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Comparison of conventional and ultrasonically assisted extractions of pharmaceutically active compounds from *Salvia officinalis*

M. Sališová [a], Š. Toma [a,*], T.J. Mason [b]

[a] *Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska dolina CH-2, SK-842 15 Bratislava, Slovak Republic*

[b] *Sonochemistry Centre, School of Natural and Environmental Sciences, Coventry University, Coventry, CV1 5FB, UK*

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Abstract

Conventional as well as ultrasonically assisted extractions of biologically active compounds from *Salvia officinalis* using 65% ethanol have been studied. Cineole, Thujone and Borneol were used as standards for the GLC–MS evaluation of the extracts. The effect of temperature, stirring and mode of sonication (ultrasonic bath or horn system) have been studied. The results indicate that ultrasonically assisted extraction with mechanical stirring at room temperature in a period of 12 h produces a substantial improvement over conventional methodology. © 1997 Elsevier Science B.V.

Keywords: Ultrasound; Extraction; *Salvia officinalis*

1. Introduction

References to the introduction of ultrasound at various stages of conventional extraction processes of different parts of plants have been known for more than three decades [1–32]. In the majority of cases positive effects of ultrasound have been reported although on occasion some degradation of the expected products has been observed [6,8,27].

Our research activities are closely connected with the research program of a small company in Slovakia, Mediplant Modra. For them the extraction of pharmacologically active compounds from *Salvia officinalis* is of great interest, since it has been shown that tincture of *Salvia officinalis* can be effectively used for the treatment of a range of oral conditions. These include inflammation of the mouth and pharynx after tonsillectomy and other surgical operations in the oral cavity, in the reduction of halitosis and in the treatment of chronic atrophic laryngitis. It has also been used in the supportive treatment with concomitant antibiotics of streptococcal angina [33,34]. A summary of the possible use of different herbal extracts in medicine can be found in several publications, one of the most recent being the book by Wijesekera [35].

The extraction of pharmacologically active compounds from *Salvia officinalis* by conventional methods (maceration, percolation) is very time consuming taking between 1 and 2 weeks. The main goal of the present work was to find out whether utilization of ultrasound could shorten this extraction time.

2. Experimental

2.1. Experiments involving the use of ultrasound

Sonochemical experiments were carried out in a 4 l ultrasonic cleaning bath (model UC 006 Dml, Teson, Vráble, 37–42 kHz, 130 W) and using an ultrasonic horn (model UUA 001, Ultragen, Nitra, 20 kHz working frequency, 300 W), the horn was operated on a 50% cycle. The efficiency of the sonication was characterized by measurement of cavitation using the KI–KI_3 test in water [36]. After 3 min sonication of 50 cm^3 of 1 M aqueous KI solution, the yield of KI_3 was 4.7×10^{-6} mol dm^{-3} and 5.1×10^{-6} mol dm^{-3}, respectively.

2.1.1. General procedure for extraction of Salvia officinalis (herba sativa)

To 15 g of dry *Salvia officinalis* (cut to about 1 cm pieces) in a 250 cm^3 Erlenmeyer flask, 125 cm^3 of 65%

* Corresponding author. E-mail: tonma@fns.uniba.sk; fax: +421-7-729-064

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ethanol was added and the flask was partially immersed in the ultrasonic bath. Temperature in the bath was controlled using a submerged cooling coil. Ultrasound was applied for 12 h and aliquots of extract (5 cm^3) were withdrawn by pipet periodically for gas chromatography (GC) analysis (see Tables 1–5). The mixture was then allowed to stand undisturbed at room temperature for 7 days (from the start of experiment) after which time the mixture was filtered and the last sample for GC was withdrawn from the filtrate.

In some experiments mechanical stirring of the reaction mixture was used in addition to sonication. For these experiments the last sample was withdrawn from the filtrate after standing for only 12 h (i.e. 24 h from the beginning of the experiment – see Table 4). The crude, almost-dry herb was transferred again to the Erlenmeyer flask and a fresh quantity of 125 cm^3 of 65% ethanol was added. The mixture was sonicated and stirred again and after 12 h a sample was withdrawn for GC analysis (36 h from the beginning of the experiment – see Table 4). After standing overnight (10 h), the extract solution was again completely removed by filtration. Once again a fresh quantity of 125 cm^3 of 65% ethanol was added to the remaining crude, almost-dry herb and the mixture was sonicated as before. After a further 10 h a sample was withdrawn for GC analysis (56 h from the beginning of the experiment – see Table 4).

Extraction incorporating an ultrasonic horn was used on the same quantity of herb in a 250 cm^3 beaker to which 125 cm^3 of 65% ethanol was added and the horn was submerged about 2–5 mm under the surface of the solution. At the beginning of the extraction the mixture was cooled to 8°C and the maximum temperature reached at the end of the sonication was 33°C (see Table 5).

Table 1
Extraction at 20°C

T (h)	Cineole (mg kg^{-1})		Thujone (mg kg^{-1})		Borneol (mg kg^{-1})	
	U	C	U	C	U	C
1	9.8	7.5	63.9	48.2	4.3	3.5
3	13.0	10.3	81.6	63.6	4.3	3.8
5	14.1	13.4	98.9	73.0	4.2	4.3
12	22.3	16.0	140.1	91.3	6.0	4.7
7 days	40.3	43.3	258.2	269.2	7.2	7.6

U: extraction with application of ultrasound.
C: control experiment, without ultrasound.

Table 2
Extraction at 30°C

T (h)	Cineole (mg kg^{-1})		Thujone (mg kg^{-1})		Borneol (mg kg^{-1})	
	U	C	U	C	U	C
1	9.3	6.8	67.1	52.8	2.9	2.5
3	16.5	10.0	118.1	71.7	3.6	3.0
5	22.0	13.6	145.5	89.2	3.9	3.3
12	30.6	18.6	201.5	127.8	4.7	3.7
7 days	36.4	39.4	250.4	247.3	4.9	4.6

U: extraction with application of ultrasound.
C: control experiment, without ultrasound.

Table 3
Extraction at 50°C

T (h)	Cineole (mg kg^{-1})		Thujone (mg kg^{-1})		Borneol (mg kg^{-1})	
	U	C	U	C	U	C
1	19.0	14.4	119.0	88.9	3.6	3.4
3	33.3	27.4	208.5	179.9	4.7	4.2
5	34.6	33.8	209.1	183.2	4.4	4.6
12	35.4	34.4	211.4	208.3	4.4	4.5
7 days	34.9	33.9	219.8	224.1	5.0	4.9

U: extraction with application of ultrasound.
C: control experiment, without ultrasound.

Table 4
Extraction at 20°C with mechanical stirring

T (h)	Cineole (mg kg^{-1})		Thujone (mg kg^{-1})		Borneol (mg kg^{-1})	
	U	C	U	C	U	C
1	14.4	13.1	95.0	84.4	5.3	3.8
3	22.7	14.0	141.9	113.9	6.3	5.3
5	27.5	19.1	185.6	127.1	7.9	6.1
12	35.8	27.9	224.8	176.6	5.6	6.5
24	33.4	29.0	232.9	192.3	8.8	8.3
With new alcohol						
36	7.4	7.4	49.5	49.3	1.8	1.6
With new alcohol						
56	1.6	2.1	10.1	14.6	0.6	1.2

U: extraction with application of ultrasound.
C: control experiment, without ultrasound.

Table 5
Extraction at 8–33°C using the horn

T (h)	Cineole (mg kg^{-1}) U	Thujone (mg kg^{-1}) U	Borneol (mg kg^{-1}) U
1	22.3	141.2	5.3
2	24.3	167.2	5.8

U: extraction with application of ultrasound.

2.2. Control experiments

These experiments were set up using the same proportions as those above but without sonication. Reactions

at higher temperatures were achieved using a thermostat and in some cases the reaction mixture was mechanically stirred (see Table 4).

2.3. GC measurements

An HP 5890 SERIES II gas chromatograph equipped with a HP 7673 autosampler, a flame-ionization detector and 10 m × 0.32 mm i.d. fused-silica capillary column (Ultra-2, HP, 0.17 μm film) was used for the chromatographic separation, and was interfaced with an HP–Chem data system (Hewlett-Packard, Palo Alto, CA). The gas flow rates (head pressure) for Helium carrier gas were 40 kPa. After injection (0.5 μl sample), the temperature programme involved an initial temperature of 40°C, then an increase to 80°C at 5°C min^{-1} and a final hold for 1 min. The injection port temperature was 250°C and the detector temperature was 250°C. Quantification was performed by comparison with a reference using Fenchone as internal standard.

Ultrasonic and control experiments were evaluated on the amount of extracted active compounds (Cineole, Thujone and Borneol) which had been used as standards. The precision of quantitative measurements of analytes have been expressed as relative standard deviations, depending on the concentration of analyzed compounds. This value, on average, is 4.2% for Cineole, 3.1% for Thujone and 8.3% for Borneol. At every temperature and under the different conditions used, two or three parallel experiments were running and every sample was injected four times. The mixture of standards were shown to be stable under the conditions of the experiment by sonication over a period of 12 h.

3. Results and discussion

The results of the experiments are summarized in Tables 1–5. From the results of extraction performed at 20°C it can be seen that applying sonication for 5 or 12 h resulted in raising the content of biologically active compounds in the extracts by approximately 40% in comparison with the control (silent) experiments (Table 1). Conducting the extraction at 30°C resulted in more pronounced effect of ultrasound. The content of biologically active compounds in this case was approximately 60% higher under the influence of ultrasound (Table 2). However, the differences between ultrasound mediated and silent extraction were found to be negligible when extractions were carried out at 50°C (Table 3).

The results in Table 4 (conventional and ultrasonic extraction using mechanical stirring) show that after 5 h there was approximately 45% more biologically active compounds in the extracts produced under sonication. The improvement induced by sonication reduced to a value of 26% after 12 h. The last entry in Table 4 shows that the maximum of bioactive compounds extractable from *Salvia officinalis* (after repeated extraction) was approximately: 41 mg kg^{-1} of Cineole, 292 mg kg^{-1} of Thujone and 11 mg kg^{-1} of Borneol. The amounts of active compounds extracted into the third portion of fresh ethanol were almost negligible. This explains why there was essentially no difference between ultrasound mediated and silent extraction after 7 days (Tables 1 and 2) or 12 h (Table 3).

4. Conclusions

From the above it can be concluded that ultrasound, introduced via an ultrasonic bath, promotes the extraction of bioactive compounds from *Salvia officinalis* and that the optimum conditions are sonication, without mechanical stirring, at 30°C for 12 h. A similar result (nearly complete extraction of active compounds) can also be achieved by performing extraction at 20°C in the ultrasonic bath with the addition of mechanical stirring. Much better results have been achieved using the ultrasonic horn where nearly 60% of active compounds can be extracted after 2 h sonication. The only difficulty encountered using this technique, however, was the inability to accurately control the extraction temperature.

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