ELSEVIER

Ultrasonics Sonochemistry 4 (1997) 135–139

Ultrasonics SONOCHEMISTRY

The use of ultrasound for the extraction of bioactive principles from plant materials

M. Vinatoru [a,*], Maricela Toma [a], Otilia Radu [a], P.I. Filip [a], D. Lazurca [b], T.J. Mason [c]

[a] *'Costin D. Nenitzescu' Institute of Organic Chemistry, P.O. Box 15 - 258, 71141 Bucharest, Romania*
[b] *R.A. Plafar, Filiala Brasov, Michael Weiss Street 3, 2200 Brasov, Romania*
[c] *Coventry University, School of Natural and Environmental Sciences, Priory Street, Coventry, CV1 5FB, UK*

Received 10 November 1996

Abstract

The paper presents our results concerning the ultrasonically assisted extraction of bioactive principles from plant material. A comparison with classical methodologies is presented and technological aspects of ultrasonically assisted extraction are discussed.

Keywords: Ultrasonic extraction; Essential oils; Dill; Fennel; Marigold; Arnica; Gentian; Camomile; Sage

1. Introduction

The use of plants to assist in the healing of wounds and the curing of disease is an ancient preoccupation of mankind. The history of essential oils began in the East in countries such as Egypt, Persia and India. Egyptian papyruses there are thousands of herbal remedies in which coriander and Castor-oil plant were used. Hebraic and Chinese manuscripts describe the medicinal benefits of over 2000 plants, offering details that are still useful today. During the Greek and Roman empires the therapy using the plants underwent considerable expansion [1–3]. As early as the 17th century apothecaries were using liquorice extracts to treat 'inflamed stomachs' just as the ancient Chinese had done centuries before. In Romania, the use of plants in medicine is also an ancient tradition. It was not however until the 19th century a variety of vegetable products were introduced in the Romanian pharmacopoeia. In 1904 the first Institute of Medicinal Plants was established in Cluj, Romania, being one of the first in the world [1]. The subsequent commercial exploitation of the production of essential oils and plant extracts began in 1940. It is estimated that from over 3400 species of flora growing in Romania more than 700 may be considered to be medicinal and aromatic plants. Many of these plants have been used for the preparation of natural materials of use for the control of agricultural pests [4].

The current world-wide interest in such traditional medicines derived from plants has been revived with the identification of an increasing number of new extracts, previously little known to Western scientists [5]. Access to such plant components depends strongly on the extraction method used and these are mostly little different from those employed in history. More modern techniques like the use of ultrasound are not yet in common usage and also not widely explored. The research reported herein is aimed at the optimisation of extraction procedures.

Often the medicinal efficacy of a plant extract does not rely on one main bioactive component but rather on a combination of the main component with small amounts of other components that have a synergistic effect. Therefore in order to obtain the most potent extract it is necessary to take into account the plant material, the solvent used for extraction and the extraction procedure employed. The existing classical techniques used to obtain bioactive extracts from plants include:

(i) direct distillation of essential oils;
(ii) water steam distillation of essential oils;
(iii) organic solvent extraction of organic compounds;
(iv) maceration with alcohol–water mixture [6];
(v) extraction with cold fat (enfleurage);
(vi) extraction with hot fat (maceration), etc. [7].

* Corresponding author. Fax: +40-1-312-1601

1350-4177/97/$17.00
PII S1350-4177(97)00018-7

Among the newer techniques used in extraction technology, the ultrasonically assisted extraction of oils [8] and other plant components has been explored. In previous papers [9,10] several aspects of ultrasonically assisted extraction of tea and the seeds of dill and fennel were described and some aspects of the influence of ultrasonic field discussed. In this paper we present more details on the possibilities of obtaining extracts at low temperatures using ultrasound as the source of energy.

2. Results and discussion

Not all of the classical extraction processes are suitable for ultrasonic enhancement. The main procedures leading to bioactive products from plants and its constituents (seeds, flowers, leaves, etc.) are: percolation, maceration, water steam distillation, Soxhlet extraction, infusion and boiling. The water steam distillation to produce essential oil for example is not amenable to ultrasonic enhancement but extraction with light solvent (e.g. petroleum ether) or with water or water–alcohol extracts (maceration) are possibilities. These methods lead to the types of extract suitable for cosmetics, pharmaceuticals as well as for food industry.

It is also necessary to establishing the appropriate place for the application of ultrasound in an extraction plant. The general operation unit scheme for solvent extraction is presented in Fig. 1.

The logical place for an ultrasonic device is in the solvent extraction unit. In the case of aqueous or alcoholic solvent this may be an ultrasonic cleaning bath or a closed reactor fitted with a horn transducer. The later type of unit could also be employed for volatile solvents like petroleum ether.

3. Experimental details

The experiments concerning ultrasonically assisted extraction were carried out in three ways:

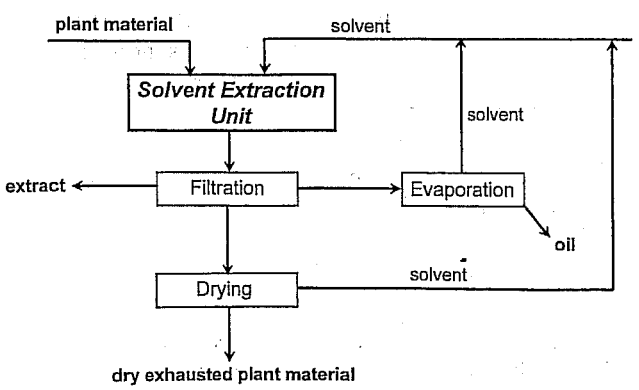

Fig. 1. General scheme for a solvent extraction unit.

(i) indirect sonication using a small cleaning bath [11];

(ii) direct sonication using small and large cleaning bath [12];

(iii) direct sonication using an ultrasonic reactor provided with a horn [13].

In all these procedures additional agitation or shaking was employed, to avoid standing waves or the formation of solid free regions for the preferential passage of the ultrasonic waves.

The solvents used in these experiments were: petroleum ether and aqueous or neat ethanol. The petroleum ether extracts would normally be used for the isolation of essential oils (after solvent removal) while the alcoholic extracts lead to tinctures.

Samples of the extracts were analyzed after different extraction times to follow the progress of the extraction. The mass balance of petroleum extracts was performed, while the alcoholic extracts were analyzed for the content of dry residue (according to the Romanian Pharmacopoeia [14]). For the classical (model) procedures the extracts were analyzed at the end of extraction time.

4. The classical (silent) procedures

Extractions using petroleum ether: For extractions from crushed seeds, leaves or entire plants involved placing samples (100 g) into a Soxhlet apparatus containing 500 cm^3 petroleum ether (boiling range 40–60°C). The extraction was continued until a sample of solvent contained only traces of plant constituents after passing through plant material [this was followed using thin layer chromatography (TLC) and gas-chromatography]. Under these conditions the total extraction time required was 4 hours for all plants investigated.

Extractions using alcohol: For ethanol (and its aqueous mixtures) a maceration procedure was employed, and the extraction time in this case was between 7 and 14 days, depending of plant type.

5. Extraction with petroleum ether using ultrasound

In the case of *indirect sonication* plant material and solvent were placed into an Erlenmeyer flask (1000 cm^3) fitted with a condenser. The flask was immersed into the cleaning bath, at four centimetres distance from the bottom of the tank, sonicated and shaken periodically. The temperature was kept constant (25°C) by a cooling coil immersed into the bath. Direct sonication was performed in a closed reactor, fitted with condenser, mechanical stirrer, ultrasonic horn, thermometer and cooling jacket. Crushed seeds, leaves or

the entire plant (100 g) were extracted with 500 cm^3 petroleum ether via indirect sonication for 0.5 hours and the yields were recorded after filtration and evaporation of solvent. In the case of petroleum ether extension of the sonication time did not produce any significant increase in the yield of essential oils.

In the *direct sonication* experiments crushed seeds, leaves or the entire plant (125 g) were extracted with 1250 cm^3 petroleum ether for 0.5 hours using an ultrasonic power of 5 W cm^{-2}. Here again the oil was obtained after filtration and evaporation of the solvent.

A comparison between the classical and ultrasonically assisted extraction of crushed dill seeds (granulation 40–60 mesh), is given in Table 1. In each case the yield of oil is based on 100 g of dill seeds.

It is clear that ultrasound improved the yield of oil which was obtained in a substantially shorter time even in the case of indirect sonication. It is also important to note that both indirect and direct sonication gave much lower amounts of heavy components (mainly waxes). This is probably the result of the shorter extraction times used in the ultrasonic processes.

The most probable mechanism for the ultrasonic enhancement of extraction is the intensification of mass transfer and easier access of the solvent to the cell material of the seeds. Although cavitational collapse in petroleum ether does not produce high energies it would be expected that it would still produce some cell disruption together with a good penetration of the solvent into the cells, through the ultrasonic jet. This is in accord with the greater yield of oil through direct rather than indirect sonication since much greater ultrasonic power is introduced in the former process. In the classical procedure the mechanism is via normal diffusion through the cell walls – a process which requires substantially longer extraction times.

6. Extractions with ethanol (neat and aqueous) using ultrasound

In these extractions the plant material (usually dry and crushed) was sonicated using only direct methods (employing either a cleaning bath or a probe system for 120 min). Very few plant materials required more than two hours of sonication. After sonication (and after the control experiments using classical extraction obtained by mixing) the mixture of plant material and solvent were allowed to stand (maturate) for 18 hours. A comparison of classical and sonic extraction procedures leading to tinctures, for six plants is given in Table 2.

Table 1
Comparison between classical and ultrasonically assisted extraction of dill seeds

Entry	Oil amount [g]	Method used	Extraction time [h]	Heavy compounds [%]
1	3.0	Soxhlet	4.0	11.95
2	3.4	us (indirect)	0.5	3.14
3	3.6	us (direct)	0.5	3.06

In total over 50 other plants have been investigated to obtain tinctures and comparative studies were performed. (A portion of each of these extracts was used for screening for potential use in the treatment of a potato disease and this will be the subject of a future publication.) The amount of dry residue obtained from the extracts depends strongly on the plant material. Thus mint leaves show only a small amount of extraction in the first half hour whereas the rest of the plants showed a rapid extraction within 15 minutes. This can be explained through the fact that mint leaves cells cannot be destroyed by simply a crushing process. Ultrasonic disruptions of the cell walls thus take some time (~30 min) after which time the release of cell content is much more rapid. Nevertheless, for all ultrasonically assisted extractions the yield from the dry residue after maturation increases. This suggests that the diffusion process continues after sonication. In almost all of the ultrasonic cases the amount of extract is similar or greater compared with the classical technique. The ultrasonic procedure thus seems to be a significant improvement when extraction time is taken into account. When the process was scaled up in a large ultrasonic cleaning bath (10 litres solvent and over 1000 g of plant material) a similar trend in extraction efficiency was observed.

Direct sonication method were performed on a scale of 1250 cm^3 solvent (70% ethyl alcohol 30% water) and 125 g plant material. The amount of dry residue after sonication and maturation is presented in Table 3 in the case of marigold flowers.

Standard (silent) extraction (4 hours, agitation, room temperature) was compared with the ultrasonically assisted method (0.5 hours, agitation, room temperature) in 96% ethyl alcohol for milled seeds of coriander, fennel and dill. The extracts were analyzed for dry residue and grease content. The latter estimation was accomplished by cooling the extract to between 0–4°C to precipitate the grease so that it could be removed by filtration. The results are presented in Table 4.

Once again the use of ultrasound improves the extraction in all cases and this is accompanied by a reduction in the amount of grease. This can be attributed to an increase in the selectivity of extraction produced by ultrasound through:

(a) a shorter extraction time;

(b) a reduction in the diffusion process which yields grease;

(c) an acceleration of the extraction process for low molecular weight compounds.

It is possible that sonochemical destruction of the walls of cells containing the volatile compounds is

Table 2
Dry residue (g/100 g extract) obtained by direct sonication in a cleaning bath

Sonication time [mins]	Mint	Camomile	Marigold	Sage	Arnica	Gentian
15	0.06	1.10	0.94	0.58	0.36	–
30	0.07	1.30	0.98	0.80	0.42	1.67
60	0.25	1.43	1.14	0.92	0.67	2.66
90	0.78	1.56	1.33	0.94	1.06	2.71
120	0.82	1.79	1.75	1.13	1.20	3.24
180	–	1.80	–	–	–	–
18 h maturation	0.91	1.91	2.20	1.15	1.50	4.68
Classical 7 days + 14 days maturation	1.02	1.73	2.25	1.02	1.75	4.75

Table 3
Dry residue (g/100 g extract) obtained by direct sonication using a probe system

Sonication time [min]	Marigold
15	–
30	1.15
60	1.74
90	1.97
120	2.25
180	–
After 18 h maturation	2.25
Classical 7 days + 14 days maturation	2.25

somewhat easier than disruption of the walls of grease cells. During this process volatile components inside the cells could produce local cavitation bubbles that contribute to disruption of the walls from the inside – a process which is far less likely with involatile grease.

The experimental set-up which was used for each of the ultrasonic extraction methods is presented in Fig. 2.

An analysis of the methods used reveal that the most effective mode of sonication is via the closed reactor fitted with a horn transducer although it is not easy to adapt such a system for pilot or industrial scale plant. When using cleaning bath system on a large scale a number of precautions are necessary. Firstly plant material agglomerates in regions within the bath so that only the outside of the material 'clump' is exposed to the ultrasonic waves. Sometimes the material sinks or, in the case of a soft plant such as camomile, it will float on the surface of the solvent. These clumps will absorb the ultrasonic energy and this can result in heating. These effects can be counteracted by the use of efficient mechanical stirring. However the speed of the stirrer has to be correctly set in order to provide efficient mixing while at the same time avoiding any decoupling effects of the ultrasonic transducers from the rapidly stirred solvent.

Since we have employed ethanol as solvent its stability under sonication was checked by gas-chromatography coupled with mass spectrometry and by monitoring possible changes in its electrical conductivity. For these experiment we have employed a Langford small ultrasonic cleaning bath in which an Erlenmeyer flask (250 cm^3) containing 75 cm^3 ethanol or aqueous ethanol mixture was immersed and temperature maintained at 25°C. In all cases a small change in electrical conductivity was observed but the GC-MS analyses (detection limits 1–2 ppm) did not reveal any chemical modification in alcohol–water mixtures between 96 and 50% by volume. (At concentrations below 50% alcohol an oxidative process was observed and ethanal was detected.)

7. Conclusions

These experiments demonstrate that the use of ultrasound in solvent extraction involving petroleum ether

Table 4
Dry residue and grease content obtained by direct sonication using a probe system

Sonication time [min]	Coriander		Fennel		Dill	
	Dry residue %	Grease %	Dry residue %	Grease %	Dry residue %	Grease %
5	0.34	0.132	0.56	0.250	–	–
15	0.45	0.146	0.77	0.278	0.26	0.123
30	0.61	0.181	0.88	0.286	0.27	0.115
Classical	0.53	0.410	1.27	0.324	0.22	0.119

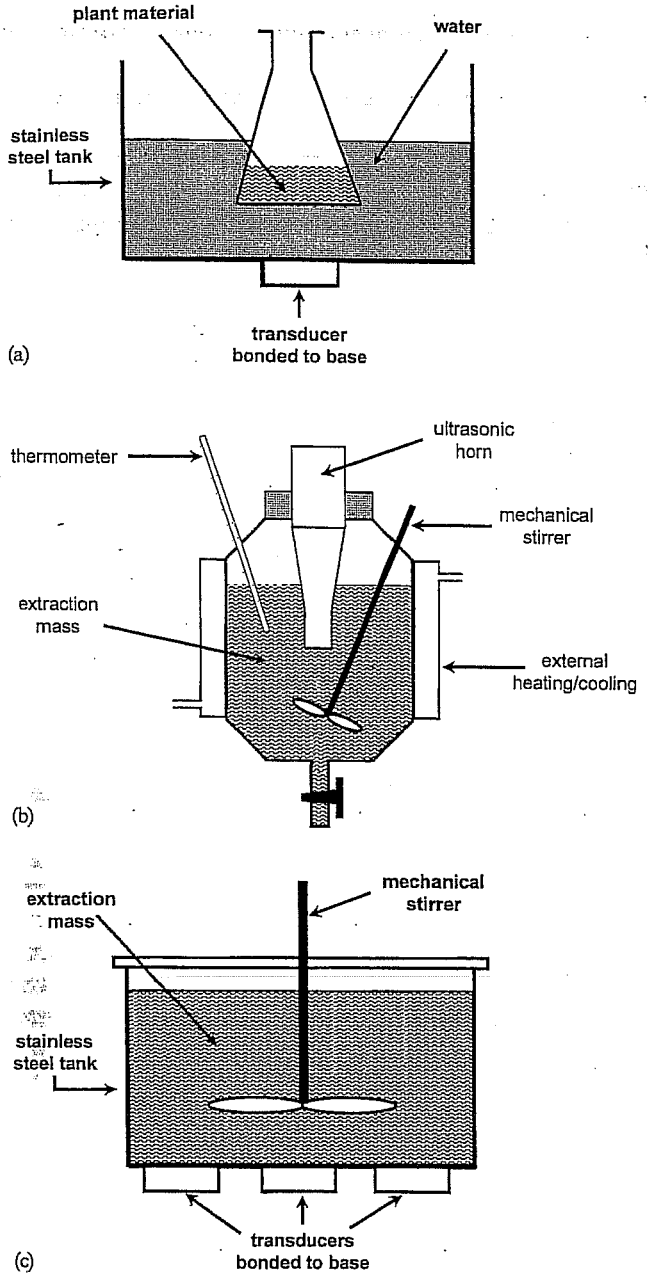

Fig. 2. (a) Indirect sonication using an ultrasonic bath. (b) Direct sonication using an ultrasonic horn. (c) Direct sonication using an ultrasonic bath.

or alcohol produces a greater yield. In the case of ethanol the temperatures used may suggest that ultrasonic technology could provide a safer procedure.

Acknowledgement

Research was performed under a Grant from the European Commission – COPERNICUS research program ERB-CIPA-CT94-0227. We also thank the Ministry of Research and Technology, Romania (Grant No. 921-96), for partial financial support.

References

[1] E. Paun, A. Mihalea, A. Dumitrescu, M. Verzea, O. Cosocariu, A Treatise of Cultivated Herbs and Aromatic Plants, Vol. I and II, Romanian Academy Publishing House, Bucharest, 1986.
[2] I. Ciulei, E. Grigorescu, U. Stanescu, Medicinal Plants, Phytochemistry and Phytotherapy, Medical Publishing House, Bucharest, 1993.
[3] V. Ceausescu, Gh. Radoias, T. Cadariu, Flavouring and Aromatics, Chemistry, Technology, Applications, Technical Publishing House, Bucharest, 1980.
[4] M. Grainge, S. Ahmed, Handbook of Plants with Pest-Control Properties, Wiley, New York, 1987.
[5] D.A. Lewis, Chemistry in Britain 28 (1992) 141.
[6] S. Ionescu, E. Savopol, Vegetal Pharmaceutical Extracts, Medical Publishing House, Bucharest, 1977.
[7] E. Guenther, The Essential Oils, Van Nostrand, New York, 1950.
[8] A. Schmall, Schweiz. Brau. Rundsch. (1953) 1; cf. Chem. Abstr. 47 (1954) 2932d; D. Thomson, D.G. Sutherland, Ind. Eng. Chem. 47 (1955) 1167; H. Rothbaecher, E. Rothbaecher, Farmacia (Romania) 18 (1) (1970) 39.
[9] T.J. Mason, Y. Zhao, Ultrasonics 32 (1994) 375.
[10] M. Vinatoru, D. Stilpeanu, S. Velea, M. Petcu, M. Vija, G. Bacneanu, C. Brinzan, Tehnologie Chimiche (Italy) 1 (1996) 76.
[11] Langford Sonomatic T75 (1.5 litres, 100 W power).
[12] Langford Sonomatic 2800 (26 litres, 600 W power).
[13] The UNDATIM Sonoreactor was used for extraction (1.5 litres, and 12 W/cm2 power) with additional stirrer.
[14] Romanian Pharmacopoeia, Medical Publishing House, Bucharest, 1993; 10th edn.