A comparison between conventional and microwave-based techniques

Extraction of Alkaloids From Species of *Senecio*

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ABSTRACT: The present work was dedicated to research into possible alternatives to the conventional Soxhlet method for pyrrolizidine alkaloids [PAs]. The intention of this work was to simplify the necessary operations and to reduce the amount of time required to perform them, without compromising the final results.

A new technique was studied for the extraction of pyrrolizidine alkaloids from species of *Senecio*, by means of closed containers subjected to controlled microwave radiation. The results which were obtained were extremely encouraging, in both qualitative and quantitative terms.

INTRODUCTION

Samples were taken of various aliquot quantities of 25 grams of dried plants of the species *Senecio paludosus* and *Senecio cordatus*. These samples were air-dried and crumbled into coarse fragments. Various aliquot quantities of *Senecio paludosus* and *Senecio cordatus* were placed in Soxhlet for extraction with methanol for a period of approximately four hours.

Meanwhile, other aliquot quantities of both species were subjected to extraction with methanol in closed containers lined with PFA and placed in a microwave unit. The operating procedures for the microwave treatment, which are indicated in Table 1 below, involved time periods ranging from 20 to 30 minutes.

Table 1.—Extraction method using methanol in a Cem MDS 2000 microwave unit, with a temperature regulator for the "P"-type alkaloids.

Type of sample	Methanol (in ml)	Controlled temperature	Maximum pressure	Number of successive extractions
1—*Senecio paludosus*	25 per extraction	65° C	16 PSI	3 x 10'
1 bis—*Senecio paludosus*	50	100° C and 80° C	68 PSI	1 x (15' at 100° C plus 5' at 80° C)
2—*Senecio cordatus*	25 per extraction	65° C	16 PSI	3 x 10'
2 bis—*Senecio cordatus*	50	100° C and 80° C	68 PSI	1 x (15' at 100° C plus 5' at 80° C)

Sampling conditions

The extract from each source was evaporated under a vacuum until dry. The residue was suspended in 2.5 percent hydrochloric acid and was then washed with diethyl ether and chloroform.

Half of the aqueous phase was found to be basic with a 25 percent solution of ammonium hydrate and was then subjected to extraction with dichloromethane. The same organic substance was then treated again in succession with 2.5 percent hydrochloric acid and 25 percent ammonium hydrate, and was then extracted again with dichloromethane. The resulting solution in dichloromethane was dried on anhydrous sodium sulfate and evaporated until dry.

In order to verify the presence of PA [i.e., pyrrolizidine alkaloid]-N-oxides, the second half of the solution, as derived from the washing with diethyl ether and chloroform, was reduced with zinc powder over the space of one full night. The resulting liquid was filtered and then subjected to the treatment described above. All of the dried residues were then weighed and dissolved in appropriate quantities of dichloromethane in order to obtain concentrations that were compatible with the CGC and CGC/MS analyzers.

Gas chromatography analyses

The introduction of a quantity of 1 µl of PA extract was performed after dilution with 1:250 dichloromethane. The analyzer was utilized under the conditions described below:

Transport gas:	Hydrogen
Flow rate:	3 ml per minute
Injection system:	Split
Split ratio:	1:30
Injector temperature:	300° C
Detector:	Flame ionization
Temperature:	300° C
Temperature of the column:	Programmable, from 120° C (for 1 minute) to 280° C (for 20 minutes) at a rate of 3° C per minute
Column:	Length, 30 m; inside diameter, 0.32 mm; melted silica capillary coated with 0.3 µ of OV-1

Gas chromatography/mass analyses

These analyses used the same capillary column as described above, under the same operating conditions with the exception of the transport gas, which in this case was helium at a flow rate of 1.6 ml per minute. The PA species were identified by means of a comparison against the spectra of pure samples and also against the spectra reported in the literature. All of the mass spectra were recorded sequentially, consistent with the gas chromatographic separation.

Results

Figures 1, 2, and 3 below show the chromatograms that were obtained for *Senecio paludosus* by means of extraction with Soxhlet and by means of single or triple extraction through the use of microwaves. Figure 4, 5, and 6 below show the analytical diagrams that were obtained for *Senecio cordatus* by means of extraction with Soxhlet and by means of single or triple extraction through the use of microwaves.

Conclusions

A comparison of the gas chromatography results obtained for the extracts which were obtained by means of the so-called "classical" technique (i.e., the Soxhlet method) and the microwave system with internal temperature control makes it possible to verify that the qualitative and quantitative composition of the respective extracts are completely analogous.

It is evident that the microwave method, as examined here, displays the noteworthy advantage of drastically reducing the time that is required to extract the PAs from the vegetable matrix, i.e., from four hours to less than half an hour. A further advantage of this method resides in its lower consumption of methanol during the extractive operations. It is probably that the noteworthy effectiveness of the microwaves with regard to the extraction yield is due to the nature of the microwave radiation itself, which not only succeeds in activating the molecules of the solvent, but also has an activating effect on the molecules of the pyrrolizidine alkaloids. In this regard the adjustment of the radiation, as achieved by controlling the temperature within the reaction vessels, appears to have major significance in connection with the reproducibility of the results.

Instruments and reagents

- A Cem MDS 2000 microwave unit with adjustable settings for the pressure and temperature inside the reaction vessel.
- Containers lined with Cem PFA Teflon (LDV, 100 ml) for reactions and extractions, utilizable at up to 200 PSI.
- A C. Erba Mega 5360 gas chromatography unit.
- A Hewlett-Packard 5988-A GC/MS gas chromatography/ mass spectroscopy unit.
- Reagent grade methanol for chromatography.
- Reagent grade hydrochloric acid, diluted with twice-distilled water to a concentration of 2.5 percent.
- Reagent grade diethyl ether.
- Reagent grade chloroform.
- Reagent grade ammonium hydrate, diluted with twice-distilled water. to a concentration of 25 percent.
- Reagent grade dichloromethane.
- Reagent grade anhydrous sodium sulfate salt.
- Reagent grade metallic zinc powder.

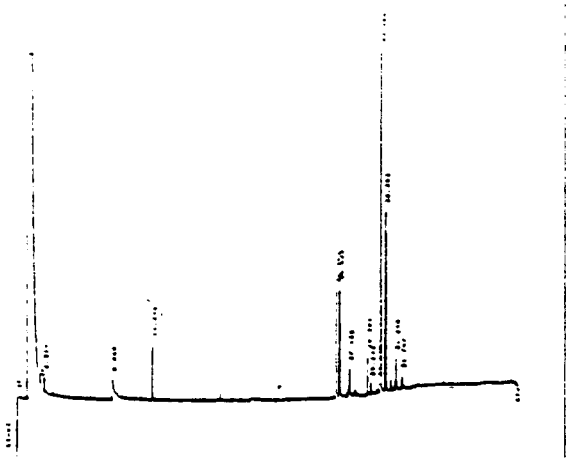

Figure 1. —*Senecio paludosus*: extraction with Soxhlet.

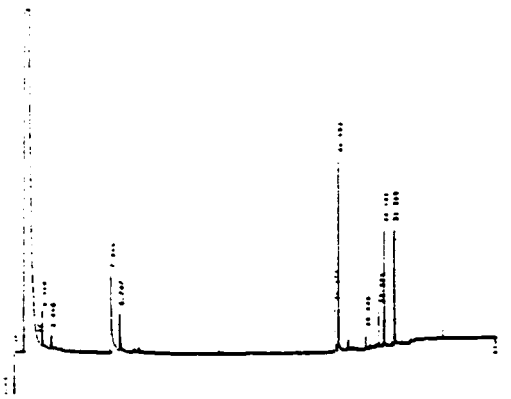

Figure 2.—*Senecio paludosus*: single extraction by means of microwaves.

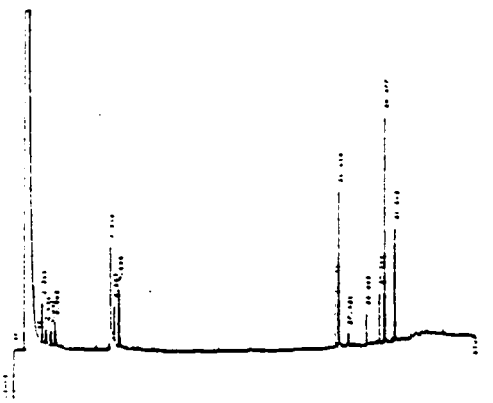

Figure 3.—*Senecio paludosus*: triple extraction by means of microwaves.

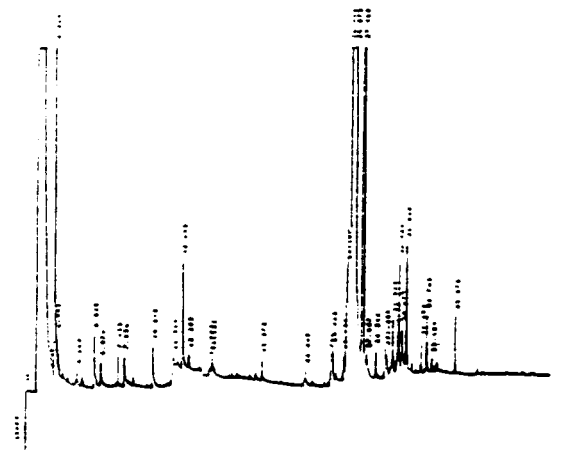

Figure 4.—*Senecio cordatus*: extraction with Soxhlet.

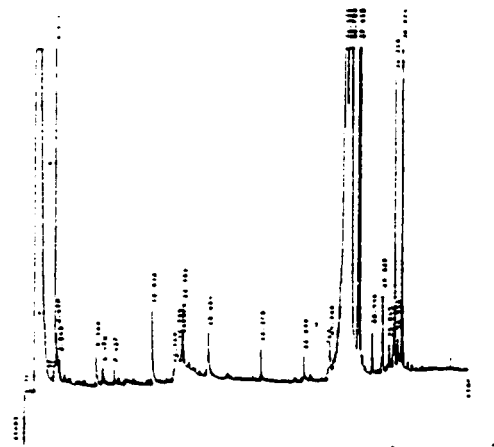

Figure 5.—*Senecio cordatus*: single extraction by means of microwaves.

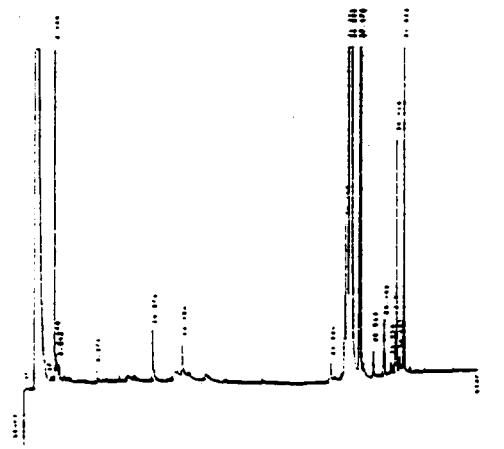

Figure 6.—*Senecio cordatus*: triple extraction by means of microwaves.