

REVIEW

Microwave assisted extraction of phytochemicals an efficient and modern approach for botanicals and pharmaceuticals

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Abstract: The final quality of pharmaceutically active herbal preparation is significantly contributed by extraction procedures. Hence in the last decade Microwave assisted extraction (MAE) has been introduced. This is an efficient and modern tool with multiple benefits as compared to the traditional methods of extraction. The benefits are in terms of reduction in cost, time of extraction, amount of solvent used, energy consumptions and low CO₂ emission. Therefore present study was planned to give brief overview on applications of microwave assisted extraction of natural products. It is also discussed that how the various parameters of microwave assisted extraction like nature of the solvent, temperature, particle size, power level of microwaves and time of irradiation influences the extract yields of plant parts. This review also emphasizes the application of MAE for increased production of phyto-medicines, sweeteners, spices and all other commercial products related to botanicals.

Keywords: Extraction, microwave assisted extraction, microwaves, pharmaceuticals, phytochemical, power level of microwaves.

INTRODUCTION

Herbal medicines have been evolving side by side of human culture. Plants are considered as natural factories for production of various phytochemicals. A large number of secondary metabolites like alkaloids, phenolics and flavonoids are synthesized by plants in addition to compounds that are needed for the reproduction and growth of plants. Advancements in natural sciences led researchers towards identification and isolation of different bioactive phytochemicals (table 1). Depending on physical nature and the properties of phyto-constituents, various methods are in use to obtain the crude extract. Among these various conventional extraction methods including infusion, digestion, decoction, percolation and maceration are commonly practiced in herbal industry for crude extraction (Siti *et al.*, 2016). Most Pharmacopeias mentioned the use of percolation and maceration to obtain plants' crude extractions. However traditional methods of extraction are time taking, like, 2-7 hours at least are required for maceration, and this process also requires a large amount of solvents (Regasini *et al.*, 2008). In case of Soxhlet extraction the targeted molecules might be decomposed due to high temperatures (Afoakwah *et al.*, 2012). Therefore there was space for invention of new techniques. Hence alternative extraction techniques like, Supercritical Fluid Extraction (SCF), Accelerated Solvent Extraction (ASE) and Ultrasonic Assisted Extraction (UAE) are invented to compensate the increasing demand

of natural products. These techniques reduced the consumption of organic solvent, enabled automation and shortened extraction time (Devgun *et al.*, 2009; Chan *et al.*, 2011).

Microwave assisted extraction is one of the advanced techniques under thought now a days. In MAE, microwave vitality is utilized to concentrate plant metabolites with the solvents. This system has demonstrated its wellbeing for the vast majority of the specimens because of the ease to handle and to understand steadiness. Exploration is continuing for functional use of microwaves for business creation of phyto-constituents, yet at the same time in early stages (Devgun *et al.*, 2009).

Principle of microwave assisted extraction

Microwaves are part of electromagnetic spectrum of light with a range of 300 MHz to 300 GHz and wavelengths of these waves range from 1cm to 1m (Mandal *et al.*, 2007). These waves are made up of two perpendicular oscillating fields which are used as energy and information carriers. First application of microwaves includes its interaction with the specific materials which can absorb a part of its electromagnetic energy and can convert it into heat. Commercial microwaves use 2450 MHz of energy for this purpose which is almost equivalent to 600-700W (Afoakwah *et al.*, 2012).

Practically, microwaves induce dipole rotation in organic molecules along with heating which causes the destruction of hydrogen bonding. This causes the traffic

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of ions which results in a heating effect due to increased kinetic energies of ions as well as friction between ions due to their continuous movements and change in directions. Destruction of hydrogen bonding also increases the penetrating efficiency of the solvents into the plant matrix (Hudaib *et al.*, 2003; Datta *et al.*, 2005; Tang, 2005).

Instrumentation

There are two different types of microwaves instruments in use (closed vessel and open vessel microwaves). In a closed vessel system, extraction is done under controlled temperature and pressure and it is the most common procedure in microwave assisted extraction, while under atmospheric pressure an open extracting vessel is used as an alternative approach for microwave assisted extractions (Kaufmann and Christen 2002).

There are four major components of both open cell and closed vessel systems:

1. Microwave generator: magnetron, it generates microwave energy.
2. Wave guide is used to propagate the microwaves from the source to the microwave cavity.
3. The applicator: where the sample is placed.
4. Circulator: this allows the microwave to move only in the forward direction (Mandal *et al.*, 2007; Vollmer, 1994).

Closed vessel systems

For acid mineralization or digestion and to obtain extract under drastic conditions, closed vessel systems are generally recommended, where they may be heated above their atmospheric boiling points (Jassie *et al.*, 1997). In closed vessel systems (fig 1), Teflon cells are used and any solvent can be chosen. But pressure inside the vessel depends upon the vapor pressure of the selected solvent. Interior temperature can also be controlled as it is directly proportional to the power level used. A maximum of 600-1000W can be attained in domestic microwaves ovens. But it is avoided as high temperatures cause destruction of thermo labile or temperature sensitive compounds. It is also important when volatile compounds are involved, in such cases cooling of vessels is important before opening but it increases the extraction time. Sometimes intermediate filtering or centrifugation is also required to separate the solid residue (Kaufmann and Christen 2002).

Followings are the advantages of closed-vessel systems:

- a) Higher temperatures are attained rapidly due to increased pressure inside the vessel
- b) Volatile compounds are not wasted in environment but remain as a part of extract.
- c) There is no or lesser risk of contamination due to closed systems.

- d) As solvent is not evaporated during heating, so a very little amount of solvent is required in this procedure
- e) The procedures like acid digestions become safer as fumes do not come out, so it becomes easy to handle such procedures (Mandal *et al.*, 2007).

Open cells

In open vessel systems (fig 2), quartz cells are used, work under atmospheric pressures and depend solely on boiling points of solvents for heating purposes or to attain maximum temperature. Solvent is heated and refluxed back through condenser, like soxhlet extraction, cellulose cartridge can also be used to carry plant sample as it helps to avoid an extra step of filtration. Open vessel system can be used to extract larger quantities of plant sample. Open vessel systems are also considered as safer in solvent handling (Letellier *et al.*, 1999; Kaufmann and Christen 2002).

Parameters of microwave assisted extraction

Whenever working with microwave assisted extraction, efficiency of the process can be enhanced by considering the solvent type, solvent to plant material ratio, extracting power of microwaves, resultant temperatures, time of extraction as well as nature of plant matrix and targeted molecule. We can have significant results only by considering above mentioned factors in microwave assisted extraction (Veggi *et al.*, 2013).

Solvent nature and volume

To optimize a protocol for microwave assisted extraction of any plant material, solvent choice is most crucial step depending on interaction of plant material and solvent molecule, solubility of target molecule in the solvent and microwaves absorbing capacity of solvent (Nyiredy, 2004). In fact choice of solvent determines that how finely and easily analytes of interest can be separated from plant matrix and unwanted plant residue. Then compatibility of the solvent selected with the next analytical technique like chromatography, spectroscopy etc. is very important, furthermore solvent used for conventional extraction methods can be used in microwave assisted extraction but it does not always guarantee to have optimized procedure for microwave assisted extraction. The Soxhlet extraction procedure of ginger gave higher yield by using hexane as compare to the MAE (Kiss *et al.*, 2000).

Solvents with high dielectric constants are considered to be better options for MAE, because the solvents which are nonpolar, are transparent to microwaves, as a result they are not heated, like *n*-hexane. If non-polar solvents are to be used according to the nature of the targeted molecule then this constrain is eliminated by adding a few drops of polar solvents to heat non-polar solvents (Tatke & Jaiswal 2011; Chen *et al.*, 2008; Mandal *et al.*, 2007; Brachet *et al.*, 2002).

Table 1: Pharmaceutical importance of Phyto-constituents

Sr. #	Phyto-constituent	Pharmaceutical importance	Reference
1	Alkaloids	Anti- cancer, pre-anesthetic in surgery, child birth, ophthalmology, in delirium, tremor, menia and parkinsonianism	Joy <i>et al.</i> , 2001; Soni <i>et al.</i> , 2012
2	Beta Carboline alkaloids	Anti-depressant, Anti-tumor, Anti-bacterial, Anti-inflammatory, Assisting detoxifying enzymes, Cardio-protective.	Patel <i>et al.</i> , 2012
3	Flavonoids	Lower blood cholesterol, Anti-oxidants, Anti-hypertensive, Anti-microbleeding, Hepato-protective, Anti-bacterial, Anti-HIV-I reverse transcriptase, Anti-dengue, Inhabitants of viral polymerase	Kumar & Pandey, 2013; Ghasemzadeh & Ghasemzadeh, 2011
4	Phenolics	Anti-cancer	Ozcan <i>et al.</i> , 2014; Raine <i>et al.</i> , 2014
5	Steroid	Anticancer, Anti asthmatic, Ant diabetic	Sheikh <i>et al.</i> , 2013
6	Tannins	Immuno-modulatory, Anti- bacterial, Anti-fungal, Hypolipidemic, Cardio-protective	Tariq & Reyaz, 2012

Extraction time

Extraction time is an essential parameter to achieve quality product. Usually, the quantity of analytes extracted is increased, by increasing the extraction time, despite of the risk of degradation. Different ranges of time are used in different experiments of microwave assisted extraction of plant metabolites. Sometimes 15-20 minutes are required and even 30 seconds have been reported for maximum production of metabolite in microwave assisted extraction (Wang *et al.*, 2007).

Nature of solvents used for extraction also contribute toward determination of extraction time. Highly polar solvents like water and methanol are tremendously heated (above their boiling points in a very short time) after longer exposure to microwaves. So thermo labile components of extracts are damaged or may be damaged. Researchers have shown that on increasing time of extraction from a certain limit, yield of extract also decreases (Mandal *et al.*, 2007). For example a clear increase of the recovery of capsaicinoids was not obtained with the increase of the extraction time indicated by the results of Barbero *et al.* (2006) while an increase in the amount of stevioside with increasing time of extraction was reported by Javad *et al.* (2014). An optimized protocol for microwave assisted extraction of a particular compound can enhance its commercial production.

Microwave power

Microwave power is inversely proportional to irradiation time. Optimum combination of time and power level can enhance the extraction of targeted molecules manifold. Longer exposure at lower power levels considered more appropriate in most of extraction cases (Javad *et al.*, 2014). It has been proven effective for a number of times e.g., under different microwave conditions, the amount of extract and a particular metabolite by microwave assisted extraction method was higher with increased power levels of microwaves from 30-150 W, it was further reported

that extraction efficiency was improved for short duration of irradiation by setting higher power levels (Shu *et al.*, 2003; Desai *et al.*, 2010). But high power with prolonged exposure always involve with the risk of thermal degradation.

Higher power levels cause sudden rupture of cells, which may cause increase in the amount of extract but that extract becomes a complex mixture from where it is difficult to isolate a single compound of interest. While in case of lower power levels, cell wall bursts at first and then cell membrane bursts slowly. It allows the step by step efflux of the plant exudates which enhances the quality of product and enable the researcher to isolate a comparatively pure product in stable form with lesser intermediate steps, provided other factors are considered carefully (Talebi, 2004; Mandal *et al.*, 2007).

Matrix characteristics

In microwave extraction of plant extracts the physical state of the material is an important factor. Usually small sized materials like 100µm to 2mm are considered to be better option because small size of the particles increases the surface area of the plant material to be extracted (Wang *et al.*, 2006). It can enhance the extraction efficiency of the solvent in two ways

- I. By increasing the contact between solvent and plant matrix
- II. By increasing the penetration power of the microwaves (Eskilsson and Bjorklund, 2000)

But problem related to lesser mesh size comes in front when filtration is to be done after MAE. Small sized particles are not filtered easily and pose problems to the extract quantity as well as quality. Such problems can be solved by centrifugation. But it was also seen that particles less than 60 meshes are not suitable for the filtration of the extracts (Bieri *et al.*, 2006). It is well reported by Kwon *et al.* (2003) that with the decrease in

Table 2: Efficacy of Microwave assisted extraction of Phyto-constituents

Medicinal Plant	Time for		Solvent optimized	Targeted Phyto-constituent	Reference
	MAE	Cold mac./soxhlet/ltrasonics			
<i>Adathoda vasica & Cymbopogan citratus</i>	210 sec	10 hrs	Water	All Phyto-chemicals	Simha <i>et al.</i> , 2016
<i>Aromatic herbs</i>	30 min	4.5 hrs	Water	Essential oil	Marie <i>et al.</i> , 2004
<i>Artemisia annua</i>	12 min	Hrs	Oil	Artemisin	Hao <i>et al.</i> , 2002
<i>Bixa orellana</i>	80 sec		Double Dist. Water	Natural Dye	Sinha <i>et al.</i> , 2013
<i>Buddleia officinalis</i>	25 min		Ethanol	Phenolics	Pan <i>et al.</i> , 2008
<i>Caraway seeds</i>			Ethanol	Terpenes	Chemat <i>et al.</i> , 2005
<i>Chinese herbs</i>			Methanol	Rutin	Zeng <i>et al.</i> , 2010
<i>Cicer arietinum</i>	20 min	3 hrs	Methanol	Saponins	Kerem <i>et al.</i> , 2005
<i>Citrus mandarium</i>	49 sec		Methanol	Phenolics	Hayat <i>et al.</i> , 2009
<i>Conicera japonica</i>	5 min		Ethanol	Chlorogenic acid	Zhang <i>et al.</i> , 2008
<i>Cortex fraxini</i>	10 min		PEG Solution	Flavone and Coumarins	Zhou <i>et al.</i> , 2011
<i>Curcuma longa</i>	4 min		Methanol	Curcumin	Mandal <i>et al.</i> , 2007
<i>Dimicarpus longa</i>	30 min	2 hrs	Ethanol	Phenolics	Pan <i>et al.</i> , 2008
<i>Flax seed</i>	3 min	3-48 hrs	Methanol	Phenolics	Beejmohan <i>et al.</i> , 2007
<i>Green coffee bean</i>	5 min		Water And Ethanol	Chlorogenic acid	Upadaya
<i>Mentha spicata</i>			70% Ethanol With Water	Flavonoid	Bimkr <i>et al.</i> , 2011
<i>Morinda citrifolia</i>	Less time	More time	Methanol		Hermiwinan <i>et al.</i> , 2007
<i>Nelumbo nucifera</i>	90 sec	2 hrs	Water	Phenolics alkaloids	Lu <i>et al.</i> , 2008
<i>Pea nut skin</i>	30 sec		Ethanol, Water	Phenolics	Tameshia <i>et al.</i> , 2010
<i>Radix astragali</i>	25 min		Ethanol	Flavonoids	Xiao <i>et al.</i> ,
<i>Salvi miltiorriza</i>	2 min		95 % Ethanol	tanshinones	Pan <i>et al.</i> , 2001
<i>Spices</i>	18 min	30 min	Ethanol And Water	Phenolics	Gallo <i>et al.</i> , 2010
<i>Stevia rebaudiana</i>			Ethanol And Water	Stevioside	Javad <i>et al.</i> , 2014
<i>Tea</i>	seconds	minutes	Water	Tea phenols	Spigno & Faweri 2009
<i>Tea</i>	0.5-8min	20 hrs	Water	Tea caffeine	Pan <i>et al.</i> , 2003
<i>Thymus vulgaris</i>	15 min	60 min	Deionized Water	Essential oils	Golmakani and Rezaei
<i>Tobacco</i>	40 min			Solanesol	Zhou & Liu 2006
<i>Tomato</i>	2.06min	2 hrs	66.2% Ethanol	Phenolics	Li <i>et al.</i> , 2012
<i>Zea mays</i>	19 min			Anthocyanins	Yang & Zhai, 2010

particle size extraction yield increases in the MAE of ginseng saponins. Water content present in plant matrix also plays its role as it is heated up and helps in enhancing the rate of microwave assisted extraction, so dry materials are heated in larger times.

Temperature

Temperature and microwave assisted extraction are interrelated strongly. Particularly in closed vessel system where temperature can raise above the boiling points of

the solvents. This also increases the pressure inside the vessel which also increases the safety concerns. If one don't has consideration of temperature factor (because due to elevated temperatures and pressures, containers can burst inside the cavity). But if this factor is under control then efficiency of microwave assisted extraction is enhanced as it increases the capacity of solvent extraction in two ways i.e.

- I. Decreasing surface tension of solvent
- II. Decreasing viscosity of the solvent

In the extraction of a number of plant metabolites, temperature was found to be an important factor (Talebi *et al.*, 2004). In an open vessel system temperature can be effectively controlled by proper combinations of extracting solvents which heat up differently (Mandal *et al.*, 2007).

The advantages of MAE over soxhlet extraction (Table II)

A potential alternative to traditional solid-liquid extraction is the MAE. Few of the potential advantages over Soxhlet are as follows:

- 1) Remarkably required less extraction time. The time of extraction generally ranging from few seconds to few minutes (15-20 min).
- 2) Less amount of solvent used. Only a few milliliter of solvent is required In MAE.
- 3) Extraction yield is improved.
- 4) Better precision and accuracy as provided by the Automation of the instrument.
- 5) Favorable for thermo labile constituents.
- 6) Heavy metals and pesticide residue which are present in minute traces can be extracted from a few milligram of plant sample.
- 7) During extraction it provides agitation, by which the mass transfer phenomenon is improved (Mandal *et al.*, 2007).

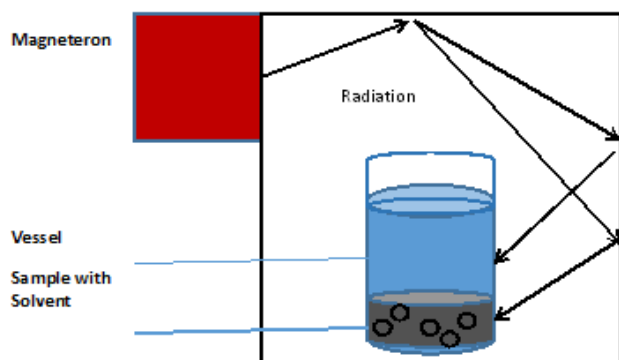


Fig. 1: Closed vessel Microwave assisted extraction system (Sticher, 2008)

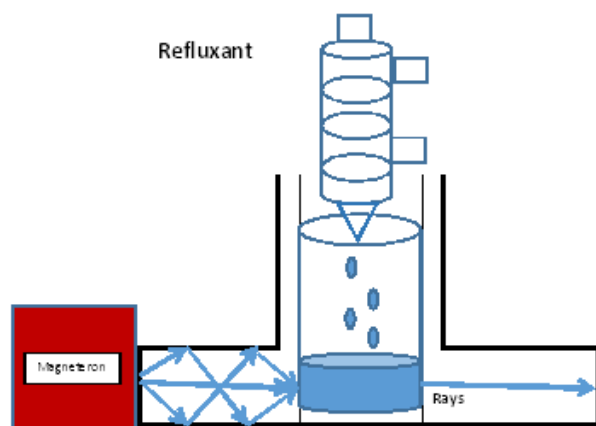


Fig. 2: Open vessel Microwave assisted extraction system (Sticher, 2008).

Soxhlet is the conventional method and the main reference for evaluating the performance of other solid-liquid extraction methods as it has long been one of the most used solid-liquid extraction techniques (Jenson, 2007).

Applications of microwave assisted extraction

Microwave assisted extraction is a reliable source of extraction of phyto-constituents. A lot of literature has been available for extraction of phenolics (Gallo *et al.*, 2010), flavonoids (Xia *et al.*, 2008), essential oils (Wang *et al.*, 2010) and glycosides (Javadet *et al.*, 2014) etc. This is also applied for the analysis of heavy metals and other pollutants present in the soil samples with decreased test time, lesser power and solvent consumptions. Microwave assisted extraction is also in use for synthesis of pharmaceuticals products. These results are guiding towards the wide range commercial applications of microwaves in the field of pharmaceuticals, food products, dentistry etc (Sadeghi *et al.*, 2017; Desrosiers *et al.*, 2009).

CONCLUSION

Microwave assisted extraction is a focused and targeted method of extraction of plant metabolites and can be easily coupled with other analytical methods like HPLC and GCMS. Its handling is further made easier by automation of the instrument. Amid the last decade MAE has risen quickly, and it has demonstrated to be effective in all viewpoints contrasted with traditional extraction procedures. It is further reasoned that the nation like Pakistan which is confronting the energy crisis problems for its economy, because we need such extraction systems for all industries which involve modern applications to reduce the overall energy usage. More research is expected to evacuate the technical barriers and to enhance the outline and scale up of the novel extraction frameworks.

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