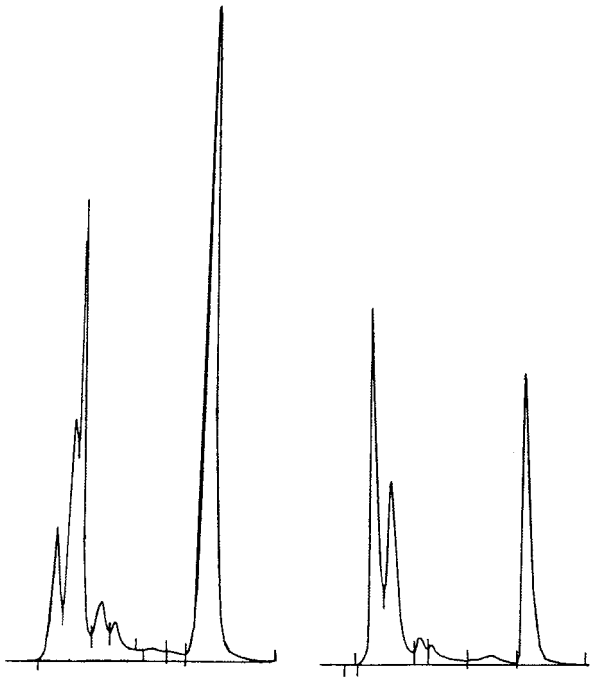

Figure 3. HPLC separation of coumarin **1** in the ethanolic (A) and in the basic aqueous extracts (B) of *Mikania glomerata*.

determinations were performed by HPLC (6–9). This extraction process was used because it is quite easy and at the same time suitable for industrial purposes. Furthermore, it must be pointed out that when the same extraction process was performed at room temperature, remarkably lower yields were always obtained (Table 1). Owing to its lower cost and toxicity, ethanol was considered as the best extraction solvent. The following step of this study was to evaluate how a preliminar treatment of the plant material could affect the overall yield of the extraction process. For this purpose, the leaves were previously dried at 50°C in a forced-ventilation oven for 45 min, and the content of coumarin in the solvent used for the extraction was compared with that detected in the same solvent when fresh leaves were used. Furthermore, the differences of coumarin content in the leaves of *M. glomerata*, collected from various areas of the State of Rio de Janeiro and in different periods of the year (October 1996 to April 1997) were evaluated for the identification of the best place and harvesting time. As reported in Table 1, the results obtained demonstrated that the fresh plant contained a higher level of coumarin compared with the dry form, indicating that the heating treatment resulted in the sublimation of part of coumarin. Furthermore, the use of raw materials collected in locations at higher altitudes, as in the cities of Vassouras and Petrópolis (RJ, Brazil), showed higher levels of coumarin. Finally, plants harvested during periods of higher temperatures (i.e., during January and February) contained higher percentages of the desired product. All the values reported in Table 1 represent the average percentages determined in January and February.

A third step of this investigation is the aim of identifying also an appropriate aqueous solvent for the extraction process to make this procedure even more feasible from

Table 1.

Evaluation of the Efficiency of the Extraction Process of Coumarin **1**, *from Mikania glomerata*

Origin	Type of Raw Material	Extraction Method	Coumarin Content (%)	Comparative Yield (%)
Campo Grande	Dry plant	Exhaustive with ethanol ref.	0.09	100
RJ-Brazil	Dry plant	Extraction with ethanol r.t.	0.03	33
	Dry plant	Extraction with hexane r.t.	0.02	22
	Dry plant	Extraction with hexane ref.	0.07	78
Jacarepagu	Fresh plant	Exhaustive with ethanol ref.	2.30	100
RJ-Brazil	Fresh plant	Extraction with ethanol r.t.	1.90	82.6
	Dry plant	Extraction with ethanol ref.	1.59	69.1
	Dry plant	Extraction with ethanol r.t.	1.35	58.9
Petrópolis	Fresh plant	Exhaustive with ethanol ref.	1.80	100
RJ-Brazil	Dry plant	Extraction with ethanol ref.	0.90	50
Vassouras	Fresh plant	Exhaustive with ethanol ref.	2.70	100
RJ-Brazil	Fresh plant	Extraction with NaOH sol. ref.	2.40	88.9
	Fresh plant	Extraction with NaOH sol. r.t.	2.00	74.1
	Fresh plant	Extraction with H_2O ref.	2.30	85.2
	Fresh plant	Extraction with H_2O r.t.	1.69	62.5

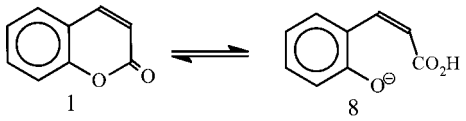

Figure 4. Base catalyzed opening of the coumarin lactonic ring.

both economical and industrial points of view. The theoretical approach to such a problem is represented by the possibility of basic catalytic opening of the lactonic ring of coumarin, in a diluted alkaline medium, leading to the ionized form of the *cis*-2-hydroxy coumaric acid, **8**, that can then again form the cyclic structure after neutralization of the medium (10–12) (Fig. 4). According to this hypothesis an aqueous basic solution was tested as extracting solvent. For this purpose, the fresh vegetal drug was used, harvested at the time and locations corresponding to the highest content of coumarin, as reported above. The extraction procedures were the same as those already standardized, replacing however the ethanol with an aqueous solution of 1% w/v sodium hydroxide. The results achieved were similar to those obtained with the ethanolic extraction. The HPLC analysis of the obtained solutions (Fig. 3) show good performance of this methodology that allows the production coumarin **1** in a selective way and with significantly high yields (Table 1) compared with the results obtained when ethanol was used as an extraction solvent. Furthermore, it must be pointed out that with the addition of diluted HCl to the aqueous basic extract, it is possible to obtain the complete precipitation of the product, with a purity level of 85% (HPLC monitoring).

A preliminar scale up of the extraction process was also carried out. For this purpose the amount of *M. glomerata* was increased to 1000 g. Similar percentages of coumarin were always obtained.

If we consider that because of the final step of precipitation, an additional purification of the product can be unnecessary in most cases, this extraction process in a basic medium appears to be remarkably less expensive compared with others or even with synthetic methods. In fact it allows for obtaining coumarin selectively, on an industrial scale, in a cost-effective manner and a reduced ecological impact.

According to the results obtained so far, it also appears possible to propose the use of this method for other plant species with even higher contents of this substance such as the *Torresea cearensis* (13) and the *Dypterix dorata* (14) as well as for the extraction of other similar lactonic systems with significant pharmacological activity. Thus, this procedure can become even more attractive in economical terms.

CONCLUSIONS

From the results obtained, it is possible to verify the feasibility of the use of *M. glomerata* as an efficient coumarin source. The proposed extractive process appears to be profitable for the production of this substance also at an industrial level. The possible use of an aqueous basic solution as an extraction solvent, the high selectivity, and relatively high yield of the extraction process, in addition to the low ecological cost of the overall procedure, showed the validity of this new methodology from different points of view. Furthermore, the possibility of changing the raw material (*M. glomerata*) to other species with higher coumarin contents increases even more the economical interest for this approach to the production o coumarin.

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