*TEKA Kom. Mot. Energ. Roln. – OL PAN, 2008, 8, 89–98*

# THE USE OF PULSATED ACOUSTIC FIELD FOR EXTRACTION OF BIOACTIVE COMPOUNDS FROM ROOTS OF VALERIAN (VALERIANA OFFICIANALIS L.)

Zbigniew Kobus, Tomasz Guz, Elżbieta Kusińska

Department of Food Engineering and Machinery, University of Life Science in Lublin,
Doświadczalna 44, 20-236 Lublin, phone: (081) 461 00 61 w.147, e-mail: zbigniew.kobus@up.lublin.pl

**Summary.** The paper presents results concerning the ultrasonically assisted extraction of bioactive compounds from roots of valerian. To lower the speed of the medium heating, pulsed ultrasonic field was applied. The investigation was accomplished by varying irradiation time, sonic power and size particles. The results were compared with classic (silent) extraction. The following were evaluated: swelling coefficient, yield of extraction and dry matter content in residue. The utilization of pulsed ultrasound proved to be a more efficient technique than the classic method of bioactive components extraction from dried valerian roots.

**Key words**: pulsed ultrasonic field, extraction, dry matter, valerian.

## INTRODUCTION

Valerian is one of basic and widely cultivated herbal plant in Poland. Its production exceeds 1000 tons a year [Jambor 2001]. The main parts of valerian used to obtain herbal extracts are rhizome and roots. They contain valepotriate compounds, valerosidatum, valerenic acid, volatile oils and others [Fernandez *et al.* 2004]. Valepotriates (valeriana-epoxy-triesters) are products that, from a chemical point of view, belong to the iridoids. Valepotriates are instable compounds and they decompose in temperatures above 35°C or under acid or alkaline in water [Bos *et al.*, 2002].

Because of rich composition the valerian roots have a very broad application. Firstly, they cause sedation and relieve insomnia [Fernandez *et al.* 2004]. Secondly, the preparations from valerian show spasmolytic acting and they are used to treat systolic states of stomach and vegetative neurosis [Leśniewicz *et al.* 2006]. Thirdly, the root oil is also used in perfumery and the tobacco flavoring industry for musky, woody and balsamic notes [Singh *et al.* 2006].

Owing to the unstable composition, the extraction from valerian roots should be performed in temperature below 35°C. As a result, the process is time-consuming and low yielding. Because of this the methods for intensification of extraction are searched for.

One possibility for intensification of the extraction in a solid-liquid system is the application of ultrasounds [Mason *et al.* 1996, Povey and Mason 1998, Mason and Lorimer 2002]. A number of papers have been published concerning the ultrasonically assisted extraction of different materials [Ebringerovà and Hromàdkovà 1997, Sališová *et al.* 1997, Vinatoru *et al.* 1999, Toma *et al.* 2001 ].

Toma *et al.* [2001] studied an influence of acoustic field at 33 kHz frequency and 1 W·cm$^{-2}$ intensity on extraction efficiency and structural changes of various plant materials: peppermint, hop, dill, pot marigold and lime. They showed an increase of the efficiency in comparison with classical extraction in almost all cases. Romdhane and Gourgon [2002] conducted the experiments upon an influence of ultrasonic field on extraction of pyrethrines from Pyrethrum flowers and oils from woad seeds. Their studies revealed that the degree of grinding and type of the raw material subjected to process had a great effect on ultrasonic extraction efficiency. Hromàdkovà and Ebringerovà [2003], using hemicellulose from buckwheat shells, observed an influence of temperature and solvent concentration on ultrasonic processing efficiency. Paniwnyk *et al*. [2001] in their study upon rutin extraction from Saphora japonica found an impact of solvent type, and Entezari *et al.* [2004] recorded an influence of acoustic field intensity on the process efficiency.

The application of power ultrasound during extraction results in a very fast increase of temperature in the treatment medium. It requires an intensive cooling of liquid to prevent losses of valuable components contained in herbs. One of the ways to limit the very fast increase of temperature is the use of a pulsing operation. This technique was applied in medicine [Crisci and Ferreira 2002, Qin *et al.* 2006]. The pulsing operation prevents the overheating of tissues during ultrasonic therapy. Apart from it, in some conditions, the pulsing operation can be even more effective in comparison with continuous ultrasounds [Leighton 1994, Mitome and Hatanaka 2002]. It depends on the intensity and frequency of ultrasounds. For example the intensity of sonoluminescence at low power (10W) in the case of pulsed ultrasound is higher than in the case of continuous ultrasounds. However, at higher power (30W) the continuous wave is more effective than the pulsing operation. This phenomenon was observed at very short ultrasonic pulses [Mitome and Hatanaka 2002]. Efficiency of pulsing operation first of all depends on the pulse length, length between pulses and the ratio of the pulse length to interval length [Henglein 1995]. At long pulse lengths the yield of the pulsed ultrasounds are as efficient as continuous ultrasounds. However, at short pulse lengths the yield decreases and there exists a critical pulse length below which there are no more chemical effects [Henglein 1995].

Until now the pulsed ultrasounds have been investigated only in sonochemical reaction. There is no information about the use of pulsing operation to obtain extracts from herbal plants in scientific literature. In this sense the aim of this work was to investigate influence of pulsed ultrasound on extraction components from valerian roots and to compare it with classical extraction. The investigation was accomplished by varying the sonication time, sonic power and size particles.

## MATERIALS AND METHODS

**Material**

Valerian roots (Fig. 1) came from a local farm situated on Lublin Upland in Poland. After drying, their samples were stored for two months until the extraction. The moisture content of dried valerian was 10%.

Fig. 1. Outward appearance of valerian roots

teka_vol8.indd 90 2008-07-01 11:47:47

**Grinding**

Dried valerian was ground in colloid mill WŻ-1. Then, the material was divided into fractions using laboratory sieving device. Two fractions of the dried material were taken for testing:

- fraction I 0.5-1 mm of particle diameter,
- fraction II 0.25-0.5 mm of particle diameter.

**Classical extraction (control)**

Classical extraction was performed in thermostat by heating of the solid-liquid mixture in Erlenmeyer's flask in the temperature of 25°C. The solution was not stirred during heating.

**Ultrasonic treatment**

Ultrasonication was carried out using the ultrasonic processor (Sonic VCX 750) at a frequency of 20 kHz (Fig. 2). The generator was a horn-type (25 mm diameter and 120 length). All experiments were performed on samples of 10 g dispersed in 100 ml of the aqueous mixtures of ethanol without additional stirring. The parameters of ultrasonic treatment were as follows:

- sonic power 3,2 $W \cdot cm^2$, 5,2 $W \cdot cm^2$ and 8,4 $W \cdot cm^{-2}$,
- length pulse 2s, interval between pulses 1s,
- times of ultrasonic treatment 10, 20 and 60 minutes that correspond with total times of extraction 15, 30 and 90 minutes, respectively.

The temperature of the medium during extraction was kept at 25±2°C.

**Swelling coefficient**

Swelling coefficient was calculated using the following formula:

$$R = \frac{m_s}{m_k}, \tag{1}$$

where:
$m_s$ – initial weight of dried valerian [g],
$m_k$ – weight of dried valerian after steeping [g].

**Yield of extraction**

Yield of extraction was calculated using the following formula:

$$E = \frac{m_e}{m_s} 100\%, \tag{2}$$

where:
$m_e$ – weight of extracted substance (dry matter) from dried valerian [g],
$m_{ss}$ – weight of dry matter in dried valerian [g].

**Dry matter determination**

Dry matter content in extract was determined by drying at 105°C till constant weight was achieved in accordance with PN-90/A 75101/03.

**Statistical processing**

Experiments were carried out in 3 replications. The achieved results were subjected to statistical processing applying variance analysis. The difference significance was tested by means of Tukey's test. All computations were made using Statistica 6.0 software.

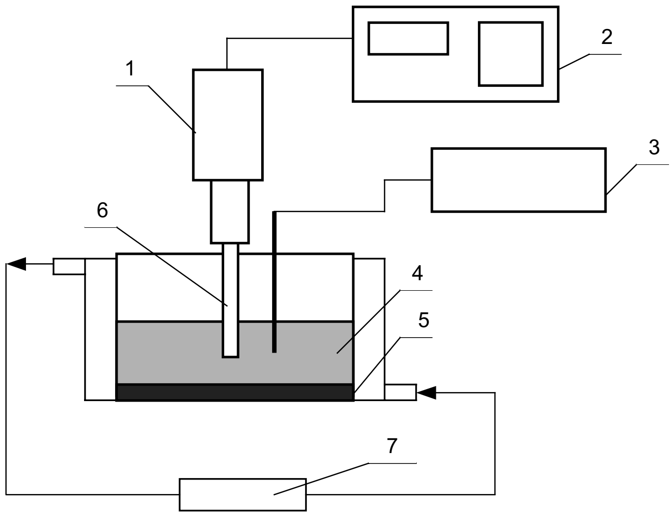


Fig. 2. Scheme of the set for ultrasonic processing: 1 – ultrasonic converter, 2 – generator with watt-meter, 3 – thermoelement, 4 – solvent, 5 – dried valerian, 6 – ultrasonic probe, 7 – cooling system

## RESULTS AND DISCUSSION

**Influence of ultrasound on heating of an extraction mixture**

Before the main experiments the preliminary investigation was conducted with an aim to evaluate the increase of temperature during the extraction treatment. The temperature of the extraction medium was directly related to the mode of ultrasonic treatment (pulsed – continuous) and the time of irradiation (Fig. 3).

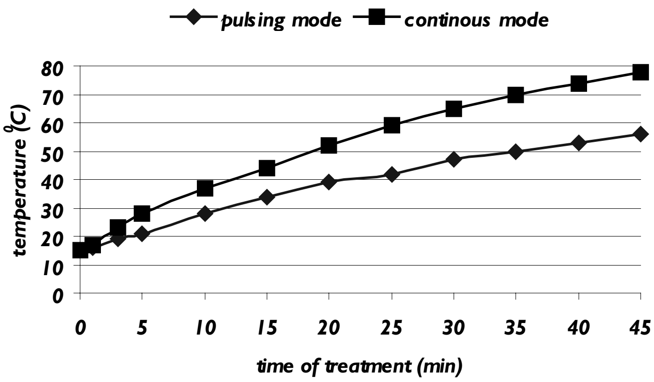


Fig. 3. Influence of sonication on the temperature of the extraction mixture

The increase of temperature during continuous mode was considerably higher in comparison with pulsed operation. After 30 minutes of continuous treatment the temperature of the mixture was about 18°C higher than in the case of pulsing operation. After 45 minutes of pulsing operation, that corresponds to 30 minutes of continuous mode, the difference decreased to 8°C. A slower increase of temperature in the case of pulsing mode allows to limit the intensity of cooling during ultrasonic extraction.

**Influence of ultrasound on the coefficient of swelling**

Extraction process from dried material involves two stages: steeping vegetal material in solvent and the mass transfer of soluble constitutes from material to solvent [Toma *et al.* 2001]. During the first stage the dried material absorbs solvent and swells. A capacity of dried material for absorption can be expressed by a coefficient of swelling. The quantity of the absorbed solvent depends on different factors such as: properties of solvent and dried material, temperature etc. Because the ultrasounds can accelerate absorption of solvent we decided to investigate the influence of pulsed acoustic field on the swelling coefficient. The results are shown in Fig. 4 and 5.

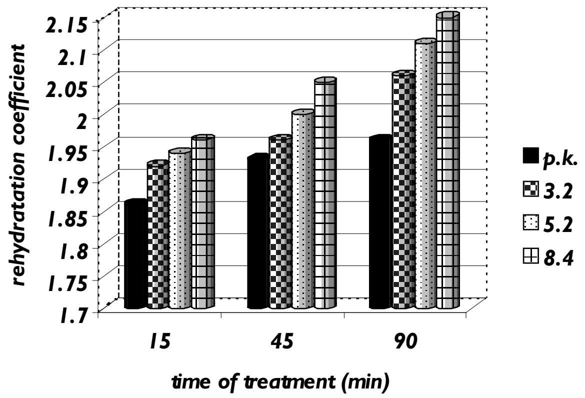


Fig. 4. Influence of sonic power and time of irradiation on swelling coefficient for fraction I

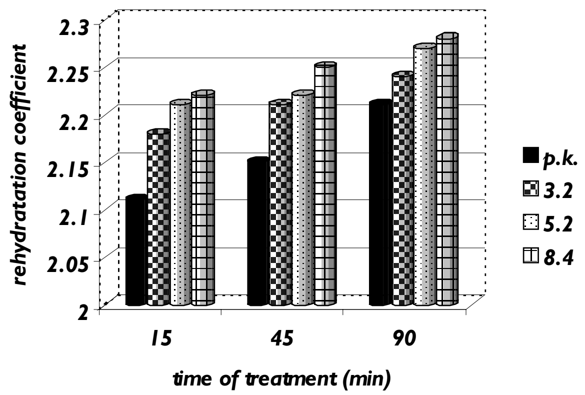


Fig. 5. Influence of sonic power and time of irradiation on swelling coefficient for fraction II

The data shows that sonication has a weak influence on absorption of solvent. In the case of 0,5-1 mm size particles the swelling coefficient increases by 3% to 7%, depending on time and intensity of ultrasound in comparison to classical steeping. An increase of grinding particles (0,25-0,5 mm size particles) results in higher swelling coefficient both for ultrasonic and classical steeping (Fig. 5). But the differences in the swelling coefficient between ultrasonic and classical procedure for both degrees of grinding are similar.

Investigators of the process of dry material swelling Toma *et al.* [2001] obtained considerably higher differences between classical and ultrasonic operation. However they used in their experimental a solvent of a significantly larger ratio of water to alcohol (25:1). It is known that alcohol is much less absorbed by dried material.

**Influence of ultrasound on the yield of extraction**

Classical extraction method was compared with ultrasonic extraction. The influence of sonic power and time of treatment on the yield of bioactive compounds from valerian roots for fraction I was seen in Fig. 6.

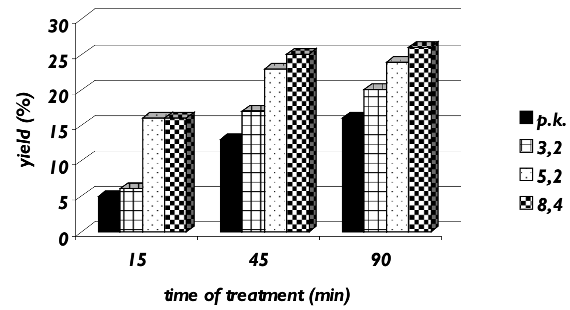


Fig. 6. Influence of sonic power and time of irradiation on yield of extraction for fraction I

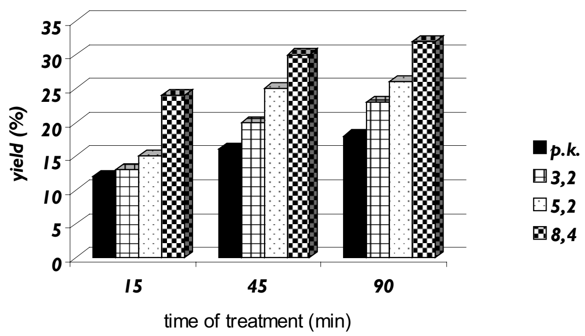


Fig. 7. Influence of sonic power and time of irradiation on yield of extraction for fraction II

There were significant differences between classical and ultrasonic extraction. The sonication effect on yield of extraction was dependent on intensity of ultrasound. The highest yield was obtained at 8,4 $W \cdot cm^{-2}$ sonic power and was 38% higher in comparison to classical extraction at the same time. The higher efficiency of the ultrasound – assisted extraction can be explained by mechanical action of acoustic field on the cell walls resulting in an increased extractability of the dry matter from valerian. Such effects were known to facilitate with improvement various components from plant materials [Romdhane and Gourdon 2002].

Fig. 7 demonstrates the effect of sonic power and time of treatment on extraction yield for fraction II.

The increase of raw material grinding resulted in higher yields both for classical and ultrasonic extraction. There were significant differences in yields during almost the whole period of irradiation and sonic power between classic and ultrasonic extraction. The highest yield was obtained for 8,4 W·cm$^{-2}$ sonic power at 90 min. time of irradiation and was 36%.

The yield of extraction is dependent among other factors on a rate of movement of solute from the saturated layer at the surface of the particle to the bulk of the solution. This rate may be described by the expression:

$$\frac{dw}{dt} = KA(C_s - C), \tag{3}$$

where:

dw/dt rate of mass transfer of solute [kmol·s$^{-1}$],

K – mass transfer coefficient [m$^2$·s$^{-1}$],

A – area of solid-liquid interface [m$^2$],

$C_S$ and C – concentration of solute at the surface of solid and in the bulk of the solution, respectively [kmol·m$^{-1}$] [Brennan *et al.* 1979].

According to equation No. 3 the rate of mass transfer from the surface of the solid is directly proportional to this area. Thus a reduction in particle size resulted in greater yields of extraction due to the increased area and a reduction in the distance the solution has to move within the solid to get to the surface.

Comparing the process of valerian extraction with results of other authors [Valachovic *et al.* 2001] we obtained higher differences between classical and ultrasonic extraction. There may be several reasons for this phenomenon. Firstly, we used very low volume of samples, that caused us to obtain higher density of ultrasonic energy. Secondly, we didn't apply circulation of solvent during classical extraction. Thirdly, we used a higher solid/liquid ratio and probably lower size particles.

**Influence of ultrasound on the dry matter content in residue**

To confirm the intensifying effect of the pulsed acoustic field during extraction the dry matter content in residues was investigated. Fig. 8 presents dry matter content in residues after alcoholic extraction for fraction I.

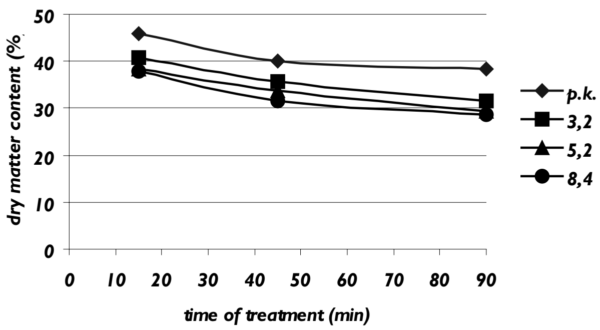


Fig. 8. Influence of sonic power and time of irradiation on dry matter content in residue for fraction I

The influence of ultrasounds on dry matter content in residue was dependent on sonic power. There were significant differences between ultrasonic and classic extraction. The highest decrease of dry matter content was obtained after 90 minutes at 8,4 W*cm$^{-2}$ sonic power.

In Fig. 9 was shown an influence of ultrasound on the dry matter content for fraction II.

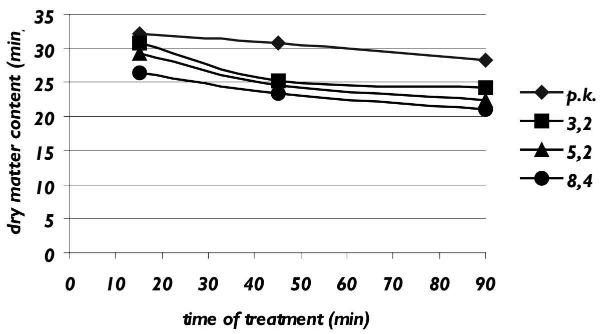


Fig. 9. Influence of sonic power and time of irradiation on dry matter content in residue for fraction II

The dry matter content in residue for fraction II was lower than in the case of fraction I. Similar trends in differences between ultrasonic and classic extraction were observed. The lowest dry matter content in residues was obtained after 90 minutes at 8,4 W·cm$^{-2}$ ultrasonic intensity.

Similar effects were also observed by other authors [Hromadkova *et al.* 2002]. They affirmed lower yield of extraction from residue obtained after ultrasonic treatment in comparison with residue obtained from classical extraction. The decrease of dry matter content in residues confirm effectiveness of pulsed acoustic field in extraction of valerian roots.

## CONCLUSION

The utilization of pulsed ultrasound has proved to be a more efficient technique than classical operation to extract bioactive components from dried valerian roots. The use of a pulsed acoustic field allowed to lower the intensity of medium cooling during the irradiation.

The pulsed ultrasound had positive effects on each step of the extraction during the preparation of medicine tincture from valerian roots. They accelerated both the swelling of dried valerian and the leaching of bioactive compounds from a matrix of solid. Because the differences between ultrasonic and classical extraction in the swelling coefficient were small, whereas the differences in yield of extraction were higher, we suppose that the pulsed ultrasound affects more the second stage of extraction.

The yield of extraction depended on the sonic power and the time of treatment. The highest yield was observed after 90 minutes at 8,4 W*cm$^{-2}$ sonic power. An decrease of size particles caused an increase of the yield of extraction both for ultrasonic and classical methods. The effectiveness of ultrasonic field was also confirmed by the decrease of dry matter content in residue.

teka_vol8.indd 96 2008-07-01 11:47:52

## REFERENCES


Bos R., Woerdenbag H., Pras N., 2002. Determination of valepotriates. Journal of Chromatography A 967, 131–146.

Brennan J.G., Butters J.R., Cowell N.D, Lilly A.E.V., 1979. Food Engineering Operations, Applied Science Publishers LTD, Essex.

Crisci A. R. and Ferreira A. L., 2002. Low-intensity pulsed ultrasound accelerates the regeneration of the sciatic nerve after neurotomy in rats. Ultrasound in med. & biol. 28 (10), 1335–1341.

Ebringerovà A. Hromàdkovà Z., 1997. The effect of ultrasound on the structure and properties of the water-soluble corn hull heteroxylan. Ultrasonics Sonochemistry 4 (4), 305-309.

Entezari M.H., Nazary S.H., Haddad Khodaparast M.H., 2004. The direst effect of ultrasound on the extraction of date syrup and its micro-organisms. *Ultrasonics Sonochemistry* 11, 137-142

Fernandez S., Wasowski C., Paladini A.C., Marder M., 2004. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. Pharmacology, Biochemistry and Behavior 77, 399–404.

Henglein A. 1995. Chemical effects of continuous and pulsed ultrasound in aqueous solution. Ultrasonic Sonochemistry 2 (2), 115-121.

Hromàdkovà Z., Ebringerovà A., 2003. Ultrasonic extraction of plant materials – investigation of hemicellulose release from buckwheat hulls. *Ultrasonics Sonochemistry* 10, 127-133.

Hromadkova Z., Ebringerova A., Valachovic P. 2002. Ultrasound – assisted extraction of water-soluble polysaccharides from the roots of valerian *(Valeriana Oficinallis L.)* Ultrasonics Sonochemistry 9 (1), 37-44.

Jambor J., 2001. The perspectives of Polish herbal products market. The Herbal News 10, 1-7.

Leighton T. G., 1994. The acoustic bubble. Academic Press, San Diego.

Lesniewicz A., Jaworska K., Żyrnicki W., 2006. Macro- and micro-nutrients and their bioavailability in polish herbal medicaments. Food Chemistry 99 (4), 670-679.

Mason T. J., Paniwnyk L., Lorimer J. P. 1996. The uses of ultrasound in food technology. Ultrasonics Sonochemistry 3 (3), 253-261.

Mason T.J., Lorimer J.P., 2002. Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing, Wiley-VCH Verlag GmbH, Weinhein.

Mitome H., Hatanaka S., 2002. Optimization of a sonochemical reactor using a pulsing operation. Ultrasonics 40 (1-8), 683-687.

Paniwnyk L., Beaufoy E., Lorimer J.P., Mason T.J. 2001. The extraction of rutin from flower buds of *Sophora japonica*. *Ultrasonics Sonochemistry* 8, 299-301

Povey M.J.W., Mason T.J., 1998. Ultrasound in food processing, Blackie Academic & Professional, London.

Qin L., Lu H., Fok P., Cheung W., Zheng Y., Lee K., and Leung K., 2006. Low-intensity pulsed ultrasound accelerates osteogenesis at bone-tendon healing junction. Ultrasound in Med. & Biol. 32 (12), 1905–1911.

Romdhane M., Gourdon C., 2002. Investigation in solid-liquid extraction: influence of ultrasound, Chemical Engineering Journal 87 (1), 11-19.

Sališová M., Toma M., Mason T.J., 1997. Comparison of conventional and ultrasonically assisted extractions of pharmaceutically active compounds from *Salvia officinalis*. Ultrasonics Sonochemistry 4 (2), 131-134.

Singh N., Gupta A.P., Singh B., Kaul V.K., 2006. Quantification of Valerenic Acid in *Valeriana jatamansi* and *Valeriana officinalis* by HPTLC. Chromatographia 63 (2006) 209–213.

Toma M., Vinatoru L., Paniwnyk L., Mason T.J., 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrasonics Sonochemistry 8 (2), 137-142.

Valachovic P., Pechova A., Mason T.J., 2001. Towards the industrial production of medicinal tincture by ultrasound assisted extraction. Ultrasonics Sonochemistry 8 (2), 111-117.

Vinatoru M., Toma M., Mason T.J. 1999: Ultrasonically assisted extraction of bioactive principles from plants and the constituents. Advances in Sonochemistry 5, 216.

Vinatoru M., Toma M., Radu O., Filip P.I., Lazurca D., Mason T.J., 1997. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrasonics Sonochemistry 4 (2), 135-139.