# PHCOG REV.: Review Article

# Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research

## Vivekananda Mandal, Yogesh Mohan, S. Hemalatha*

*Pharmacognosy Research Laboratory, Department of Pharmaceutics,*
*Institute of Technology, Banaras Hindu University, Varanasi 221005, India.*
*E-mail address: pharmafriend@rediffmail.com, dryogesh_bhu@rediffmail.com, hemalatha111@rediffmail.com (S. Hemalatha)*
*Corresponding author, Tel.: +91 9415256481 (O); +91 0542 2575810 (R); fax: +91 0542 2368428*

**ABSTRACT**

In recent years, the use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step. This review highlights the importance of extraction step in setting up respectable standards for herbal medicine worldwide. High and fast extraction performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave assisted extraction (MAE) technique. A brief theoretical background of microwave heating and the basic principles of using microwave energy for extraction have been presented for better understanding. Discussions on the main parameters influencing the extraction efficiency (namely solvent nature and volume, extraction time, microwave power, matrix characteristics and temperature) and different statistical optimization strategies are also highlighted. Finally the potential applications of this new method with comparison of its performance to that of classical techniques are also elucidated.

*Key words:* Microwave assisted extraction (MAE), thermolabile, microwave heating, dielectric properties, optimization, taguchi design, RSM

## INTRODUCTION

The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high activity profile drugs. The potential natural anticancer drugs like vincristine, vinblastine and taxol can be the best example (1). Recent years have shown a growing popularity and faith in the use of herbal medicine worldwide. This may be because of the realization that modern synthetic drugs have failed to provide a "cure all" guarantee to most of the human diseases with often producing undesirable side effects, which at the end turnout to be more problematic than the actual disease itself. The herbal medicine provides a ray of hope through its cocktail of phyto-compounds, which are believed to act in a synergistic manner, providing excellent healing touch with practically no undesirable side effects, provided its quality is assured off.

The modernization of herbal medicine has also raised quite a more than a few eyebrows in matters related to safety and quality of herbal medicine. In other words, the standardization and quality aspect of herbal medicine becomes a high profile issue. At present, however quality and safety related problems seems to be overshadowing the potential genuine benefits associated with the use of herbal medicine. The problem roots to the lack of high performance, reliable extraction, analytical techniques and methodologies for establishing a standard therapeutic functionality for herbal medicines. Extraction forms the first basic step in

medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants (2).

Yet the extraction step remains often a neglected area, which over the years has received much less attention and research. An efficient or incomplete technique means considerable constraint on the throughput of any method and involves a significant additional workload to staff (3). The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents (4). The fact that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an absolute necessity (5). Keeping in pace with such requirements recent times has witnessed the use and growth of new extraction techniques with shortened extraction time, reduced solvent consumption, increased pollution prevention concern and with special care for thermolabile constituents. Novel extraction methods including microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized solvent extraction (PSE) have drawn significant research attention in the last decade. If these techniques are explored scientifically, can prove out to

be an efficient extraction technology for ensuring the quality of herbal medicines worldwide.

For the past 126 years, Soxhlet extraction has been the most respected among all other conventional techniques (6). It serves a dual purpose of (a): extraction step for the isolation of phyto-constituents and (b): as a well established model for the comparison of new extraction alternatives. One of the major significant shortcomings of Soxhlet extraction is the lengthy extraction time that can be 8, 16, 24 hours or more (7), which results in consumption of considerable time and heat energy. The lengthy time requirement makes it more labor-intensive and limits the number of samples that can be processed which may not be entertained from commercial aspects. Use of large amount of organic solvents requires an additional recovery step and subsequent evaporation to concentrate the extract, resulting in more cumbersome process and also being detrimental to environment

Although many reports have been published on application of microwave heating for extraction of organic compounds and pesticide residue from environmental samples (6-10), microwave has only recently been applied to extraction of plant materials. Very few publications in scientific journals do exist related to this area. So extraction of phyto-constituents by microwave provides a vast scope of research exploration. Some excellent review articles dealing with the application of microwave concept to agricultural and environmental samples have already been published (11-15). This review basically deals with the application of microwave as an extraction tool for the extraction and isolation of phyto-constituents. The paper also provides a short theoretical background of microwave heating and the basic principles of using microwave energy for extraction of phyto-constituents, together with the brief description of the commercially available set up. Then the main parameters influencing the actual extraction conditions are discussed with the presentation of original experimental conditions with different optimization strategies that can be adapted. Finally, the performance of the technique is compared to that of other classical methods. The overall objective behind this review is to highlight the unique capabilities, advantages and use of this modern technique thus enabling the readers to predict its promising future.

## MICROWAVE THEORY

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X- ray and infrared rays in the electromagnetic spectrum (16). In modern day science microwaves serves two major purpose – communication and as energy vectors. The latter application is the direct action of waves on materials that has the ability to convert a part of the absorbed electromagnetic energy to heat energy. Microwaves are made up of two oscillating perpendicular field's i.e. electric field and magnetic field and the former is responsible for heating (16). Unlike conventional heating which depends on conduction – convection phenomenon with eventually much of the heat energy being lost to the environment. Whereas in case of MAE, heating occurs in a targeted and selective manner with practically no heat being lost to the environment

as the heating occurs in a closed system. This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet (1). The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic conduction and dipole rotation, which in most cases occurs simultaneously (6,16). Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field. Heating is affected only at a frequency of 2450 MHz. The electric component of the wave changes $4.9 \times 10^4$ times per second (8). Every time the solvent molecules tries to align itself with the electric field to keep itself in the same phase, but with the electrical component of the wave changing at such a rapid speed, the molecules fails to realign itself and starts vibrating which generates heat through frictional force. With frequency greater than 2450 MHz the electrical component even changes at a much higher speed as a result the molecules doest not get sufficient time to even start to align itself with the external field as a result no heating occurs. If the frequency is less than 2450 MHz the electrical component changes at a much lower speed and the molecules get sufficient time to align itself with the electric field, thus there occurs no heating. The above mechanisms clearly indicate that only dielectric material or solvents with permanent dipoles only do get heated up under microwave. The efficiency with which different solvents heat up under microwave depends on the dissipation factor (tanδ), which is indeed the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules (8). The dissipation factor is given by the equation:

$$\tan \delta = \varepsilon'' / \varepsilon',$$

where $\varepsilon''$ is the dielectric loss which indicates the efficiency of converting microwave energy into heat. $\varepsilon'$ is the dielectric constant which is the measure of the ability to absorb microwave energy. Table 1 lists the dielectric constants and dissipation factors for solvents commonly used in MAE. The table shows that both ethanol and methanol will undergo lesser microwave absorption than water due to their lower $\varepsilon'$ value but the overall heating efficiency for both the solvents will remain higher than water (due to increased tan δ value). Whereas on the other hand hexane and other less polar solvents like chloroform will remain transparent to microwave, thus producing no heat.

### Extraction principle

Even though dried plant material is used for extraction in most cases, but still plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect, evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell (17). The pressure pushes the cell wall from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the ruptures cells

to the surrounding solvent thus improving the yield of phyto-constituents. This phenomenon can even be more intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave (higher tan δ value). Higher temperature attained by microwave radiation can hydrolyze ether linkages of cellulose, which is the main constituent of plant cell wall, and can convert into soluble fractions within 1 to 2 min. The higher temperature attained by the cell wall, during MAE, enhances the dehydration of cellulose and reduces its mechanical strength and this in turn helps solvent to access easily to compounds inside the cell (18). In order to study cell damage during the MAE experiments, tobacco leaf samples were examined by scanning electron microscopy (19). Scanning electron micrographs of the untreated sample, heat-reflux extraction sample and MAE sample revealed that there were no structural difference between heat-reflux extraction and those of untreated samples, except few slight ruptures on the surface of the sample. However, the surface of the sample was found greatly destroyed after MAE. This observation suggests that microwave treatment affects the structure of the cell due to the sudden temperature rise and internal pressure increase. During the rupture process, a rapid exudation of the chemical substance within the cell into the surrounding solvents takes place. This mechanism of MAE based on exposing the analytes to the solvent through cell rupture is different from that of heat-reflux extraction that depends on a series of permeation and solubilization processes to bring the analytes out of the matrix. Destructive changes in the plant tissue of fresh orange peel due to microwave treatment was also observed using scanning electron micrographs (20). These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the release of chemicals. Evidence has also been presented that during the extraction of essential oils from plant materials (21), MAE allows the desorption of compounds of interest out of the plant matrix. This occurs due to the targeted heating of the free water molecules present in the gland and vascular systems; this leads to localized heating causing dramatic expansion, with subsequent rupture of their walls, allowing essential oil to flow towards the organic solvent (22). The effect of microwave energy is strongly dependent on the dielectric susceptibility of both the solvent and solid plant matrix (8). Most of the time, the sample is immersed in a single solvent or mixture of solvents that absorb microwave energy strongly (8). Temperature increases penetration of the solvent into the matrix and constituents are released into the surrounding hot solvent. However in some cases only selective heating of sample matrix is brought about by immersing the sample in a microwave transparent solvent (hexane, chloroform). This approach is particularly useful for thermolabile components to prevent their degradation (6,8,16,17).

Although microwave energy has tremendous heating potential, use of microwave extraction technology have only recently appeared in analytical laboratories. In 1975, Abu-

Samra et al. were the first researchers ever to use a microwave domestic oven in the laboratory, performing trace analysis of metals from biological samples (6). It took almost as long as 10 years for the first publication to appear in 1986 (6,16). Ganzler et al. have developed extraction protocols for lipids, antinutritives and pesticide from soils, seeds foods and feeds in a few milliliters of solvent, irradiated for 30 s up to 7 times in a domestic oven (1140 W). Since then microwave digestion methods have been developed for extraction of different sample types such as plants, biological, environmental, geological and metallic matrices.

## INSTRUMENTS

There are two types of commercially available MAE systems: closed extraction vessels and focused microwave ovens (7,23). The former performs extraction under controlled pressure and temperature. The latter is also named as focused microwave assisted Soxhlet or solvent extraction (FMASE), in which only a part of the extraction vessel containing the sample is irradiated with microwave. However, both the above-mentioned systems are available as multi-mode and single- mode or focused systems (24). A multimode system allows random dispersion of microwave radiation within the microwave cavity, so every zone in the cavity and sample it contains is evenly irradiated (fig. 1). Single mode or focused systems allows focused microwave radiation on a restricted zone where the sample is subjected to a much stronger electric field than in the previous case (fig. 1). Even a modified multimode domestic microwave oven operates as an open vessel extraction system (fig. 2)

Principle elements of a microwave device

Both multi- mode and focused microwave devices comprise four major components:

(a) Microwave generator: magnetron, which generates microwave energy

(b) Wave guide: which is used to propagate the microwave from the source to the microwave cavity

(c) The applicator: where the sample is placed and

(d) Circulator: this allows the microwave to move only in the forward direction.

However, the applicator in case of multi- mode system can be a closed cavity inside which microwaves are randomly dispersed. Uniform distribution of microwave energy inside the cavity can be achieved by using beam reflectors or turntable that makes heating of the sample independent of the position. In focused microwave systems, the extraction vessel is however kept directly in a microwave waveguide and that acts as the applicator. The bottom few inches of the vessel are directly exposed to the microwaves, whereas the upper region of the vessel remains cool as glass is transparent to microwaves and hence does not get heated up in the process. This results in an effective condensing mechanism inherent in the design.

The advantages of closed-vessel systems can be summarized as follows.

a)  They can reach higher temperatures than open vessel systems because the increased pressure inside the vessel raises the boiling point of the solvents used. The higher

temperatures in turn decrease the time needed for the microwave treatment.

b) Loss of volatile substances during microwave irradiation is virtually completely avoided.

c) Less solvent is required. Because no evaporation occurs, there is no need continually to add solvent to maintain the volume. Also, the risk of contamination is avoided as a result there is little or no risk of airborne contamination.

d) The fumes produced during an acid microwave extraction are contained within the vessel, so no provision for handling potentially hazardous fumes need to be made.

By contrast, closed-vessel systems are subject to several shortcomings, as follows.

a) The high pressures used pose safety (explosion) risks.

b) The amount of sample that can be processed is limited

c) The usual constituent material of the vessel, PTFE (polytetraflouro ethylene), does not allow high solution temperatures.

d) The single-step procedure excludes the addition of reagents or solvents during operation.

e) The vessel must be cooled down before it can be opened after the treatment to avoid loss of volatile constituents

Atmospheric pressure (open-vessel) microwave sample preparation can be even more effective than closed-vessel methods. The use of atmospheric pressure provides substantial advantages over pressurized vessels, as follows.

a) Increased safety results from operating at atmospheric pressure with open vessels

b) The ability to add reagents at any time during the treatment.

c) The ability to use vessels made of various materials, including PTFE, glass and quartz.

d) The ease with which excess solvent can be removed.

e) The ability to process large samples.

f) The absence of any requirement for cooling down or depressurization.

g) The low cost of the equipment required.

h) The ability to go through leaching cycles until quantitative removal of the target species is achieved.

i) Fully automatic operation.

j) Open-vessel operation is more suitable with thermolabile species as it uses low temperatures relative to closed-vessel systems.

Despite their many advantages, open-vessel systems are also subject to several shortcomings, as follows.

a) The ensuing methods are usually less precise than those developed using closed-vessel systems.

b) The sample throughput is lower, as most open systems cannot process many samples simultaneously, while closed-vessel systems can handle 8-14 samples at a time.

c) The operation times required to obtain results similar to those of closed-vessel systems are usually longer.

### FACTORS AFFECTING MAE

### Solvent nature and volume

A correct choice of solvent is fundamental for obtaining an optimal extraction process. Solvent choice for MAE is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent (6). Preferably the solvent should have a high selectivity towards the analyte of interest excluding unwanted matrix components. This is important particularly in the extraction of pesticide and organic pollutants from soil sample. Another important aspect is the compatibility of the extracting solvent with further chromatographic analytical steps. MAE can also be performed with the same solvent as used for the conventional extraction methods. However, the optimal extraction of solvents for MAE cannot always be deduced from those used in conventional procedures. MAE of ginger using hexane gave lesser yield than the Soxhlet extraction procedure (25). On the other hand use of ethanol as the extracting solvent gave significantly higher yield than Soxhlet ethanol extraction. This can be accounted due to the difference in dielectric properties of the solvent. Hexane is transparent to microwave and so does not heats up under microwave, whereas ethanol has good microwave absorbing capacity and hence heats up faster and can enhance the extraction process. Thus Dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. The separation efficacy and selectivity of MAE towards separation of color pigments from *Capsicum annum* was investigated using 30 extracting solvent mixtures by spectral mapping technique (26). It was established that both the efficacy and selectivity of MAE depend significantly on the dielectric constant of the extracting solvent mixture. Generally, in most cases mixtures of solvents with good heating efficiency under microwave (high tanδ value) are used and aqueous methanol and ethanol serves the purpose to its best. A methanol- water (90:10) mixture combination proved out to be a significant factor in the MAE of paclitaxel from *Taxus baccata,* evaluated using factorial design (27). Among the various ethanolic concentration studied, use of 95% (v/v) ethanol showed the best optimum results in MAE of tanshinones from *Salvia miltiorrhiza* (28). Small amount of water in the extracting solvent can penetrate easily into the cells of the plant matrix and facilitate better heating of the plant matrix. This in turn increases the mass transfer of the active constituents into the extracting solvent. 80% (v/v) aqueous methanol was found to be the optimum extracting solvent composition for the extraction of chlorogenic and geniposidic acid in MAE of *Eucommia ulmodie* performed under two indexes orthogonal experimental design conditions (29). Several more such use of high microwave absorbing solvent composition are reported in the MAE of coumarin, anthraquinones, safflower yellow and phenolic compounds (30-33). Sometimes mixtures of high and low microwave absorbing solvent composition were found to produce optimum results. Ethanol is a relatively good absorber ($\varepsilon'$ = 25.7) of microwave energy and not a good extraction solvent for solanesol (19). However, hexane is a good extraction solvent but not a good absorber of microwave energy ($\varepsilon'$ = 2.0). Therefore, ethanol and hexane were mixed in different ratios for MAE process. The ratio of hexane to ethanol of 1:3 gave the best percentage extraction of solanesol from tobacco leaves among combinations of hexane

to ethanol tested. When the solvent was only constituted of ethanol, the percentage extracted of solanesol decreased, since ethanol has low solubility for solanesol. Hexane is generally used for the extraction of volatile oil. MAE of essential oils has been patented by Pare in 1991 (16). Inner glandular and vascular systems of the plant material are highly susceptible to microwave irradiation owing to their high natural moisture content (35). Rapid internal heating of these structures brings about effective cell rupture, releasing the analytes into the cold solvent. Solvent free MAE (SFMAE) has been designed for the extraction of volatile oil from several aromatic herbs where the natural moisture content of the plant material serves as the heating source and no extracting solvent are used (35,36).

Volume of the extracting solvent is also a critical factor. The overall knowledge is that the solvent volume must be sufficient to ensure that the plant matrix is always entirely immersed in the solvent throughout the entire irradiation time. There exist many varying reports regarding the volume of solvent to be used with respect to the amount of sample. Generally, a higher ratio of solvent volume to solid matrix may be effective in conventional extraction methods. However, in MAE a higher ratio may yield lower recoveries, which may be due to inadequate stirring of the solvent by microwaves (17). Response surface and contour plots were used to study the effects of extraction of solid: liquid ratio on MAE of pectin (37). It was established that lower volume of solvent led to higher yield of pectin when $p^H$ was about 1. But the above fact may not always be true as there exist reports which claim just the reverse, as in the case of MAE of artemisnin from *Aretimisia annua* L. it was reported that higher extraction rate can be achieved by more amount of solvent (38). MAE of flavonoids from S. medusa dried cell cultures increased with increasing liquid/solid ratio from 25:1 (ml/gm) to 100:1 (ml/gm) (39), but too much of extracting solvent will mean more energy and time to condense the extraction solution in the later step and purification process. Thus, the liquid/solid ratio of 50: 1 (ml/gm) was found suitable to reach the high yield of flavonoids from the dried cell cultures. The amount of plant materials and the volume of extraction solvent used in the microwave assisted extraction reported before were generally ranged between a laboratory scale of milligram and milliliter (39). However in many applications a ratio 10: 1(ml/mg) to 20:1 (ml/mg) was found to be optimum (27,28,29,40,41). The heating efficiency of the solvent under microwave should also be given due consideration as the evaporation of the solvent will depend how rapidly it heats up under microwave. Thus a careful optimization of this parameter is of primary importance in MAE.

### Extraction time

As in other extraction technique, time is another parameter whose influence needs to be taken into account. Generally, by increasing the extraction time, the quantity of analytes extracted is increased, although there is the risk that degradation may occur. Often 15 – 20 min is sufficient, but even 40 sec have been demonstrated to have given excellent recovery (29,37). MAE of polyphenols and caffeine was found

to increase up to 4 min and later decreased with the increase of time (41). In the extraction of artemisnin an overall high of 92% extraction was achieved with 12 min after which extraction yield dropped down (38), as over exposure may lead to thermal degradation of effective constituents. Similar reports also exist in case of MAE of *Salvia militiorrhiza*. But some extraction reports also reveal that varying extraction time does not significantly improves recovery. To determine the time needed to obtain complete recovery, extractions of samples of peppers were performed for different lengths of time. Extraction times of 5, 10, 15, and 20 min were evaluated. The results indicated that, a clear increase of the recovery of capsaicinoids was not obtained with the increase of the extraction time (42). Therefore, 5 min was selected as the extraction time, since this was sufficient to extract all the capsaicinoids present in fresh samples of peppers. A proper study on optimization of extraction time is vital because extraction time may vary with different plant part used. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermolabile constituents

### Microwave Power

Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. Amount of ginsenosides extracted by MAE method under different microwave conditions were studied (56). In general, the extraction efficiency was improved by raising microwave power from 30 to 150 W. During short extraction time (1 and 2 min), recovery was enhanced with increased microwave power. The difference of the ginsenosides extracted between 30 and 150 W appeared to be more significant with short extraction time compared to long extraction time. High power with prolonged exposure always involves the risk of thermal degradation. Reports on the other hand also exist which shows that varying of power from 400 W to 1200 W had no significant effects on the yield of flavonoids extraction (39). However it was seen that extraction time was shortened by 45 min when using 1200 W. In the extraction of embelin, the effect of power level was studied at 300 W and 450 W for 80 sec duration (18). At higher power level settings the extraction pattern was same whereas purity reduced substantially. Rapid rupture of cell wall takes place at higher temperature when kept at higher power, as a result together with the desired analytes impurities are also leached out into the solvent. Whereas at low power levels the cell wall rupture might take place gradually this enables selective MAE. In closed vessel systems, the chosen power settings depends on the number of samples to be extracted during a single extraction run, as up to 12 vessels can be treated in a single run (6). The power must be chosen correctly to avoid excessive temperature, which could lead to solute degradation and overpressure inside the vessel.

### Matrix characteristics

The plant particle size and the status in which it is presented for MAE can have a profound effect on the recoveries of the compounds. The particle sizes of the extracted materials are

generally in the range of 100 μm – 2 mm (17). Fine powder can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave. One of the disadvantages associated with the use of finer particles is difficulty of separation of the matrix from the solvent after microwave irradiation. Generally, centrifugation or filtration is applied to satisfy the above purpose and use of very fine particles may pose some technical problems. In the MAE of ginseng saponins, extraction yield increased with the decrease in particle size (40), but it was also seen that particles less than 60 meshes are not suitable for the filtration of the extracts. A similar report also exists for MAE of cocaine (43). However, there exists an interesting report for MAE of alkaloids from fruits of *Macleaya cordata* that reveals no significant differences in extraction efficiencies between pulverized and non-pulverized fruits (44). The contents of alkaloids in final extracts was however different after further purification process. The status of the plant matrix presented for MAE also needs to be evaluated during the extraction process. Sample pretreatment prior to MAE can bring about effective and selective heating of the plant matrix. The plant matrix may be selectively heated by microwave with the extracting solvent surrounding the sample transparent to microwave. This approach as already explained earlier can be used for the extraction of thermolabile constituents. In MAE of ginger, higher yields were obtained with samples being pretreated with ethanol or water (25). Samples pretreated with solvents with higher microwave absorbing capacity when coupled with extracting solvents like ethanol or methanol bring about heating by at least two competing mechanisms namely, direct heating from the interaction of microwaves with ethanol and heating from the diffusion of excess heat resulting from the interaction of the microwaves with the pretreated matrix. Water soaked samples (4 ml/gm) were used for the extraction of paclitaxel which resulted in better recovery (27). In many cases the natural moisture content of the matrix improves the extraction recoveries, as in the case of extraction of essential oil (34,35). In some cases soaking of the dried plant material in the extracting solvent prior to MAE has resulted in improved yield. This phenomenon is called pre-leaching extraction. Pre-leaching time at room temperature before MAE for 2 min influenced the percentage extraction of tanshinones (28), reaching the highest extraction when pre-leaching time of 45 min was allowed. Allowance of Pre-leaching time at room temperature before MAE slightly influenced the percentage extraction of glycyrrhizic acid from licorice root (45). Increase of pre-leaching time from 4 min to 90 min saw the extraction of polyphenols from green tea leaves increase by 1.53%, while the extraction of caffeine increased by 0.49% (41). These results show that pre-leaching strategy adds up to the extraction efficiency of MAE.

### Temperature

Microwave power and temperature are very interrelated to each other and needs to be given special attention particularly when working with closed vessel system. In closed vessel systems, temperature may reach well above the boiling point of the solvent (16). Table 2 shows the temperature attained by some commonly used microwave solvents under pressurized conditions (16). This elevated temperature does indeed result in improved extraction efficiencies since desorption of analyte from active sites in the matrix will increase. Additionally solvents have higher capacity to solubilize analytes at higher temperature while surface tension and solvent viscosity decreases with temperature, which will improve sample wetting and matrix penetration respectively. Increase in temperature is also associated with increase in pressure in closed systems, which can raise safety concerns. Temperature was found to be a significant factor in the extraction of paclitaxel (27). Temperature can be effectively controlled in open vessel system by proper combinations of extracting solvents which heat up differently. Thorough study of different MAE investigations and from the personal experience of the authors, we present a brief schematic MAE flow chart (fig. 3) for open vessel extraction systems, performed under atmospheric pressure.

### OPTIMIZATION STRATEGIES in MAE

As MAE is influenced by many factors as described and with these factors severely interacting with one another a statistical optimization strategy needs to be adopted for determination of the optimum operating conditions. An orthogonal $L_9$ array design was used for the extraction optimization of *E.ulmodies* (29). Orthogonal array design is a powerful optimization strategy given by Taguchi, where the influence of individual factors on the experiment output and interaction within the factors can be studied in the shortest possible number of experimental trials (46). A full evaluation of four factors with three levels each will require 81 experimental trials with classical approach, but the same objective can be achieved with only 9 trials by using Taguchi $L_9$ array. Design of experiments using Taguchi approach can be effectively used for product and process designs, study the effects of multiple factors on the performance, and solve production problems by objectively laying out the investigative experiments. Taguchi method improves the quality of products and process, which is achieved when a higher level of performance is consistently achieved (47). The highest possible performance is obtained by determining the optimum combination of design factors. The consistency of performance is obtained by making the product / process insensitive to the influence of the uncontrollable factor. Several multivariate approaches had been used as optimization designs. In microwave assisted Soxhlet extraction for olive seeds three variables (namely cycle number, irradiation power and time) at three levels were studied using a central composite design based on the Box and Wilson procedure (48). A $2^4$ factorial design followed by response surface methodology was adapted for the optimization of factors (namely extraction time, temperature, methanol concentration in water and sample solvent loading ratio) involved in extraction paclitaxel (29). A full two level factorial design allowing four degrees of freedom and involving 11 randomized runs including 3 center

*Table 1: Dissipation factor and dielectric constants for some solvents commonly used in MAE.*

| Solvent | Dielectric constant[a] ($\varepsilon'$) | Dielectric loss ($\tan\delta$) $\times 10^{-4}$ |
|---|---|---|
| Acetone | 20.7 | |
| Acetonitrile | 37.5 | |
| Ethanol | 24.3 | 2500 |
| Hexane | 1.89 | |
| Methanol | 32.6 | 6400 |
| 2- propanol | 19.9 | 6700 |
| Water | 78.3 | 1570 |

a: determined at 20°C

*Table 2: Boiling temperatures and temperatures under microwave at 175 psig of different solvents*

| Solvent | Boiling temperature °C | Temperature at 175 psig |
|---|---|---|
| Dichloromethane | 39.8 | 140 |
| Acetone | 56.2 | 164 |
| Methanol | 64.7 | 151 |
| Hexane | 68.7 | nh |
| Ethanol | 78.3 | 164 |
| Acetonitrile | 81.6 | 194 |
| 2- propanol | 82.4 | 145 |
| Petrol ether | 35-80 | nh |
| Acetone/hexane (1:1) | 52 | 156 |

nh = no heat under microwave

*Table 3: Open vessel MAE performed with domestic (modified/unmodified) microwave set up*

| Plant material | Analyte | Solvent | Extraction time | Country | Reference |
|---|---|---|---|---|---|
| *Cuminum cyminum* and *Zanthoxylum bungeanum* | Essential oil | Solvent free extraction | 30 min | China | 36 |
| Whole plant of *Nothapodytes foetida* | Camptothecin | 90% methanol | 7 min | India | 51 |
| Fresh stems and leaves of *Lippia alba* | Essential oil | Solvent free extraction | 30 min | Colombia | 52 |
| Dry fruits of *Macleaya cordata* | Sanguinarine and chelerythrine | 0.1 molelit$^{-1}$ HCl aqueous solution | 5 min | China | 44 |
| Dried roots of *Salvia miltiorrhiza* | Diterpenes like tanshinones | 95% ethanol | 2 min | China | 28 |
| Dried bark of *Eucommia ulmodies* | Geniposidic acid and chlorogenic acid | methanol water mixture | 40 sec | China | 29 |
| Tobacco leaves | Solanesol | hexane:ethanol (1:3) | 40 min | China | 19 |
| Licorice root | Glycyrrhizic acid | 50-60% ethanol with 1-2% ammonia | 4 – 5 min | China | 45 |
| Dried apple pomance | Pectin | Hcl solution | 20.8 min | China | 37 |
| Dried berries of *Embelia ribes* | Embelin | acetone | 80 sec | India | 18 |
| Curcuma rhizomes | Curcumol, curdione and germacrone | water | 4 min | China | 53 |
| Green tea leaves | Polephenols and caffeine | 50% ethanol water mixture | 4 min | China | 41 |
| *Artemisia annua* L. | Artemisnin | #6 extraction oil | 12 min | China | 38 |

*Table 4: Closed vessel MAE performed on different plant materials*

| Plant material | Analyte | Solvent | Extraction time | Country | Reference |
|---|---|---|---|---|---|
| *Pastinaca sativa* fruits | Furanocoumarins | 80 % methanol | 31 min | Poland | 54 |
| Flowering tops of *Melilotus officinalis* L. | Coumarin and melilotic acid | 50% aqueous ethanol | 10 min | Italy | 30 |
| *Hypericum perforatum* and *Thymus vulgaris* | Phenolic compounds | aqueous HCl | 30 min | Czech republic | 33 |
| *Capsicum annum* powders | Pigments | acetone: water (1:1) | 120 sec | Portugal | 26 |
| Dried ginger roots | Ginger | ethanol water mixture | 60 – 120 sec | Venezuela | 25 |
| Roots of *Panax ginseng* | Saponin | 60% ethanol | 30 sec | South Korea and Canada | 40 |
| Dry needles of *Taxus baccata* | Paclitaxel | 90% methanol | 15 min | Iran | 27 |
| Leaves of *Olea europeaea* | Oleuropein and related biophenols | ethanol:water (80:20) | 8 min | Spain | 49 |
| *Cicer arietinum* L. (chickpea seeds) | Saponin | ethanol | 20 min | Israel | 55 |
| *Panax ginseng* roots | Ginsenosides | ethanol | 15 min | Taiwan | 56 |
| Roots of *Morinda citrifolia* | Anthraquinones | 80% ethanol in water | 15 min | Thailand | 31 |
| Olive seeds | Edible oil | hexane | 20-25 min | Spain | 48 |
| *Capsicum frutescens* | Capsaicinoids | ethanol | 5 min | Spain | 42 |

and extractant composition) influencing the extraction of oleuropein and related biophenols from olive leaves (49). Central composite design with response surface strategy was also adapted for pectin extraction from apple pomance. Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing the design process. RSM stems from science discipline in which physical experiments are performed to study the unknown relation between a set of variables and the system output, or response for which only a few experiment values are acquired (46,50). In the 'conventional design' approach, a design is improved by evaluating its 'response' and making design changes based on experience or intuition. This approach does not always lead to the desired result, that of a 'best' design, since the design objectives are often in conflict. It is therefore not always clear how to change the design to achieve the best compromise of theses objectives. The improvement procedure that incorporates design criteria into a mathematical framework is referred to as design optimization. Some of the statistical software's used for the optimization purpose are STATGRAHICS, MINITAB, STATGRAPHICS PLUS, DOE software.

**Advantages over Soxhlet extraction**

MAE has been considered as a potential alternative to traditional solid – liquid extraction. Some of its potential advantages over Soxhlet are highlighted below

1) Significant reduction of extraction time. Extraction time usually ranging from few seconds to few minutes (15 – 20 min)
2) Reduced solvent usage. In MAE only a few milliliter of solvent is required
3) Improved extraction yield
4) Automation provides better accuracy and precision
5) Suitable for thermolabile constituents
6) Can even extract minute traces of constituents including heavy metals and pesticide residue from a few milligram of plant sample
7) Provides agitation during extraction, which improves the mass transfer phenomenon.
8) Instrumental set up like Soxwave combines both the features of Soxhlet and advantages of microwave, thus making extraction even more attractive.

**APPLICATIONS AND COMPARISON WITH CONVENTIONAL TECHNIQUES**

Application of MAE for open and closed vessel systems have been tabulated separately in Table 3,4. Several studies have been reported on the comparison of MAE (mainly in terms of recoveries and duration) with other conventional techniques. In most of the cases Soxhlet has been used as the control experiment. MAE of anthraquinones in pure ethanol at 60°C for 30 min gave a recovery of 65% which was comparable to that which resulted from 3 day maceration in pure ethanol at room temperature (31). Recovery was further enhanced to

*Table 4: Closed vessel MAE performed on different plant materials*

| Plant material | Analyte | Solvent | Extraction time | Country | Reference |
|---|---|---|---|---|---|
| *Pastinaca sativa* fruits | Furanocoumarins | 80 % methanol | 31 min | Poland | 54 |
| Flowering tops of *Melilotus officinalis* L. | Coumarin and melilotic acid | 50% aqueous ethanol | 10 min | Italy | 30 |
| *Hypericum perforatum* and *Thymus vulgaris* | Phenolic compounds | aqueous HCl | 30 min | Czech republic | 33 |
| *Capsicum annum* powders | Pigments | acetone: water (1:1) | 120 sec | Portugal | 26 |
| Dried ginger roots | Ginger | ethanol water mixture | 60 – 120 sec | Venezuela | 25 |
| Roots of *Panax ginseng* | Saponin | 60% ethanol | 30 sec | South Korea and Canada | 40 |
| Dry needles of *Taxus baccata* | Paclitaxel | 90% methanol | 15 min | Iran | 27 |
| Leaves of *Olea europeaea* | Oleuropein and related biophenols | ethanol:water (80:20) | 8 min | Spain | 49 |
| *Cicer arietinum* L. (chickpea seeds) | Saponin | ethanol | 20 min | Israel | 55 |
| *Panax ginseng* roots | Ginsenosides | ethanol | 15 min | Taiwan | 56 |
| Roots of *Morinda citrifolia* | Anthraquinones | 80% ethanol in water | 15 min | Thailand | 31 |
| Olive seeds | Edible oil | hexane | 20-25 min | Spain | 48 |
| *Capsicum frutescens* | Capsaicinoids | ethanol | 5 min | Spain | 42 |

and extractant composition) influencing the extraction of oleuropein and related biophenols from olive leaves (49). Central composite design with response surface strategy was also adapted for pectin extraction from apple pomance. Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing the design process. RSM stems from science discipline in which physical experiments are performed to study the unknown relation between a set of variables and the system output, or response for which only a few experiment values are acquired (46,50). In the 'conventional design' approach, a design is improved by evaluating its 'response' and making design changes based on experience or intuition. This approach does not always lead to the desired result, that of a 'best' design, since the design objectives are often in conflict. It is therefore not always clear how to change the design to achieve the best compromise of theses objectives. The improvement procedure that incorporates design criteria into a mathematical framework is referred to as design optimization. Some of the statistical software's used for the optimization purpose are STATGRAHICS, MINITAB, STATGRAPHICS PLUS, DOE software.

**Advantages over Soxhlet extraction**

MAE has been considered as a potential alternative to traditional solid – liquid extraction. Some of its potential advantages over Soxhlet are highlighted below

1) Significant reduction of extraction time. Extraction time usually ranging from few seconds to few minutes (15 – 20 min)
2) Reduced solvent usage. In MAE only a few milliliter of solvent is required
3) Improved extraction yield
4) Automation provides better accuracy and precision
5) Suitable for thermolabile constituents
6) Can even extract minute traces of constituents including heavy metals and pesticide residue from a few milligram of plant sample
7) Provides agitation during extraction, which improves the mass transfer phenomenon.
8) Instrumental set up like Soxwave combines both the features of Soxhlet and advantages of microwave, thus making extraction even more attractive.

**APPLICATIONS AND COMPARISON WITH CONVENTIONAL TECHNIQUES**

Application of MAE for open and closed vessel systems have been tabulated separately in Table 3,4. Several studies have been reported on the comparison of MAE (mainly in terms of recoveries and duration) with other conventional techniques. In most of the cases Soxhlet has been used as the control experiment. MAE of anthraquinones in pure ethanol at 60°C for 30 min gave a recovery of 65% which was comparable to that which resulted from 3 day maceration in pure ethanol at room temperature (31). Recovery was further enhanced to
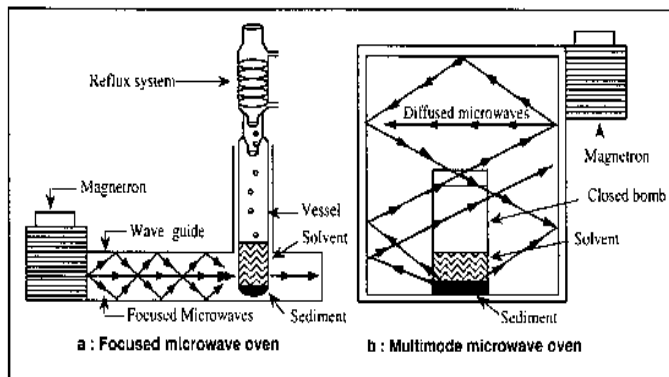
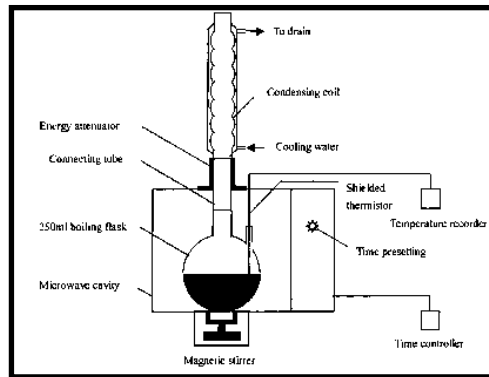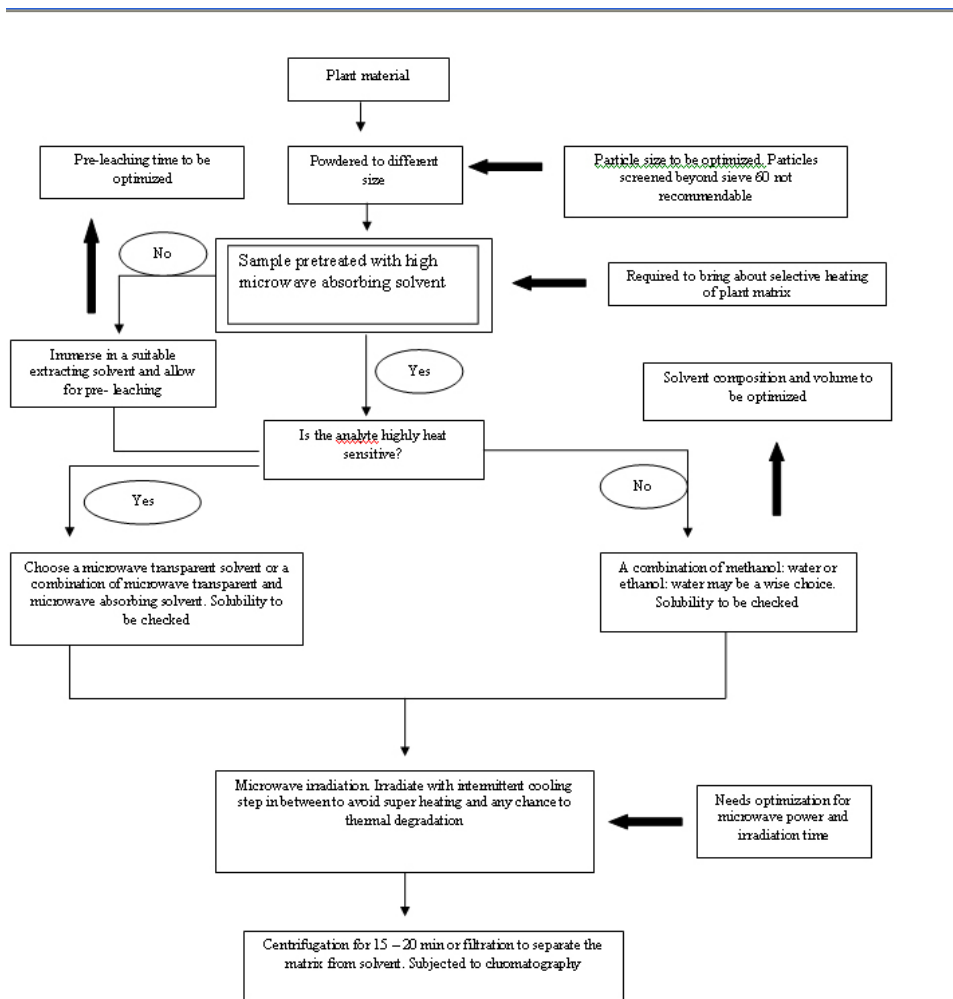*Fig. 1: Monomode (a) versus multimode systems (b)*



*Fig. 2: Scheme of a modified multimode domestic microwave oven (open vessel extraction)*



*Thicker arrows indicates the steps which needs proper optimization to determine the optimum extraction conditions to attain the maximum yield*

*Fig. 3: Extraction scheme for open vessel MAE system*

points was used for a screening study of the behavior of the three variables (namely irradiation time, irradiation power 96% when ethanol- water mixture was used as the extracting solvent; the results were then comparable to 4 hrs of Soxhlet extraction. MAE of artemisnin for 12 min gave much higher yield than that of Soxhlet extraction for 12 hrs (38). In the extraction of capscicinoids from peppers, conventional stirring extraction took minimum of 15 min to obtain extractions of more than 95% of the quantities of capsaicinoids that are obtained within 5 min of MAE (42). Extraction time for solvent free microwave extraction (SFME) of essential oils from *Zingiber officinale* and *Illicium verum* took only 30 min whereas hydro distillation method (HD) took 80 min (35). The electricity consumption cost for SFME was only 0.04KWh$^{-1}$ whereas for hydro distillation it was 0.75 KWh$^{-1}$. Extraction of essential oil from aromatic herbs requires energy of 4.5KWh$^{-1}$, whereas SFME requires only 0.25 KWh$^{-1}$. At the same time, the calculated quantity of $CO_2$ rejected in the atmosphere is dramatically more in the case of HD (3600 gm $CO_2$ per gm of essential oil). HD requires an extraction time of 270 min for heating 6 kg of water and 500 gm of plant material to the extraction temperature, followed by evaporation of water and essential oil (34). The SFME method required heating for 30 min only of the plant matter and evaporation of the *insitu* water and essential oil of the plant material. MAE of paclitaxel showed considerable reduction of time (15 min vs. 16 hr) and solvent consumption (10 ml vs. 100 ml) (27). In terms of selectivity, MAE using aqueous methanol as solvent, yielded extracts that could be directly analyzed by HPLC without the need for further purification step, whereas purification is an unavoidable aspect of conventional extraction. Reduction in extraction time was reported for MAE of oleuropein from olive leaves (8 min versus 24 hr at 40°C) than when compared to conventional techniques (48). Four different extraction methods were compared for extraction of sanguinarine and chelerythrine from fruits of *Macleay cordata* (44), and it was seen that maceration and MAE reported to have produced significantly higher values with 30 min and 5 min as the extraction time respectively. Ultrasound and percolation gave lesser yield with 30 min and 50 min as the extraction time respectively. Highest percentage of Solanesol reached 0.91% in 40 min of MAE under optimal NaoH concentration; on the other hand heat reflux produced 0.87% extraction in 180 min (19). Similar reports related to reduction of extraction in extraction time and solvent volume has been reported in many more MAE of natural products.

## CONCLUSION

Medicinal plant research is aimed at the isolation and identification of naturally occurring substances. Chemical analysis of extracts from plant material will play a central role in the development and modernization of herbal medicine. The majority of extraction procedures for the determination of plant metabolites are developed in such a way that the final extract introduced into the GC and HPLC columns contains only the analytes with all interferences removed. This is one area where conventional techniques have spelled utter disaster. MAE has risen rapidly in the last decade, and for most applications it has proven to be effective in all aspects compared to traditional extraction techniques. The need for development of existing methods of separation and introduction of new techniques of high resolution and effectiveness must be seriously felt. In all probability, such developments will give rise to the discovery of new effective compounds from phyto- pharmaceutical sources. More research is needed to improve the understanding of extraction mechanism, remove technical barriers, improve the design and scale up of the novel extraction systems for their better industrial applications.

## REFERENCES

1. C.W. Huie. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. *Anal. Bioanal. Chem*. **373:** 23-30 (2002).
2. G. Romanik, E. Gilgenast, A. Przyjazny, J. Namiesnik and M. Kaminski. *J. Biochim. Biophys. Methods*. Article in press. (2007)
3. R.M. Smith. Before the injection- modern methods of sample preparation for Techniques of preparing plant material for chromatographic separation and analysis. *J. Chromatogr. A*. **1000:** 3-27 (2003).
4. M.D. Luque de Castro and L.E. Garcia-Ayuso. Soxhlet extraction of solid matrices: an outdated technique with a promising innovative future. *Anal. Chim. Acta*. **369:** 1-10 (1998).
5. S. Nyiredy. Separation strategies of plant constituents- current status. *J. Chromatogr. B*. **812:** 35-51 (2004).
6. M. letellier, H. Budzinski, L. Charrier, S. Capes and A.M. Dorthe. Optimization by factorial design of focused microwave assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. *J. Anal. Chem*. **364:** 228-37 (1999).
7. Pastot, E. Vazquez, R. Ciscar and M. De la Guardia. *Anal. Chim. Acta*. **344:** 241-49 (1997).
8. O. Zuloaga, N. Etxebarria, L.A. Fernandez and J.M. Madariaga. Optimization and comparison of microwave assisted extraction and Soxhlet extraction for the determination of polychlorinated biphenyls in soil samples using an experimental design approach. *Talanta*. **50:** 345-57 (1999).
9. R. Sanghi and S.S. Kannamkumarath. Comparison of extraction methods by Soxhlet, sonicator and microwave in the screening of pesticide residues from solid matrices. *J. Anal. Chem*. **59(11):** 1032-36 (2004).
10. S. Karthikeyan, R. Balasubramanian and S.W. See. Optimization and validation of a low temperature microwave assisted extraction method for analysis of polycyclic aromatic hydrocarbons in airborne particulate matter. *Talanta*. **69:** 79- 86 (2006).
11. C.S. Eskilsson and E. Bjorklund. Analytical scale microwave assisted extraction. *J. chromatogr. A*. **902:** 227-50 (2000).
12. O. Nara. An ecofriendly extractor. *Talanta*. **68:** 522-26 (2006).
13. V. Camel. Microwave assisted solvent extraction of environmental samples. *Trends Anal. Chem*. **19(4):** 229-47 (200).
14. M. Al-Harahsheh and S.W. Kingman. Microwave assisted leaching- a review. *Hydrometallurgy*. **73:** 189-03 (2004).
15. R.E. Haque. Microwave energy for mineral treatment process- a brief review. *Int. J. Miner. Process*. **57:** 1-24 (1999).
16. M. Letellier and H. Budzinski. Microwave assisted extraction of organic compounds. *Analusis*. **27:** 259-71 (1999).
17. L. Wang and C.L. Weller. Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci. Technol*. **17:** 300-12 (2006).
18. C. Latha. Microwave assisted extraction of embelin from *Embelia ribes*. *Biotechnol. Lett*. **9243:** 10529 (2006).
19. H. Zhou and C. Liu. Microwave assisted extraction of solanesol from tobacco leaves. *J. Chromatogr. A*. **1129:** 135-39 (2006).
20. M.Kratchanova, E. Pavlova and I. Panchev. The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carbohydr. Polym*. **56:** 181-86 (2004).
21. M.D. Luque de Castro, M.M. Jimenez-Carmona and V. Fernandez. Towards more rational techniques for the isolation of valuable essential oils from plants. *Trends Anal. Chem*. **18(11):** 708-16 (1999).
22. L.E. Garcia-Ayusa, J. Velasco, M.C. Dabarganes and M.D. Luque de Castro. Determination of the oil content of seeds by focused microwave assisted Soxhlet extraction. *Chromatographia*. **52:** 102-08 (2000).
23. J.L. Luque-Garcia and M.D. Luque de Castro. Focused microwave assisted Soxhlet extraction: devices and applications. *Talanta*. **64:** 571-77 (2004).
24. J.L. Luque-Garcia and M.D. Luque de Castro. Where is microwave based analytical treatment for solid sample pre-treatment going?. *Trends Anal. Chem*. **22(2):** 90-99 (2003).
25. M.J. Alfaro, J.M.R. Belanger, F.C. Padilla and J.R.R. Pare. Influence of solvent, matrix dielectric properties and applied power on the liquid-phase microwave assisted extraction of ginger (*Zingiber officinale*). *Food Res. Int*. **36:** 499-504 (2003).

26. G.A.C. Kiss, E. Forgacs, T.C. Serati, T. Mota, H. Morais and A. Ramos. Optimisation of the microwave assisted extraction of pigments from paprika (*Capsicum annum* L.) powders. *J. Chromatog. A*. **889:** 41-49 (2000).

27. M. Talebi, A. Ghassempour, Z. Talebpour, A. Rassouli and L. Dolatyari. Optimization of the extraction of paclitaxel from *Taxus baccata* L. by the use of microwave energy. *J. Sep. Sci.* **27:** 1130-36 (2004).

28. X. Pan, G. Niu and H. Liu. Microwave assisted extraction of tanshinones from *Salvia miltiorrhiza* bunge with analysis by high performance liquid chromatography. *J. Cromatogr. A*. **922:** 371-75 (2001).

29. H. Li, B. Chen, Z. Zhang and S. Yao. Focused microwave assisted solvent extraction and HPLC determination of effective constituents in *Eucommia ulmodies* Oliv. *Talanta*. **63:** 659-65 (2004).

30. E. Martino, I. Ramaiola, M. Urbano, F. Bracco and S. Collina. Microwave assisted extraction of coumarin and related compounds from *Melilotus officinalis* L. as an alternative to Soxhlet and ultrasound assisted extraction. *J. Chromatogr. A*. **1125:** 147-51 (2006).

31. S. Hemwimon, P. Pavasant and A. Shotiprule. Microwave assisted extraction of antioxidative anthraquinones from roots of Morinda *citrifolia*. *Sep. Purif. Technol*. Article in press (2007).

32. L. Chan, L. Ding, H. Zhang, J. Hi, Y. Wang, X. Wang, C. Qu and H. Zhang. Dynamic microwave assisted extraction coupled with on-line spectrophotometric determination of safflower yellow in *Flos carthami*. *Anal. Chim. Acta*. **580:** 75-82 (2006).

33. D. Sterbova, D. Matejicek, J. Vlcek and V. Kuban. Combined microwave assisted isolation and solid-phase purification procedures prior to the chromatographic determination of phenolic compounds in plant materials. *Anal. Chim. Acta*. **513:** 435-44 (2004).

34. M.E. Lucchesi, F. Chemat and J. Smalja. Solvent free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydrodistillation. *J. Chromatogr. A*. **1043:** 323-27 (2004).

35. Z. Wang, L. Wang, T. Li, X. Zhon, L. Ding, Y. Yu, A. Yu and H. Zhang. Rapid analysis of the essential oils from dried *Illicum verum* Hook. and *Zingiber Officinale*. Rosc. by improved solvent free extraction microwave with three types of microwave absorption medium. *Anal. Bioanal. Chem*. **386:** 1863-68 (2006).

36. Z. Wang, L.Ding, T. Li, X. Zhou, L. Wang, H. Zhang, L. Liu, Y. Li, Z. Liu, H. Wang, H. Zeng and H. He. Improved solvent free microwave extraction of essential oil from dried *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim. *J. Chromatogr. A*. **1102:** 11-17 (2006).

37. S. wang, F. Chan, J. Wu, Z. Wang, X.Liao and X. Ha. Optimization of pectin extraction assisted by microwave from apple pomance using response surface methodology. *J. Food Eng*. Article in press (2007).

38. J. Hao, W. Han, S. Huang, D. Xue and X. Deng. Microwave assisted extraction of artemisnin from Artemisia annua L. *Sep. Purif. Technol*. 28: 191-196 (2002).

39. M. Gao, B. Song and C. Lin. Dynamic microwave assisted extraction of flavonoids from *Saussurea medusa* Maxim. Cultured cells. *Biochem. Eng. J*. **332:** 79-83 (2006).

40. J. Kwon, G. Lee, J.M.R. Belanger and J.R.J. Pare. Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave assisted process. *Int. J. Food Sci. Technol*. **38:** 615-22 (2003).

41. X. Pan, G. Niu, and H. Liu. Microwave assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process*. **42:** 129-33 (2003).

42. G.F. Barbero, M. Palma and C.G. Barroso. Determination of capsaicinoids in peppers by microwave assisted extraction high performance liquid chromatography with fluorescence detection. *Anal. Chimca. Acta*. **578:** 227-33 (2006).

43. S. Bieri, Y. Ilias, C. Bicchi, J.L. Veuthey and P. Christen. Focused microwave assisted extraction combined with solid phase micro extraction and gas chromatography- mass spectrometry for the selective analysis of cocaine from coca leaves. J. Chromatogr. A. **1112:** 127-32 (2006).

44. F. Zhang, B. Chen, S. Xiao and S. Yao. Optimization and comparison of different extraction techniques for sanguinarine and chelerythrine in fruits of *Macleaya cordata* (Wild) R. Br. *Sep. Purif. Technol*. **42:** 283- 283- 90 (2005).

45. X. Pan, H. Liu, G. Jia and Y.Y. Shu. Microwave assisted extraction of glycyrrhizic acid from licorice root. *Biochem. Eng. J*. 5: 173-77 (2000).

46. R.K. Roy. Design of Experiments using Taguchi Approach: 16 Steps to Product and Process (J. Willey and Sons, 2001). ISBN: 0471361011.

47. D.M. Osborne and R.L. Armacost. Review of techniques for optimizing multiple quality characteristics in product development. *Computers Ind. Engng*. **31(1/2):** 107-10 (1996).

48. L.E. Garcia-Ayuso, M.D. Luque de Castro. A multivariate study of the performance of a microwave assisted Soxhlet extractor for olive seeds. *Anal. Chim. Acta*. **382:** 309-16 (1999).

49. R. Japon-Lujan, J.M. Luque-Rodrigue and M.D. Luque de Castro. Multivariate optimization of the microwave assisted extraction of oleuropein and related biophenols from olive leaves. *Anal Bioanal Chem*. **385:** 753-59 (2006).

50. A review of robust optimal design and its application in dynamics. *Comput. Struct*. **83:** 315-26 (2005).

51. D.P. Fulzele and R.K. Satdive. Comparison of techniques for the extraction of the anticancer drug camptothecin from *Nothapodytes foetida*. *J. Chromatogr. A*. **1063:** 9-13 (2005).

52. E.E. Stashenko, B.E. Jaramillo and J.R. Martinez. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Columbia and evaluation of its in vitro antioxidant activity. *J. Chromatogr. A*. **1025:** 93-103 (2004).

53. C. Deng, J, Ji, N. Li, Y. Yu, G. Duan and X. Zhang. Fast determination of curcumol, curdione and germacrone in three species of curcuma rhizomes by microwave assisted extraction followed by headspace solid phase microextraction and gas chromatography- mass spectrometry. *J. Chromatogr. A*. **1117:** 115-20 (2006).

54. M. Waksmundzka-Hajnas, a. Petruczynik, A. Dragan, D. Wianowska, A.L. Dawidowicz and I. Sowa. Influence of the extraction mode on the yield of some furanocoumarins from *Pastinaca sativa* fruits. *J. Chromatogr. B*. **800:** 181-87 (2004).

55. Z. Kerem, H. German-Shashoua and O. Yarden. Microwave assisted extraction of bioactive saponins from chickpea (*Cicer arietinum* L.). *J. Sci. Food Agric*. **85:** 406-12 (2005).

56. Y.Y. Shu, M.Y. Ko and Y.S. Chang. Microwave assisted extraction of ginsenosides from ginseng root. *Microchem. J*. **74:** 131-39 (2003).

**\*\*\*\*\*\***