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An overview of the ultrasonically assisted extraction of bioactive principles from herbs

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Abstract

This paper presents a review of the ultrasonically assisted extraction of bioactive principles from herbs. Much of the work was carried out under European community grants under the COPERNICUS programme and in a COST D10 network. Some aspects of classical and non-conventional extraction procedures are also presented and briefly discussed. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Vegetal materials are invaluable resources, useful in daily life as food, food additives, flavors, fragrances, pharmaceuticals, colors or directly in medicine. This use of plants has a long history all over the world and, over the centuries, humanity has developed better methods for the preparation of such materials. Nowadays, there is a renaissance of interest in natural remedies, in part due to some disillusionment with modern medicine and drugs that either do not perform entirely to expectation or are accompanied by unwanted side effects. Natural remedies have the advantage that they have passed the proof of time. On the other hand some synthetic drugs even though they have been used for over 100 years, may still need more time to be proven to be absolutely harmless.

Perhaps anyone not familiar with the problems related to medicinal herbs might consider this subject unimportant, or of only minor importance. Others may feel that, in a period in which synthetic and highly efficient drugs are available for treatment of various diseases, medicinal herbs have become archaic and represent only a centuries-old tradition. Others however consider them a form of natural remedy to oppose modern drug consumerism.

A herbal extract could be defined as the compounds and/or compound mixtures obtained from fresh or dried plants, or parts of plants: leaves, flowers, seeds, roots and barks, by different extraction procedures. Characteristically, the active constituents are obtained together with other materials present in the vegetal mass. The extraction of bioactive components from vegetal materials is part of *phytopharmaceutical* and *food* technology.

It is obvious that herbal preparations are of great medicinal interest, and for this reason herbal preparations, which include herbal extracts, were introduced in the pharmacopoeias of numerous countries, Table 1 [1].

Medicinal and aromatic plants provide an inexhaustible resource of raw materials for the pharmaceutical, cosmetics and food industries and more recently in agriculture for pest control [2]. People have learned to increase the power or usefulness of herbs by preparing medicinal compounds from them, by preserving them so that they are always available, and by finding new ways to release their active constituents. Among modern methods used to release the bioactive constituents from herbs is ultrasonically enhanced solvent extraction. The use of ultrasound to enhance the extraction yield is a technique that started in the 1950s with laboratory scale experiments [3].

To make an extract, first of all it is important to identify the desired herb and the part of it which contains useful constituents, but this is not so simple. Around 350 years ago, one of the most famous English

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Table 1
Entries on herbal drugs in pharmacopoeias

Country	Number of herbal drugs monographs
French pharmacopoeia	190
Switzerland pharmacopoeia	180
USSR pharmacopoeia	140
Polish pharmacopoeia	125
British codex	125
Belgian pharmacopoeia	120
Holland pharmacopoeia	120
Hungarian pharmacopoeia	110
Romanian pharmacopoeia	105
Italian pharmacopoeia	105
German pharmacopoeia	85
British pharmacopoeia	80
Scandinavian pharmacopoeia	80
International pharmacopoeia	45

herbalist Culpeper, described in a wonderful book several rules for gathering plant parts for herbal preparations [4]. His rules are still applicable today and readers will find them useful in the gathering of leaves, flowers, seeds, roots, barks and other parts.

Actually, when research work for developing new extraction techniques starts up, the first thing to be done is to gather the required herbs. However for chemists not familiar with botany this could prove to be a very difficult task. Indeed, the chemical content of herbs strongly depends on the gathering time. For industrial processing medicinal and aromatic herbs, the harvesting time as well as the preserving methods are of crucial importance and therefore a precise technique must be setup for this purpose [5].

2. A short history of plants and plant extract uses

The history of vegetal materials use is as old as humanity. The first use was for nutrition, initially from natural and later from cultivated flora. Soon, the medicinal properties of herbs were discovered. Several herbs, such as lavender, hyssop, thyme, etc., were introduced into agriculture for their essential oils or their medicinal compounds.

Egyptian papyruses describe thousands of recipes showing that coriander and castor oils were used for medicinal application, cosmetics and preservatives. Hebrew and Chinese manuscripts describe over 2000 herbs, with details that are useful today. In the Greek and Roman empires there was an expanding process for the therapeutic use of herbs, mainly for their essential oil content. Galenus described, in the second century, in around 30 papers and recipes, herbal extracts for medicinal purposes which are still up to date. "Hungarian water", known since 1380 was the first alcoholic extract from rosemary, and was, for five centuries, extensively used in Europe. Paracelsus, in the 16th century made an important contribution to the study of aromatic and medicinal herbs, introducing aromatic and medicinal herbs to a "hot bath" (this is still in use today in Romania at the Ana Aslan Geriatric Institute, Otopeni). In the 19th century French researchers extended investigations into the field of herbal products, setting up procedures to obtain the desired extracts driven by the growing demand for perfumes.

In Romania the use of medicinal herbs has been known since antiquity. For example the herb 'Motherwort' (*Leonurus cardiaca)* was mentioned by Herodotus (5th century B.C.) in his writings about people living north of the Danube river [6]. In 1700 in Bucharest, the hospital 'Coltea' included a pharmacy selling medicinal herbs. In the 19th century, herbal products were introduced in the Romanian pharmacopoeia and in 1904 the first institute of medicinal herbs was established in Cluj city [7].

It is well known that plants provide perfect laboratories for the synthesis of medicinal compounds. Some of these compounds were introduced into industry e.g. aspirin, discovered initially as salicylic acid in willow bark and leaves, which has become one of the most used industrial drugs. According to Hippocrates for healing an ill person first use psychotherapy, then if there are no results use phytotherapy and, only if these two therapies failed use, surgery. Nowadays after phytotherapy, modern medicine introduces chemotherapy (synthetic drugs) as another step before surgery.

The interest in medicinal and aromatic herbs has revived with the identification of new compounds and the discovery of new applications of various herbal constituents.

The expression "folk medicine", even though it may have pejorative overtones, actually means centuries of empirical experiments and over a hundred years of scientific research, gathered and transmitted by oral and written forms throughout many generations to the present day. It is our duty to use this information and to bring it to light by using modern methodologies, one of these being the ultrasonically assisted extraction of bioactive principles.

3. Extraction procedures

To obtain extracts from vegetal materials several methods are available:

1. Distillation:
   (a) direct essential oil distillation;
   (b) water steam distillation;
   (c) water and steam distillation.
2. Solvent extraction:
   (a) solvent extraction (percolation);
   (b) maceration with solvent;

   (c) boiling with water (infusion);
   (d) extraction with cold fat (enfleurage);
   (e) extraction with hot fat.
3. Cold compression, which is the usual method for the natural oil industry.
4. Non-conventional extraction techniques:
   (a) supercritical fluid extraction;
   (b) vortical (turbo) extraction;
   (c) extraction by electrical energy;
   (d) ultrasonically assisted extraction.

3.1. Distillation

Distillation means that vegetal materials are mixed (or not) with water followed by heating or by the introduction of water steam. The resulting vapors are cooled and collected in a separator and the essential oil separates from water. This yields a crude essential oil that should be further distilled. This is the main procedure for obtaining essential oils, but some other extraction techniques such as extraction with light solvents can offer similar or better extracts [8,9].

During distillation it is obvious that the use ultrasonic energy is futile. A distillation unit provided with an ultrasonic source will produce more rapidly boiling centers, but no collapsing bubbles. Therefore this kind of unit will be useful only to enhance boiling with little, if any, improvement in yield.

However, ultrasound can be successfully employed to enhance extraction when low boiling point solvents are used, and the temperature of the extraction mixture is kept below its boiling point. An example showing how ultrasound can help solvent extraction of essential oils from different vegetal materials is given in Table 2 [8].

It is interesting to note that at longer extraction times, [7 days maceration (M) + 30 min ultrasonic extraction (US) + 240 min reflux (R)] the concentration of limonene decreases even when ultrasound is used. This is quite strange since the amount of oil is greater than that obtained via classical or ultrasound extraction. It seems that maceration prevents more limonene being extracted but the mechanism not yet understood. One of the most important observations in this case is that ultrasonically assisted extraction leads to non-detectable amounts of heavy components.

3.2. Solvent extraction

Solvent extraction procedures, excluding fat extraction, are more amenable to ultrasonic treatment. This can be achieved simply by the introduction of an ultrasonic transducer into the extraction unit. This is possible because in almost all cases solvent extraction uses cold solvent (percolation can be done with cold as well as with hot solvent).

Table 3 contains results showing the effect of ultrasound on dry residue yield for several medicinal and aromatic plants [9]. Table 3 shows that when using sonication the content of dry residue (which measures total extract) is either similar to or greater that that obtained by classical or maceration methods.

In another example of ultrasonically assisted solvent extraction, the authors follow the individual components of sage extraction [10], Table 4. These studies clearly show that a probe system is a better sonication technique for the extraction of cineole, thujone and borneol. Even when using a cleaning bath as the ultrasonic device for extraction, the yield of extracted compounds rose to near completion.

3.3. Non-conventional extraction techniques

Extraction with supercritical fluid is one of the newer extraction techniques that can offer very good yields. For this procedure the most often used supercritical fluid is carbon dioxide. A typical scheme for a supercritical extraction unit is given in Scheme 1. From the bottle 8, the carbon dioxide gas after passage through a heat exchanger and filter (4 and 7) is compressed in the compressor 3, and cooled again until reaching the liquid state when it is introduced into the extraction unit 1. After completing the extraction, the liquid is transferred though a throttle 5 into the separation unit 2. Here the liquid carbon dioxide is heated to reach the gaseous

Table 2
Comparison between different extraction methods of 100 g of dill seeds, using hexane

Method used	Oil amount (g)	Extraction time	Components		
			Limonene %	Carvone %	Heavy comp. %
CE	3.00	240 min	40.79	47.29	0.09
US	3.40	30 min	49.63	48.15	–
US	3.40	60 min	51.22	45.84	–
M + US	3.50	7 days M + 30 min US	25.08	66.84	0.05
M + US	3.50	7 days M + 60 min US	20.77	65.40	0.34
M + US + R	3.55	7 days M + 30 min US + 240 min R	20.72	65.69	0.10
M + US + R	3.55	7 days M + 60 min US + 240 min R	20.28	65.92	0.34

CE: classical extraction (Soxhlet); US: ultrasonic extraction; M: maceration; R: reflux.

Table 3
Dry residue (g/100 g extract) obtained by direct sonication

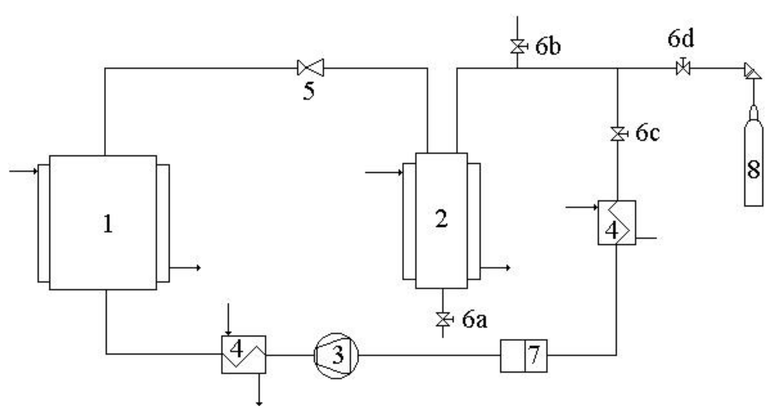
Sonication time (min)	Mint[a]	Camomile[a]	Sage[a]	Arnica[a]	Gentian[a]	Marigold[a]	Marigold[b]
15	0.06	1.10	0.58	0.36	–	0.94	–
30	0.07	1.30	0.80	0.42	1.67	0.98	1.15
60	0.25	1.43	0.92	0.67	2.66	1.14	1.74
90	0.78	1.56	0.94	1.06	2.71	1.33	1.97
120	0.82	1.79	1.13	1.20	3.24	1.75	2.25
180	–	1.80	–	–	–	–	–
18 h maturation	0.91	1.91	1.15	1.50	4.68	2.20	2.25
Classical 7–14 days	1.02	1.73	1.02	1.75	4.75	2.25	2.25

[a] Cleaning bath.
[b] Probe system.

Table 4
Comparison of different ultrasonic extraction procedures for sage

Extraction procedure	Component extracted (mg/kg)		
	Cineole	Thujone	Borneol
Probe (2 h)	24.3	167.2	5.8
Cleaning bath[a]	16.5	118.1	3.6
Cleaning bath + stirrer[a]	22.7	141.9	6.3
Silent control experiment[b]	14.0	176.6	6.5

[a] For these procedures data were collected at 3 h.
[b] Best silent results.



Scheme 1. Schematic diagram of supercritical fluid extraction unit.

state, removed, and from the bottom of the vessel the extract is drawn off through the valve 6a.

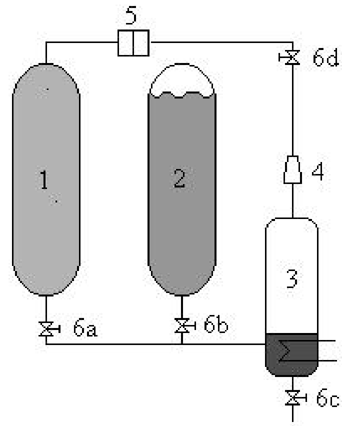
An example of the use of supercritical extraction techniques is given in Table 5 [11]. From this example one can see that using different pressures of supercritical fluid a selective extraction can be performed.

A technique resembling supercritical fluid extraction is that developed by Wilde [12], using tetrafluoroethane as the working solvent. In Scheme 2 a simplified diagram of a possible extraction unit is given. This new extraction technique combines the advantages of supercritical fluid extraction with those of normal solvent extraction. Using different combinations of fluoroethane with organic solvents, selective extractions can be performed. This technique is suitable for fragrance extraction, giving better yields and a good quality essential oil.

Table 5
Supercritical extraction (selected examples)

Herb, air dried	Main constituents	Molecular weight	Extractable at (bar)
Caraway seeds	Limonene	136	70
	Carvone	160	70
	Triglycerides	>600	90
Peppermint leaves	Menthol	158	70
	Menthone	156	70
Cannabis	Cannabidiol	314	85
	Cannabinol	310	90
	Tetrahydrocannabinol	315	90

The vortical (turbo) extraction procedure uses a high speed stirrer that induces hydrodynamic cavitation, enhancing thereby the extraction yield, Table 6 [13]. It is



1 = fluid tank
2 = extraction vessel
3 = extract concentrator
4 = compressor
5 = heat exchanger
6a-6d = valves

Scheme 2. Schematic diagram of a tetrafluoroethane extraction unit.

obvious that using a high speed stirrer, the contact between vegetal material and solvent is improved and therefore the diffusion process through the cell walls is increased. Moreover, during vortical extraction hydrodynamic cavitation bubbles are produced and their collapse acts in a similar way to the effect of ultrasonic devices. If the vegetal material is well milled, the differences between classical and vortical extraction diminish.

Electrical discharges within the extraction mixture were also claimed to increase the extraction yield, as suggested for the first time by Issaev and Mitev [14] and performed by the same authors [15] for *Cytisus laburnum* (broom). This extraction technique is represented in Scheme 3. The alkaloid yield during the extraction of *Rauwolfia* by electrical discharge was increased by 25%, according to Boiko and Mizineko [16]. All authors working with this type of apparatus noted that during electrical discharges, cavitation bubbles are produced and that this technique therefore has similarities to ultrasonic extraction.

Table 6
Effect of milling degree and type of extraction on the dry residue and tannins content of tincture of tormentil

Extraction method	Milling degree	Dry residue (%)	Tannins (g)
Maceration	2–5 mm	4.40	2.15
Vortical extraction	”	4.72	2.35
Maceration	Up to 1 mm	4.75	2.28
Vortical extraction	”	5.00	2.45
Maceration	0.1–0.5 mm	5.05	2.45
Vortical extraction	”	5.12	2.60

Rotation speed of vortex: 14,000–20,000 RPM.

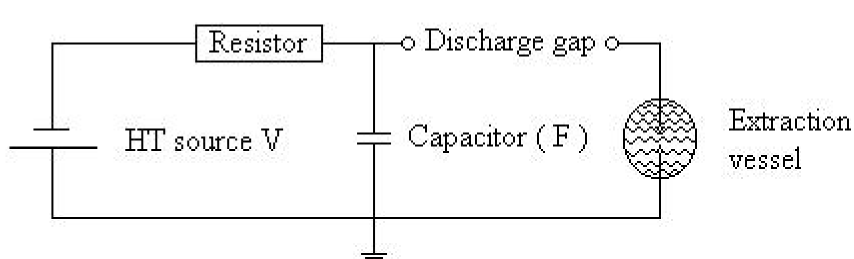
3.4. Ultrasonic extraction

A number of papers have been published dealing with the ultrasonically assisted extraction of different vegetal materials. One of the first citations concerning ultrasonic extraction (1952) was related to hop extraction in an aqueous medium and showed that ultrasonic extraction was comparable with the boiling extraction process [17]. It was shown that during ultrasonic extraction it was possible to save some 30–40% of hops in the production of beer [18].

Several references concerning ultrasonically assisted extraction are summarized in Table 7. It is worth noting that when high frequency ultrasound is employed, the extraction yield did not increase significantly however the degradation of the herb constituents was diminished. In the case of low frequency sonication degradation becomes more important, especially when alkaloids are being extracted. This effect could be employed as a tool to help in the extraction of medicinal compounds by using lower frequencies to assist in the degradation of toxic alkaloids during the process.

4. Extraction mechanisms

Vegetal tissue consists of cells surrounded by walls (Fig. 1). The extraction mechanism involves two types of physical phenomena: diffusion through the cell walls and washing out (rinsing) the cell contents once the walls are broken. Both phenomena are significantly effected by ultrasonic irradiation. Some cells exist in the form of glands (external or internal) that are filled with essential



Scheme 3. Schematic diagram of an electrical discharge extraction unit.

Table 7
Examples of ultrasonically assisted extraction

Ultrasonic frequency (kHz)	Extracted drugs	Remarks	References
2400	Cinchona bark	No yield improvement (too high frequency US)	[19]
400	Peanuts (oil)	Ultrasonic yield increased when hexane is used as solvent	[20]
500	Belladonna leaves	Similar yield as maceration, no decomposition of alkaloids, for short time sonication	[21]
25	Rauwolfia roots	US time 15 min; maceration 8 h for the same yield	[22]
20–40	*Datura stramonium* (thorn apple)	US offer 9% greater alkaloids in 1 h, but 40 kHz are more effective	[23]
20	Cinchona bark	US extraction gives 15% more alkaloids in 1.5 h comparing with 7 h Soxhlet.	[24]
1000	Nux vomica seeds	1.2% and 0.95% US alkaloids extraction yield in 20 min, compared with 0.64% and 0.94% in 8 h[a]	[25,26]
800	Digitalis leaves	US lead to similar or better yield, but decrease the glycoside yield due to H_2O_2 formation	[27]
20	Berberine	US extraction 50% greater in 0.5 h, compared with alkaline extraction for 24 h	[28]
1000	*Urtica dioica*	US extraction gives better results after 5 min, using 1 W/cm^2	[29]
800	*Inula helenium* and *Telekia speciosa*	No degradation of inulin, good yield for shorter time (10–40 min)	[30]
1000	*Amarantus retroflexus*	5 min sonication do not affect the extracted amino acids composition	[31]

[a] First figure refers to strychnine, the second to brucine.

oil. A characteristic of such glands (when external) is that their skin is very thin and can be very easily destroyed by sonication. This explains why the extraction of essential oil, as well as fat oil, is facilitated by sonication. For internal glands, it is the milling degree of the vegetal material which plays an important role and this is illustrated in Table 8 [32].

It is obvious that reducing the size of the vegetal material particles will increase the number of cells directly exposed to extraction by solvent and thus exposed to ultrasonically induced cavitation. This effect can be utilized by milling the material before extraction. It should be borne in mind however that powerful sonication can itself serve to mill the vegetal material. External essential oil glands are already exposed directly to the cavitating solvent and consequently are readily disrupted.

Table 8
Influence of milling degree on the extraction of clove flowers[a]

Extraction time (min)	Extraction technique	Milling degree	Eugenol extracted (g/100g)
30	Silent	not milled	4.10
30	Silent	0.1–0.5 mm	25.20
30	US	not milled	4.22
30	US	0.1–0.5 mm	32.66

[a] Extraction solvent ethyl alcohol 96%, cleaning bath with stirring. Silent method involve only stirring.

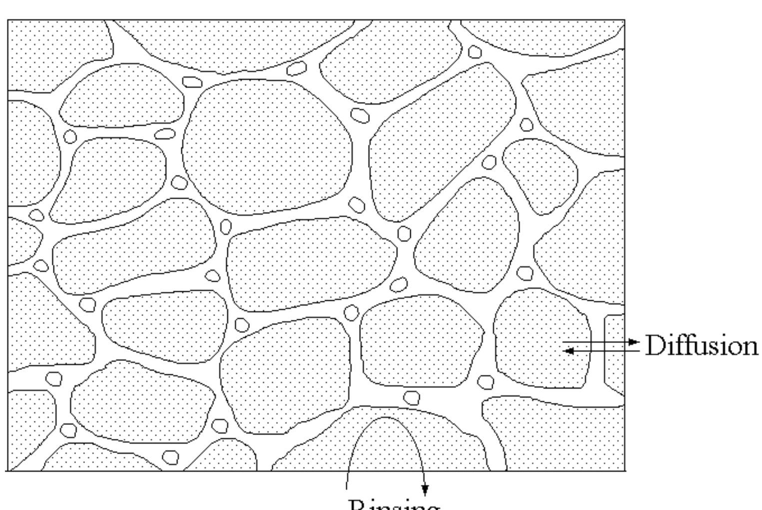


Fig. 1. Schematic diagram of vegetal cell structures.

The ultrasonic breakdown of vegetal cells using normal ultrasonic extraction devices such as a cleaning bath or probe system, may not be the only mechanistic hypothesis for extraction improvement, especially when dried vegetal material is used. This is because solvent extraction from dried material is a two stage process involving:

(i) steeping vegetal materials in solvent to facilitate swelling and hydration processes;
(ii) the mass transfer of soluble constituents from the material to solvent by diffusion and osmotic processes.

Ultrasound can facilitate swelling and hydration and so cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhancing mass transfer. Both steps have been shown to be sensitive to sonication as can be seen in Fig. 2 and Table 9 [33].

Ultrasound increases the swelling index i.e. the water uptake by the vegetal material during sonication. The extractive value is much greater under sonication, compared with mechanical stirring. An increase in the swelling of vegetal tissue can, in some cases, break the cell walls which favors the washing out process.

The mechanical effect of ultrasound during extraction has been demonstrated in the case of marigold leaves when sonicated at two different ultrasonic frequencies (20 and 500 kHz). The external oil glands (excretion hairs) were observed and the lower frequency sonication was found to destroy all excretion hairs as well as part of the leaves, whereas higher frequency left the leaf tissue unaffected (Figs. 3–5) [33].

5. Laboratory and large scale ultrasonic extraction

To perform ultrasonically assisted extraction is not difficult on a laboratory scale using a simple cleaning bath (Figs. 6 and 7). Using such equipment it is possible to obtain good extraction yields by direct or indirect extraction [34]. In both cases it is preferable to use a mechanical stirrer and to cool the extraction mixture

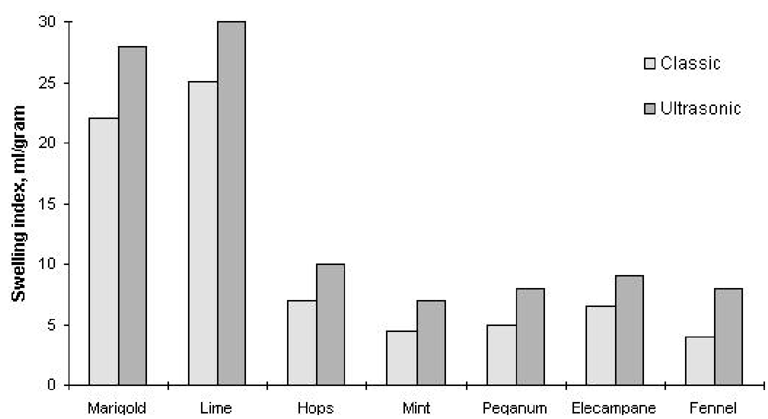


Fig. 2. The enhancement of vegetal material swelling induced by ultrasonic treatment [29].

Table 9
Comparison of extractive value of sonicated and silent methods for some vegetal species [29]

Vegetal species	Method	ETH	ETHW	WATER	GLYW	EETH
Fennel *Foeniculum vulgarae*	Classic	10.2	7.4	8.2	13.4	12.2
”	1/2 h US	13.7	7.3	11.0	13.4	16.0
”	1 h US	14.3	7.5	15.6	15.2	19.3
Hops *Humulus lupulus*	Classic	20.4	27.0	24.0	22.5	4.5
”	1/2 h US	22.8	31.0	28.3	28.0	6.3
”	1 h US	27.0	32.0	29.5	31.0	7.2
Marigold *Calendula officinalis*	Classic	16.0	20.0	30.0	22.5	9.2
”	1/2 h US	16.5	22.0	30.5	27.8	9.5
”	1 h US	18.0	23.0	31.4	28.0	9.7
Peganum harmala	Classic	16.2	16.2	21.4	–	14.8
”	1/2 h US	17.3	17.0	22.0	–	15.6
”	1 h US	19.0	18.6	22.0	–	16.8
Mint *Mentha piperita*	Classic	8.5	21.3	26.4	24.6	3.2
”	1/2 h US	9.3	26.3	26.5	25.2	3.6
”	1 h US	9.7	27.2	26.8	26.7	4.2

ETH: ethanol–water (94 v/v), ETHW: ethanol–water (70 v/v), WATER: water, GLYW: glycerol–water (3.5 v/v) and ETEH: ethyl ether.

Fig. 3. Pot marigold petal sonicated at 20 kHz. All excretion hairs were destroyed by ultrasound.

Fig. 4. Control: nonsonicated, shown a high density of excretion hairs.

Fig. 5. Pot marigold petal sonicated at 500 kHz. Only top of excretion hairs are destroyed.

since the absorption of ultrasonic energy can cause warming. By indirect sonication, only small amounts of vegetal material can be extracted, whereas using the direct procedure, large amounts of vegetal material can be employed.

Another device that could be used for ultrasonically assisted extraction is the probe system (Fig. 8). This requires a glass vessel provided with a stirrer and a cooling jacket. In some cases, when soft vegetal material is employed, it is not possible to perform extractions with such a device using the normal ratio of vegetal material/solvent of 1/10 (for certain herbs this can be 1/5). This is because the vegetal material is too concentrated and dampens the transfer of ultrasonic energy. If the same volume of solvent is used but the vegetal mass is divided into two portions the extraction can be carried out in two stages. In the second the decanted solvent from stage 1 (containing the extract from the first stage) is used.

A possible industrial reactor for vegetal mass extraction is given (Fig. 9). Following on from the results obtained during the research work done under an EU COPERNICUS programme (ERB-CIPA-CT94-0227-

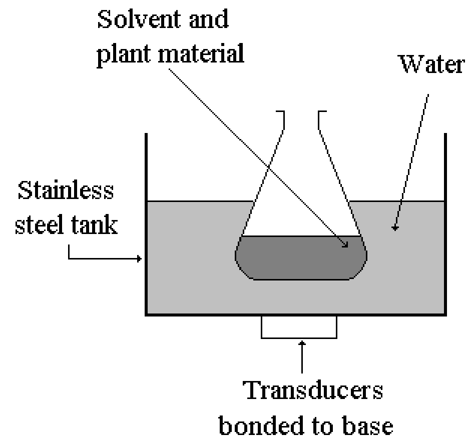


Fig. 6. Experimental setup for indirect extraction using a cleaning bath.

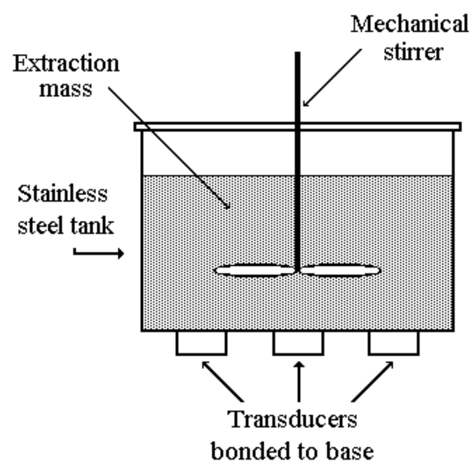


Fig. 7. Experimental setup for direct extraction using a cleaning bath.

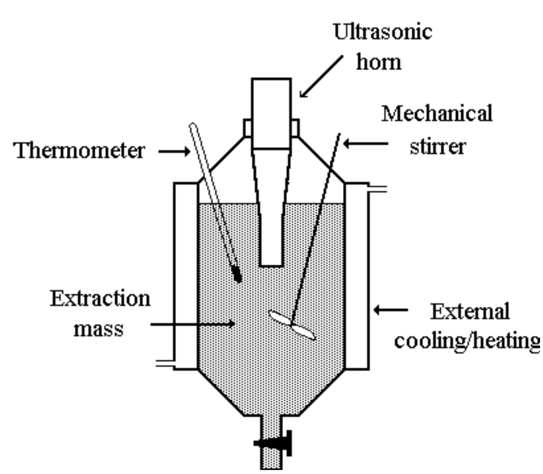


Fig. 8. Experimental setup for direct extraction using an ultrasonic horn.

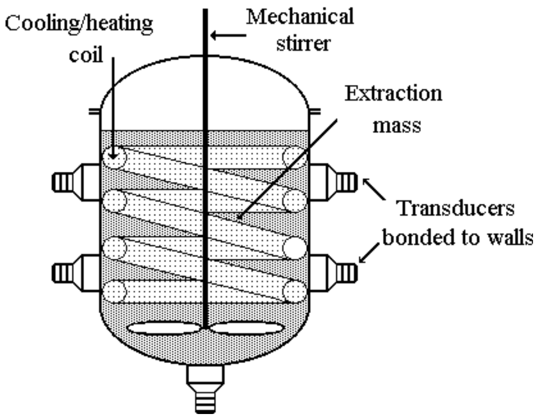


Fig. 9. Possible setup for an ultrasonic extraction reactor.

1995), the Romanian team designed and built the first industrial ultrasonic reactor dedicated to the solvent extraction of herbs. The reactor is of 1 m^3 capacity (700–850 l, working capacity) and operates in the PLAFAR factory, Brasov, producing mainly ethyl alcohol extracts for the Romanian market (Fig. 10) [35].

Twelve experiments with different herbs were performed on an industrial scale using classical methods involving 28 days of maceration with continuous stirring in ethyl alcohol at 45°C. Better extraction yields were obtained using similar herb/alcohol mixtures under sonication after 6 h. The dry residue of the ultrasonic extract was 50% greater than that obtained classically, and the targeted components also seem to be better extracted, as can be seen in the IR spectra of both extracts (Fig. 11).

A particularly good result using the ultrasonic reactor involved St. John's wort herb extraction in ethyl alcohol where a very good yield was obtained in a much shorter time than under classical maceration or percolation procedures.

Fig. 10. Industrial ultrasonic reactor.

6. New and emerging research involving sonochemical extraction

6.1. Allelopathy

Allelopathy can be defined as the ways in which some plants can use their own chemicals to control other plant species in the neighborhood. From the time when the COPERNICUS research programme started, over 50 different herbs and several plants have been extracted and compounds isolated from them. The extraction is simple and fast using the ultrasonic technique and, after isolation of compounds, each could be tested for its allelopathic properties. To illustrate such work the inhibitory effect of the essential oil obtained from winter savory on the germination of wheat seeds is shown in Fig. 12 [36].

It is evident that at the correct concentration of winter savory oil, the seedling growth is completely inhibited, compared with the control. Natural compounds from herbs have already proved to be useful for the control of agriculture pests e.g. Collego is used to control vetch from rice and soybean cultures in the USA. This is obtained from a mushroom (*Colletotrichum gloeosporides*) which secretes a molecule which is toxic towards vetch [37].

6.2. New phytopharmaceutical technology

In order to establish ultrasonic extraction as a new industrial technology it is important to identify and put into place those parameters important for scale-up. As a first step in this process a commercial product should be identified. One of the first experiments performed to this end involved St. John's wort herb extraction at industrial level. This revealed that the hypericine content of the extract reached the desired level in a much shorter time than classical maceration or percolation methodology, at room temperature.

The sonochemical approach will be of great benefit to phytopharmaceutical technology as it is used more extensively for the rapid and efficient extraction of plants.

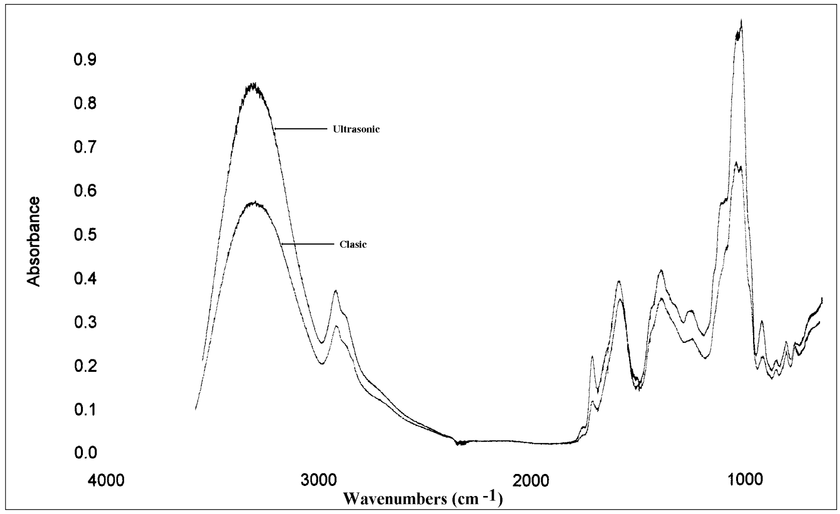


Fig. 11. Infrared spectra of classic and ultrasonic industrial extract.

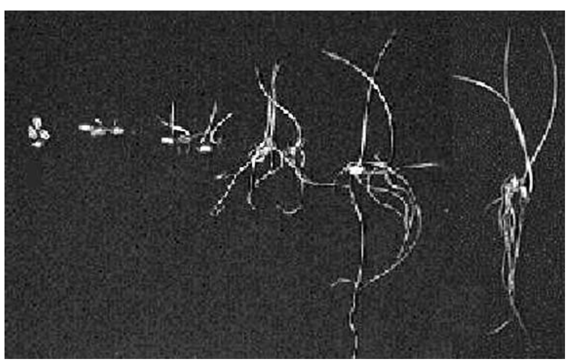


Fig. 12. Inhibitory effect of winter savory essential oil on wheat seedlings.

7. Conclusions

Two main conclusions can be drawn from the information presented in this paper:

1. Ultrasound has been proven to assist solvent extraction and should prove to be a powerful tool for the phytopharmaceutical extraction industry.
2. Ultrasonically assisted extraction is a versatile technique that can be used both on a small and large scale.

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