International Journal of Applied and Natural Sciences (IJANS)
Vol.1, Issue 1 Aug 2012 8-26
© IASET

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

MODERN EXTRACTION METHODS FOR PREPARATION OF BIOACTIVE PLANT EXTRACTS

ANKIT GUPTA, MADHU NARANIWAL & VIJAY KOTHARI
Institute of Science, Nirma University, Ahmedabad-382481, Gujrat, India

ABSTRACT

Extraction is the first crucial step in preparation of plant formulations. Modern methods of extraction are effective in advancing the development of traditional herbal remedies. The development of modern sample-preparation techniques with significant advantages over conventional methods for the extraction and analysis of medicinal plants is likely to play an important role in the overall effort of ensuring availability of high-quality herbal products to consumers worldwide. Sample preparation is of utmost importance to the development of analytical methods for the analysis of constituents present in the botanicals and herbal preparations. In this article principle behind operation of various extraction methods, factors influencing method performance, research progress, strength and weakness of different extraction approaches are discussed. Emphasis is put on the methods which are solvent and energy saving, and suitable for thermolabile phytocompounds.

KEYWORDS: Efficacy, Extraction Efficiency, Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), Ultrasonication Assisted Extraction (UAE).

INTRODUCTION

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Tonthubthimthong et al., 2001). In 1985 Farnsworth et al. identified 119 secondary plant metabolites which were used as drugs. Out of 255 drugs which are considered as basic and essential by the World Health Organization (WHO), 11% are obtained from plants and a number of synthetic drugs are also obtained from natural precursors. Phytochemicals are known to possess antioxidant (Wong et al., 2009), antibacterial (Nair et al., 2005), antifungal (Khan and Wassilew, 1987), antidiabetic (Singh and Gupta, 2007, Kumar et al. a, 2008), anti-inflammatory (Kumar et al. b, 2008), antiarthritic (Kumar et al. c, 2008), and radio-protective activity (Jagetia et al., 2005), and due to these properties they are largely used for medicinal purpose. The development of drug resistance and the undesirable side effects of certain antibiotics have led to the search for new antimicrobial agents, mainly among plant kingdom, in order to find leads with unique chemical structures which may exert a hitherto unexploited mode of action. The phytochemical investigation of a plant may involve following steps: authentication and extraction of the plant material, separation and isolation of the constituents of interest, characterization of the isolated compounds and quantitative evaluation (Evans, 2008).

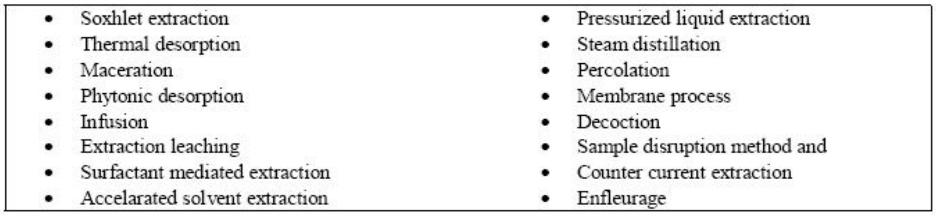
Considerable effort has been made by researchers to find efficient extraction methods in order to get high efficiency and efficacy. Efficiency refers to the yield of extraction, whereas efficacy refers to the potency (magnitude of bioactivity / the capacity to produce an effect) of the extract. For isolation of biological components, extraction from plant is one of the more sustainable approaches (Jadhav et al., 2009). For obtaining better quality and high efficiency of extraction from herbs, one has to optimize the methods for better efficiency. A strong positive linear correlation (r = 0.96) between extraction efficiency and total antibacterial activity was found during investigation on plant seed extracts (Kothari, 2010). The analytical procedures have several critical steps like sampling, sample preparation, quantification, statistical evaluations, etc. (Pawliszyn, 1997). The need for selection of most appropriate extraction methodology is evident from the fact that when different methods are applied on same plant material with same solvent, extraction efficiency can vary significantly (Table 1). In addition, the method selected as the most appropriate one also needs to be standardized so as to achieve acceptable degree of reproducibility.

It should be noted that choice of appropriate solvent is of essential importance along with application of a compatible extraction method. For selection of solvents *'like dissolves like'* principle is applicable. Thus polar solvents will extract out polar substances and non-polar material will be extracted out by non-polar solvents. Solvent extraction is the most popular method of extraction.

Table 2 lists some solvents suitable for extraction of particular classes of plant compounds. Hydroalcoholic solvent mixture (mixture of alcohol and water in varying proportions) is generally considered to give high extraction yields (Table 1), which is owing to their expanded polarity range. Sample preparation is the crucial first step in analysis of herbs, because it is necessary to extract the desired chemical components from the herbal material for further separation and characterization (Huie, 2002). For the extraction of therapeutically desired active constituents various solvents such as water, ethanol, chloroform, ethyl acetate, methanol, etc. are commonly used. Sometimes mixtures of solvents are also used to get better extraction efficiency (Table 3-4).

The development of modern sample preparation techniques has significant advantages over conventional methods (1) in terms of reduction in organic solvent consumption and in minimizing sample degradation. They also result in the elimination of undesirable and insoluble components from the extract. The modern methods includes microwave assisted extraction (MAE), ultrasonication assisted extraction (UAE), supercritical fluid extraction (SFE), solid phase micro extraction (SPME), Soxhwave, etc. Latter is a combination of Soxhlet with microwaves. This combines rapid heating capacity of microwaves with the simplicity of Soxhlet. Here solvent recovery is also possible, which is not the case in ordinary MAE. However it has not found widespread use yet. Classical methods are fairly simple, standard and continue to have widespread use, but these methods can also be insufficient and slow, consume large quantities of organic solvents, and cause degradation of heat labile constituents. While using conventional methods, quality related problems viz. lack of consistency, safety, and efficacy are also the issues. Furthermore, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, and selectivity are also the benefits of modern processes (Kothari *et al.,* 2010).The purposes of standardizing extraction procedures for production of crude drugs are to obtain the therapeutically desired portion and to eliminate the inert material by treatment with selective solvents and methods. With the increasing demand for herbal medicinal products, and natural products for health care all over the world, herbal manufacturers aim at using the most appropriate extraction technologies to produce extracts of defined quality with least batch-to-batch variation, which can also help in scale-up of extraction. Standardization of extraction procedures contributes significantly to the final quality of the herbal drug. To have a complete idea of the bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology to achieve the broadest possible range of phytochemicals. The selection of method to isolate active components with best yield and highest purity from natural sources is mainly dependent on the nature of compounds and raw material which is going to be processed [Kothari *et al.,* 2009].

Box 1. Conventional Methods Used to Recover Natural Products (Handa *Et Al.,* 2008)



- Soxhlet extraction
- Thermal desorption
- Maceration
- Phytonic desorption
- Infusion
- Extraction leaching
- Surfactant mediated extraction
- Accelarated solvent extraction
- Pressurized liquid extraction
- Steam distillation
- Percolation
- Membrane process
- Decoction
- Sample disruption method and
- Counter current extraction
- Enfleurage

EXTRACTION TECHNOLOGY

A typical extraction process may contain following steps (Handa *et al.,* 2008):

1. Collection and authentication of plant material & drying
2. Size reduction
3. Extraction
4. Filtration
5. Concentration
6. Drying & reconstitution

Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material **:** solvent ratio etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through selective use of solvents.

ADVANCED EXTRACTION METHODS

Microwave Assisted Extraction (MAE)

Upon absorption by a material, electromagnetic energy of microwaves is converted to heat energy. 2450 MHz (2.45 GHz) is the most commonly used frequency for commercial microwave instruments, which has an energy output of 600-700 W (Jain *et al*., 2009). MAE is a simple, environment friendly and economical technique for the extraction of biologically active compounds from different plant materials (Hemwimon *et al.,* 2007). Samra *et. al.* had first time used microwave domestic ovens for the treatment of biological samples for metal analysis in 1975 (Letellier and Budzinski, 1999). The application of MAE for plant materials was first reported by Ganzler and co-workers in 1986 (Kaufmann and Christen, 2002).

Microwaves possess electric and magnetic fields which are perpendicular to each other. The electric field causes heating via two simultaneous mechanisms, namely, dipolar rotation and ionic conduction. Dipolar rotation is due to the alignment on the electric field of the molecules possessing a dipole moment in both the solvent and the solid sample. This oscillation produces collisions with surrounding molecules leading to liberation of thermal energy into the medium. With a frequency of 2.45 GHz, this phenomenon occurs 4.9×10^9 times faster and thus the resulting heating is very fast. Indeed, larger the dielectric constant of the solvent (Table 4), more rapid the heating is. Consequently, unlike classical conductive heating methods, microwaves heat the whole sample simultaneously. In the case of extraction, the advantage of microwave heating is the disruption of weak hydrogen bonds promoted by the dipole rotation of the molecules (Kaufmann and Christen, 2002).

Components of the sample absorb microwave energy in accordance to their dielectric constants (Ahuja and Diehl, 2006). When plant material is immersed inside a microwave transparent solvent, the heat of microwave radiation directly reaches to the solid without being absorbed by the solvent, resulting in instantaneous heating of the residual moisture in the solid. Heating causes the moisture to evaporate and creates a high vapour pressure that breaks the cell wall of substrate and releases the content into solvent. Solvents employed for most MAE operations are those with a high dielectric constant and capacity to strongly absorb microwave energy, however, the extraction selectivity, and the ability of the medium to interact with microwaves can be modulated by using mixtures of solvents. It is not uncommon to use binary mixture of solvents (Table 3), with only one solvent capable of absorbing microwave (Camel, 2001). Though polar solvents are usually believed to be better than non-polar ones (Jagetia *et al*., 2005; Kothari *et al.,* 2010; Proestos and Komaitis, 2007) ‘Broken cell-wall theory’ places microwave transparent solvents above the microwave absorbing ones (Kothari *et al.,* 2009). Addition of water to the solvent may lead to increased yields. Microwave transparent solvents like acetone proved to be best for extraction of phenolic compounds (Proestos and Komaitis, 2007). In case of methanol:chloroform mixture, former provides better overall heating efficiency because of its high dissipation factor. Because of low polarity chloroform remains transparent (Kothari *et al.,* 2009; Proestos and Komaitis, 2007). Microwave transparent solvents (e.g., hexane) are particularly suitable for extraction of thermolabile components (Mandal *et al.*, 2008).

MAE can be practiced in two different modes- one is *closed vessel operation*, that is under controlled (elevated) pressure and temperature, another is *open vessel operation* performed at atmospheric pressure. These technologies are named as pressurized microwave assisted extraction (PMAE) and focused microwave assisted extraction (FMAE), respectively (Chemat and Esveld, 2001). In closed vessel system the solvent may be heated much above their atmospheric boiling point. Both extraction speed and efficiency are enhanced in this procedure (Kaufmann and Christen, 2002). In closed vessels the temperature may be elevated by simply applying the correct pressure. The closed vessel system is most suitable for volatile compounds. In open vessel system the maximum temperature is determined by the boiling point of the solvent used (Camel, 2001). Compared to closed vessel extractions, open cells offer increased safety in sample handling and, furthermore, they allow larger amounts to be extracted (Kaufmann and Christen, 2002). Open cells can accommodate multiple extraction vessels at a time. Advantage of improved mass transfer due to agitation is available in both modes of MAE (Mitra, 2003; Sarker, 2006). Though superheating has been indicated to occur during microwave processes (Chemat and Esveld, 2001), MAE is not likely to suffer from thermal degradation of phytoconstituents by superheating because superheating is reported to occur in homogenous systems, and not in heterogeneous ones -in which MAE falls.

The ability of microwave radiation to heat solid material effectively can be used for obtaining essential oils. This yields essential oils consisting of relatively low volatile fractions as compared to hydro distillation (Handa *et al.*, 2008). MAE is highly effective for obtaining extracts under mild conditions. MAE has shorter extraction time, lesser solvent requirement, improved purity of the extract, low cost, and better extraction yield in comparison to Soxhlet extraction. Therefore it has been considered as a potential alternative to conventional methods (Ahuja and Diehl, 2006; Chemat and Esveld, 2001). Microwaves have been reported to cause little or no quality deterioration when applied to substances of plant origin such as ascorbic acid, where as moist heat application resulted in quality deterioration (Sasaki *et al.*, 1998). MAE has been shown to be faster than the reflux method for extraction of phenolic compounds.

Curcumin can be extracted in lesser time with better precision through MAE than conventional methods (Mandal *et al.*, 2007). MAE is an alternative technique for extraction of tanshinones from root of *Saliva miltiorrhiza Bunge*, it provides higher extraction efficiency in shorter time and is less labour intensive (Pan *et al.*, 2002). A kinetic study of the effect of solvent composition, solvent volume, extraction temperature, and matrix characteristics on the MAE of leaves of rosemary and peppermint revealed that for a sample matrix which contains water as a component, the use of pure, microwave transparent solvents such as hexane could result in the rapid extraction of essential oil components. This was because of direct interaction of microwaves with the free water molecules present in the cell, which caused rupture of the cell and release of essential oil into organic solvent used (hexane). More effective microwave heating for leaves of rosemary and peppermint can be achieved by optimizing the plant material**:**solvent ratio (Huie, 2002). A higher microwave temperature and a short extraction time are more effective in extracting antioxidative phenolic compounds from tomato using MAE (Li *et al*., 2012). Extract prepared by MAE showed highest phenolic and tannin content and also had the highest scavenging activity. MAE was found superior in term of extraction efficiency and particularly for extraction of phenolic and tannin content extraction and a significant increase (20%) in antioxidant activity was also noticed followed by UAE. Both microwave and sonication assisted extraction proved to be effective in increasing the yield of phenolics and tannins and in increasing the potential of antioxidant activity (Thomas *et al*., 2012). MAE was proven as a potential alternative to traditional methods for extraction of phenols such as chlorogenic acids from green coffee beans and prepared extracts were also showing a good radical scavenging activity (Upadhyay *et al*., 2012). While extracting mitragynine (an alkaloid) from leaves of *Mitragyna speciosa* plant, MAE gave highest alkaloid fraction amount and highest amount of mitragynine was present in the extract prepared by UAE (Orio *et al*., 2012).

Extracts prepared by MAE method from seeds of *Manlikara zapota, Annona squamosa, Tamarindus indica, Phoenix sylvestris, Citrus limon, Carica papaya,* and *Tricosanthes dioica* have shown significant antibacterial and/or antioxidant activity, with extraction efficiency in the range 2-15 % (Kothari *et al.,* 2010 ; Kothari, 2011; Kothari and Seshadri, 2010]. For *A. squamosa* seeds extraction efficiency of 17% [in chloroform-methanol mixture] was reached with a total microwave exposure of just 50 s (Kothari *et al.,* 2009). MAE was used to extract paclitaxel in methanol-water mixture from Iranian yew trees (Ahuja and Diehl, 2006). MAE has been applied for preparation of plant extracts while evaluating them for antibacterial (Kothari and Seshadri, 2010;Kothari, 2010; Kothari, 2011), and antioxidant (Dai and Mumper, 2010; Kothari *et al.,* 2010 ; Kothari and Seshadri, 2010 ; Kothari, 2010) activity.

Ultrasonication Assisted Extraction (UAE)

UAE involves application of high-intensity, high-frequency sound waves and their interaction with materials. UAE is a potentially useful technology as it does not require complex instruments (Fig 1) and is relatively low-cost. It can be used both on small and large scale (Dai and Mumper, 2010). UAE involves ultrasonic effects of acoustic cavitations. Under ultrasonic action solid and liquid particles are vibrated and accelerated and, because of that solute quickly diffuses out from solid phase to solvent (Cares et al., 2009). Several probable mechanisms for ultrasonic enhancement of extraction, such as cell disruption, improved penetration, and enhanced swelling, capillary effect, and hydration process have been proposed (Huaneng et al., 2007). If the intensity of ultrasound is increased in a liquid, then it reaches at a point at which the intramolecular forces are not able to hold the molecular structure intact, so it breaks down and bubbles are created, this process is called cavitation (Baig et al., 2010). Collapse of bubbles can produce physical, chemical and mechanical effects which result in the disruption of biological membranes to facilitate the release of extractable compounds and enhance penetration of solvent into cellular materials and improve mass transfer (Cares et al., 2009; Metherel et al., 2009). The beneficial effects of sound waves on extraction are attributed to the formation and asymmetrical collapse of microcavities in the vicinity of cell walls leading to the generation of microjets rupturing the cells. The pulsation of bubbles is thought to cause acoustic streaming which improves mass transfer rate by preventing the solvent layer surrounding the plant tissue from getting saturated and hence enhancement of convection (Kadkhodaee and Kakhki: http://confbank.um.ac.ir/modules/conf_display/saffron/pdf/p55.pdf). Skin of external glands of plant cell wall is very thin and can be easily destroyed by sonication, and this facilitates release of essential oil contents into the extraction solvent, thus resulting in reduced extraction time and increased extraction efficiency (Huie, 2002).

Extraction of the tea solids from dried leaves with water using ultrasound gave 20% improvement in extraction yield. UAE also proved better for extraction of carnosic acid by using different solvents viz. ethanol, ethyl acetate, and butanone and also reduced the extraction time (Baig *et al*., 2010). In general, solvent type has a significant effect on both extraction rate and the final yield of total isoflavones but in one study when UAE was used for the extraction of isoflavones from the stem of *Pueraria lobata* (Willd.), the increased extraction rate and yield was obtained for all types of solvents. The higher the electrical power input in the range of 0-650 W, the higher the extraction yield was observed (Huaneng *et al*., 2007). UAE provides better extraction of the vanillin in shorter time period for different solvents as compared to the Soxhlet method (Jadhav *et al*., 2009). Ultrasonic treatment on commercial scale could be reliable and simple by applying ultrasound to the pre-leached mixture for short time period (Jadhav *et al*., 2009). UAE of resveratrol from grapes was considered to be very effective. The degradation of resveratrol from grapes may be negligible within a certain extraction time period with the use of UAE (Cho *et al.*, 2006). UAE has retaining effect on extraction of protein and pectin, which improve the sensory quality of tea. UAE was found appropriate for the extraction of aroma compounds and glycosidic aroma precursors (Xia *et al*., 2006). UAE was also used for extraction of oil from soybean (Li *et al*., 2004), rapeseed (Ibiari *et al*., 2010), and *Monopterus albus* (Abdullah *et al*., 2010). Studies concerning effect of different solvents and their mixture, effect of solvent volume, sonication power, and sonication time indicated that UAE has the potential to improve extraction efficiency and reduce processing time, and during processing the oil composition was also not affected by the use of ultrasound. UAE gives the highest extraction yield of some flavonoids such as- tectoridin, iristectorin B, iristectorin A, tectorigenin, iris-tectorigenin A, and total isoflavones, in lesser time in comparison to maceration and Soxhlet extraction (Sun *et al*., 2011). Important functional components from grape seeds were extracted by UAE. Extraction variables, particularly extraction time and temperature, strongly influence the UAE of total phenolics, antioxidants, and anthocyanins from grape seeds (Ghafoor *et al*., 2009). Ultrasound-assisted extraction was reported as a simpler and more effective alternative to conventional extraction methods for the isolation of ginsenosides (saponins) from various types of ginseng. Sonication-assisted extraction of ginseng saponins was about three times faster than the traditional extraction method. The ultrasonic extraction was not only more efficient but also convenient for the recovery and purification of the active ingredients**.** The sonication-assisted extraction can be carried out at lower temperatures which are favorable for the thermally unstable compounds (Wu *et al*., 2001). Sonication proved to be the most powerful tool for extraction of certain phytochemicalshypericin, pseudohypericin, hyperoside, rutin, quercitrin, and hyperforin- from *Hypericum perforatum L.* when extraction efficiency of UAE was compared with that of conventional maceration, indirect sonication, Soxhlet extraction, and accelerated solvent extraction (ASE) (Smelcerovic *et al*., 2006). Application of UAE significantly accelerated the analyte (nicotine from pharmaceutical and plant formulations) extraction. Each extraction step takes up to 24 h in conventional cold extraction technique, whereas UAE took less than 20 min to achieve the same extraction efficiency, and the consumption of environmentally harmful organic solvent is also lowered.

Ultrasound has the main advantage of shorter reaction/preparation time, usage of small amounts of material, efficient and minimum expenditure on solvents, and the increase in sample throughput. It is very useful for the isolation and purification of bioactive principles (Ishtiaq *et al.*,2009). One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (> 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Handa *et al*., 2008).

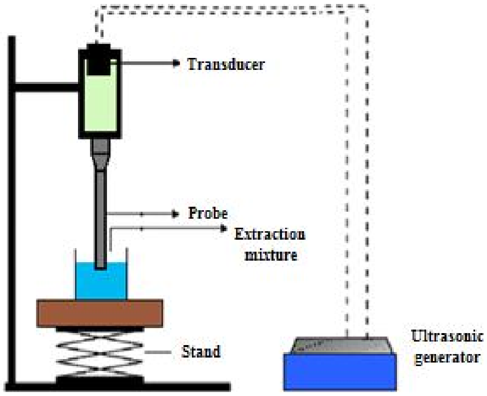


Fig 1. Schematic Representation of UAE Setup [adapted from Jadhav *et al*., 2009**]**

Supercritical Fluid Extraction (SFE)

SFE can be used to extract certain compounds from plants at temperature near to ambient, thus preventing the substance from incurring in thermal denaturation. SFE is an old technique of solvent extraction but its commercial application happened slowly due to the sophisticated and expensive high pressure equipment and technology required (Tonthubthimthong et al., 2001). SFE is currently a well-established method for extraction and separation because its design and operating criteria are now fully understood (Li et al., 2010). The favourable transport properties of fluids near their critical points allow deeper penetration into solid plant matrix and more efficient and faster extraction than with conventional organic solvents. The extraction is carried out in high-pressure equipment in batch or continuous manner. In both cases, the supercritical solvent is put in contact with the material from which a desirable product is to be separated. Generally cylindrical extraction vessels are used for sample preparation (Handa et al., 2008). In batch processing solid is placed into extraction vessel and the supercritical solvent is fed in until the target extraction conditions are reached. And in semi batch processing the supercritical solvent is fed continuously through a high pressure pump at a fixed flow rate, to precipitate solute from supercritical solution one or more separation stages are used. Supercritical fluid technology is now recognized as an effective analytical technique with efficiency comparable to existing chemical analysis methods. SFE is favourably applicable for the qualitative and quantitative identification of constituents of natural products, including heat-labile compounds (Mohameda and Mansoor, 2002).

SFE is used for extraction of volatile or aroma compounds, such as essential oils, and caffeine from the plant materials. Many factors such as temperature, pressure, sample volume, cosolvent addition, and flow and pressure control are important during extraction by SFE. In practice conditions somewhat above the critical temperature and pressure for a particular substance are usually applied and these supercritical fluids exhibit properties intermediate between those of the liquid and gaseous phases (Evans, 2008). The fluid possesses properties bounded by the extremes of the gaseous and liquid states, and these properties may be adjusted with alteration of the applied pressure and temperature (Kroon and Raynie, 2010). Under suitable conditions, any fluid can reach its supercritical state. The possibility of using supercritical fluids as extraction solvents is directly linked to their density. A supercritical fluid is referred to as a dense gas, a fluid above its critical temperature (T_C) and critical pressure (P_C) to a certain extent. To be supercritical, the reduced temperature Tr (i.e. T/T_C) must not exceed 1.2 or 1.3, whereas the reduced pressure Pr (i.e. P/P_C) may be as high as allowed by technological limits (Handa *et al.*, 2008). For water, the critical conditions for temperature (T_C) and pressure (P_C) are 374ºC and 220 atmosphere respectively, and for carbon dioxide T_C is 30.9ºC and P_C is 73.8 atm (Evans W.C., 2008).

Several solvents can be used for SFE, such as, hexane, pentane, butane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbons (Reverchon and Marco, 2006). Carbon dioxide ($CO_{2)}$ is the most commonly used extraction solvent in SFE (Handa *et al.*, 2008). CO_2 alone is non selective but its capacity and selectivity of extraction can be improved by using a co solvent or modifier. After the extraction co-solvent can easily be removed. CO_2 is generally the most desirable solvent in SFE, because its critical temperature is only 304 K, which makes it attractive for the extraction of heat–labile compounds. In addition CO_2 is an inert, safe (non-flammable, non-explosive), inexpensive, noncorrosive, odourless, colorless, clean solvent that leaves no solvent residue in the product; it is also non-toxic and is generally accepted as a harmless ingredient in pharmaceuticals and food, and it is easily removable from the extracted oil by simple expansion. Moreover, carbon dioxide has a low surface tension and viscosity, and high diffusivity which make it attractive as a supercritical solvent (Handa *et al.*, 2008; Tonthubthimthong *et al.*, 2001). Carbon dioxide is cheap, environment friendly and generally recognized as safe by regulatory authorities. Design of processes using supercritical solvents is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating conditions. Therefore, phase equilibrium engineering plays a key role in the synthesis and design of these processes (Herrero *et al*., 2009).

Sometimes in place of CO_2, argon is used because it is inexpensive and more inert then CO_2. The component recovery rates generally increase with increasing pressure or temperature. The highest recovery rate in case of argon was obtained at 500 atm and 150°C. Due to interaction between modifiers (co-solvents) and matrix, plant matrix swell, this is an important factor in enhancing extraction recovery.

Advantages of SFE over conventional methods can be summarized as (Handa *et al.*, 2008; Ahuja and Diehl, 2006):

1. Extraction of constituents at low temperature, which avoids damage from heat
2. No solvents residue
3. Environment friendly
4. Better diffusivity
5. Low viscosity of supercritical fluid, which allow more selective extractions
6. Fast extraction

SFE conditions could be fine tuned for selective extraction of an antioxidant fraction with almost no residual aroma from rosemary plants. It has also been shown by operating under subcritical temperature and pressure that CO_2 can be used as solvent for the selective extraction of essential oils and diterpene glycosides from plants of medicinal interest (Huie, 2002). Supercritical CO_2 behaves like a lipophilic solvent but has advantage over liquid solvents that its selectivity and solvent power is adjustable and can be set to values ranging from gas-like to liquid-like. SFE can produce superior quality product without any artefacts, and a better retention of the original flavour or fragrance. For extraction of essential oil low CO_2 density (0.25-0.50 g cm^3), is preferable. But for the extraction of non- oil compounds a high CO_2 density can be used [58]. Use of CO_2 (methanol as co-solvent) to extract nimbin in an extractor made from a piece of high pressure HPLC tubing has been reported. Effect of operating conditions during extraction viz. CO_2 flow rate; CO_2 pressure; extractor temperature, and weight of neem sample were investigsted. Nimbin extraction yields of approximately 0.35 kg nimbin/kg of nimbin in seeds using only supercritical CO_2 extraction were obtained. The extraction yield was approximately 0.175 mg nimbin/g of neem seeds and the neem seeds were assumed to contain about 0.5 mg of nimbin/g of neem seeds. The best extraction conditions were found to be at 308 K temperature, 23 MPa pressure, and a flow rate of 1.24 ml/min. The specific extraction rate was not found to be a function of sample size for a range of samples studied (Tonthubthimthong *et al.*, 2001).

SFE conditions were optimized for extraction of active ingredients from *Curcuma zedoaria*, the density of CO_2 and the fluid volume passing through the plant matrix were found to be most important factors in increasing the extraction efficiency, whereas increasing the temperature has little effect on extraction efficiency (Huie, 2002). The rate of extraction of volatile active components such as essential oils from matrices by SFE seems to be governed by analyte-matrix interaction rather than the bulk solubility of the analyte in the pure CO_2, the extraction rate were found to increase greatly due to addition of an organic modifier (Huie, 2002).

Extraction of grape seed oil by SFE with CO_2 offers certain advantages concerning the quality of the extracted oil and the efficiency of the process. The oil obtained by extraction with supercritical carbon dioxide is free of organic solvent, processing time was also lower than during conventional solvent extraction. Complete removal of organic solvents from the extract, otherwise is a time and energy consuming process in present-day oil technology (Aleksovski *et al*., 1998). Supercritical CO_2 extraction had been applied for extracting seed oils from fennel seeds too (Reverchon and Marco,2006).

SFE has been demonstrated to be more effective than other conventional techniques for the extraction of antimicrobial compounds. A clear trend in food and natural products analysis, as well as in other fields of analytical science, is the hyphenation of sample treatment procedures with analytical tools in order to produce effective on-line couplings. Some of these couplings have involved the use of SFE (Herrero et al., 2009).

We recently performed a comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds (Kothari *et al*., 2012). Extracts from seeds of five different plants- *Annona squamosa* (Annonaceae), *Manilkara zapota* (Sapotaceae), *Phoenix sylvestris* (Palmae), *Syzygium cumini* (Myrtaceae), and *Tamarindus indica* (Cesalpiniaceae)- were prepared in water, methanol, and ethanol by employing five different methods of extraction viz. Soxhlet method, Ultrasonication, extraction by continuous shaking at room temperature, and microwave assisted extraction- with and without intermittent cooling. All these extracts were compared with respect to extraction efficiency, total phenol content, total flavonoid content, antioxidant capacity, and antibacterial activity. Soxhlet method proved best in terms of high extraction efficiency, and extraction of phenolic compounds. Microwave assisted extraction with intermittent cooling (MAE), room temperature extraction by shaking (ERT), and ultrasonication assisted extraction (UAE) proved good at extracting antibacterial compounds from plant seeds. Latter also proved effective at extracting antioxidant compounds. Methanol proved most suitable solvent for extraction of flavonoids. Following these experiments, we have also been able to extract antifungal (particularly against *Malassezia furfur*) phytocompouds from plant seeds using MAE (Ramanuj et al., 2012).

FINAL COMMENTS

Medicinal plants are important for discovery and identification of new therapeutic compounds. Extraction method plays an important role in separation and characterization of different phytochemicals from herbs, and screening plant extracts for novel leads. Conventional methods are exhaustive and require more time, power, sample and solvent consumption is higher than their modern counterparts (Table 5). The recovery, stability and overall quality of extract also can be improved by selection of a better method. Modern methods can be optimized for extraction of a particular compound (or a certain class of plant metabolites) and the extract can be directly used for gas chromatography (GC) or high pressure liquid chromatography (HPLC). Among the modern methods, MAE has been proposed as more amenable to be coupled with subsequent separation and characterization operations. The on-line continuous sampling dynamic microwave-assisted extraction (on-line CSDMAE) coupled with high-performance liquid chromatographic separation and determination of the lignans in Wuweizi and naphthoquinones in Zicao is one such example (Gao *et al.,* 2012). Compared with the conventional extraction methods, such as off-line continuous microwave-assisted extraction, ultrasound-assisted extraction and Soxhlet extraction, this method is claimed to be quicker and more effective. Optimization of microwave assisted extraction for the characterization of olive leaf phenolic compounds by using high-

performance liquid chromatography (HPLC) coupled to electrospray time-of-flight mass spectrometry (ESI-TOF-MS) and electrospray ion trap tandem mass spectrometry (ESI-IT-MS2) has also been reported recently (Taamalli et al.,2012). As compared to the conventional method, MAE is shown to be a better alternative for the characterization of phenolic compounds from olive leaves due to its efficiency and speed.

Modern methods are amenable to high degree of automation, and several parameters can be controlled at a time. Sample and solvent consumption are also reduced by selecting the most appropriate method. A good extraction can be achieved in shorter period of time, and the recovered extract can have improved yield and quality than that prepared by a conventional method. Methods such as SFE, MAE, and UAE are better suited for the extraction of heat labile and volatile compounds, which is not the case with the conventional methods. Former are more promising for industrial applications due to their enhanced efficiency, specificity and selectivity. Further innovations such as a Soxhwave under reduced pressure (which will bring together advantages like rapid heating due to microwave, fast boiling of solvents due to reduced pressure, and scope of solvent recovery) will in future make extraction of thermolabile plant constituents much more efficient and rapid. However, this will require surmounting novel fabrication and engineering challenges. At present such a device is not available at reasonable price for widespread use throughout the globe. At present, open-vessel MAE seems to be the most convenient, simple, and rapid option for extraction of thermolabile phytoconstituents, as it does not demand any sophisticated machinery (except a simple microwave oven), as opposed to methods like ultrasonication or SFE.

REFERENCES

1. Abdullah S, Mudalip SK, Shaarani SM, Pi NA. 2010. Ultrasonication extraction of oil from Monopterus albus: effect of different ultrasonic power solvent volume and sonication time. J. Appl. Sci. 10: 2713-2716.
2. Ahuja, S., and Diehl, D. 2006. Sampling and Sample prepration. In: Comprehensive Analytical Chemistry,Vol. 47 (Eds.), S Ahuja, and N Jespersen, Oxford, UK: Elsevier (Wilson & Wilson) Chap-2, pp.15-40.
3. Aleksovski S, Sovova H, Urapova B, Poposka F. 1998. Supercritical CO2 extraction and Soxhlet extraction of grape seeds oil. Bulletin of the Chemists and Technologists of Macedonia, 17: 129–134. Baig S, Farooq R, Rehman F. 2010. Sonochemistry and its industrial applications. World Appl. Sci. J. 10: 936-944.

4. Balandrian MFJ, Kjoke A, Wuretle E. 1985. Natural plant chemicals: source of industrial and medicinal materials. Sci. J. 228:1154-1160.
5. Camel V. 2001. Recent extraction techniques for solid matrices-supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. The Royal Soc. Chem. Analyst. 126:1182-1193.
6. Cares MG, Vargas Y, Gaete L, Sainz J, Alarcon J. 2009. Ultrasonically assisted extraction of bioactive principles from Quillaja Saponaria Molina. Physics. Procedia. 3: 169-178.
7. Chemat F, Esveld E. 2001. Microwave assisted heterogeneous and homogeneous reactions. Fifth international electronic conference on synthetic organic chemistry (ECSOC-5). http://www.mdpi.org/ecsoc-5.htm. (last accessed Jan 2, 2012)
8. Cho YJ, Hong JY, Chun HS, Lee SK, Min HY. 2006. Ultrasonication assisted extraction of resveratrol from grapes. J. Food. Eng. 77: 725-730.
9. Cowan MM. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.
10. Dai J, Mumper RJ. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 15: 7313-7352.
11. Evans, W.C. 2002. General methods associated with the phytochemical investigation of herbal products. In Trease and Evans Pharmacognosy (15 ed.), New Delhi: Saunders (Elsevier), pp.137-148.
12. Gao S, You J, Wang Y, Zhang R, Zhang H. 2012. On-line continuous sampling dynamic microwave-assisted extraction coupled with high performance liquid chromatographic separation for the determination of lignans in Wuweizi and naphthoquinones in Zicao. J. Chromatogr B. [Epub ahead of print].
13. Ghafoor K, Choi YH, Jeon JY, Jo IH. 2009. Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape (Vitis Vinifera) seeds. J. Agric. Food Chem. 57: 4988–4994. doi :10.1021/jf9001439.
14. Handa SS, Khanuja SPS, Longo G, and Rakesh DD. 2008. Extraction technologies for medicinal and aromatic plants. Trieste: ICS UNIDO.
15. Hemwimon S, Pavasant P, Shotipruk A. 2007. Microwave assisted extraction of antioxidative arthraquinones from roots of Morinda citrifolia. Sep. Purif. Technol. 54: 44-50.
16. Herrero M, Mendiola JA, Cifuentes A, Ibanez E. 2009. Supercritical fluid extraction: Recent advances and applications. J. Chromatogr A. doi:10.1016/j.chroma.2009.12.019.
17. Houghton PJ, Raman A. 1998. Laboratory Handbook for the Fractionation of Natural Extracts. London: Chapman and Hall.
18. Huaneng X, Yingxin Z, Chaohong H. 2007. Ultrasonically assisted extraction of isoflavones from stem of Pueraria Lobata (Willd.) Ohwi and its mathematical model. Chin J. Chem. Eng. 15: 861-867.
19. Huie CW. 2002. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem. 373: 23-30.

20. Ibiari NN, Enin SAAEl, Attia NK, Diwani GEL. 2010. Ultrasonic comparative assessment for biodiesel production from rapeseed. J. Am. Sci. 6: 937-943.
21. Ishtiaq F, Farooq R, Farooq A, Siddique M, Shah H, Hassan MU, Shaheen MA. 2009. Application of ultrasound in pharmaceutics world. Appl. Sci. J. 6: 886-893.
22. Jadhav D, Rekha BN, Gogate PR, Rathod VK. 2009. Extraction of vanillin from vanilla pods: A comparison study of conventional Soxhlet and ultrasound assisted extraction. J. Food Eng. 93: 421–426.
23. Jagetia GC, Venkatesh P, Baliga MS. 2005. Influence of seed extracts of S. cumini on mice exposed to different doses of γ radiation. J. Radiat. Res. 46: 59-65.
24. Jain T, Jain V, Panday R, Vyas A, Shukla SS. 2009. Microwave assisted extraction for phytoconstituents - An overview. Asian J. Res. Chem. 2: 19-25.
25. Kadkhodaee, R., Kakhki, A.H. Ultrasonic extraction of active compounds from saffron. http://confbank.um.ac.ir/modules/conf_display/saffron/pdf/p55.pdf.
26. Kaufmann BA, Christen P. 2002. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. Phytochem. Anal. 13: 105-113.
27. Khan, M., Wassilew, S.W. 1987. Natural pesticides from the neem tree and other tropical plants. (Eds) Schmutterer, H. and Asher, K. R. S., Germany: Digitalverlag GmbH, pp.645-650.
28. Kothari V, Punjabi A, Gupta S. 2009. Optimization of microwave assisted extraction of Annona squamosa seeds. The Icfai J. Life sci. 3: 55-60.
29. Kothari V, Pathan S, Seshadri S. 2010. Antioxidant activity of M. zapota and C. limon seeds. J. Nat. Remedies. 10[2]: 175-180.
30. Kothari V, Seshadri S. 2010. Antioxidant activity of seed extract of Annona squamosa and Carica papaya. Nutri. Food Sci. 40: 403-408.
31. Kothari V, Seshadri S. 2010. In vitro antibacterial activity of Manikara zapota, Annona squamosa, and Tamarindus indica. Biol. Res. 43: 165-168.
32. Kothari V, Shah A, Gupta S, Punjabi A, Ranka A. 2010. Revealing the antimicrobial potential of plants. Int. J. Biosci. Tech. 3: 1-20.
33. Kothari, V. 2010. Screening of various plant products/extracts for antimicrobial and antioxidant properties and to investigate correlation of the latter with phenolic content of sample. Ph.D. thesis, Nirma University, pp.59.
34. Kothari V. 2011. In vitro antibacterial activity in seed extracts of Phoenix sylvestris Roxb (Palmae), and Tricosanthes dioica L (Cucurbitaceae). Curr. Trends Biotechnol. Pharm. 4: 993-997.
35. Kothari V, Gupta A, Naraniwal M. 2012. Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds. J. Nat Remedies 12(2):162-173.
36. Kroon, J.J., Raynie, D.E. 2010. Supercritical fluid extraction. Handbook of Sample Prepration, (Eds) Janusz Pawliszyn, New Jersey: John Wiley & Sons Inc. pp.191.

37. Kumar A, Krishan MRV, Aravindan P, Jayachandran T, Deecaraman M, Ilavarasan R, Padmanabhan A. 2008. Anti diabetic activity of Syzygium cumini seed and its isolate compounds against streptozotocin induced diabetic rats. J. Med. Plants. Res. 2: 246-249.
38. Kumar A, Krishan MRV, Aravindan P, Jayachandran T, Deecaraman M, Ilavarasan R, Kumar RM, Padmanabhn A. 2008. Anti inflammatory activity of Syzygium cumini seed. Afr. J. Biotechnol. 7: 941-943.
39. Kumar A, Padmanabhan A, Krishnan MRV. 2007. Central nervous system activity of Syzygium cumini seeds. Pak. J. Nutr. 6: 698-700.
40. Kumar KE, Mastan SK, Reddy KR, Reddy GA, Raghunandan N, Chaitanya G. 2008. Anti arthritic property of methanolic extract of Syzygium cumini seed. Int. J. Integr. Biol. 4: 55-60.
41. Leonelli C, Mason TJ. 2010. Microwave and ultrasonic processing: Now a realistic option for industry. Chem. Eng. Process. 49: 885-900.
42. Letellier M, Budzinski H. 1999. Microwave assisted extraction of organic compounds. EDP Sciences, Wiley-VCH, Analysis. 27: 251-271.
43. Li H, Deng Z, Wu T, Liu R, Loewen S, Tsao R. 2012. Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes, J. Food Chem. 130: 928–936.
44. Li H, Pordesimo L, Weiss J. 2004. High intensity ultrasound-assisted extraction of oil from soybeans. Food Res. Int. 37: 731-738.
45. Li H, Wu J, Rempel CB, Thiyam U. 2010. Effect of operating perameterson oil and phenolic extraction using supercritical CO2. J. Am. Oil Chem. Soc. 87: 1081-1089.
46. Mandal V, Mohan Y, Hemalatha S. 2008. Microwave assisted extraction of curcumin by sample solvent dual heating mechanism using Taguchi L9 orthogonal design. J. Pharm. Biomed. Anal. 46: 322-327.
47. Mandal V, Mohan Y, Hemeletha S. 2007. Microwave assisted extraction: An innovative and promising extraction tool for medicinal plant research. Pharmacog. Rev. 1:7-18.
48. Mendham, J., Denney, R.C., Barnes, J.D., and Thomas, M.J.K. 2005. Vogel's Textbook of Quantitative Chemical Analysis (6th Ed.) Delhi: Person Education pp. 298.
49. Metherel AH, Taha AY, Izadi H, Stark KD. 2009. The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples [flaxseed]. Prostaglandins Leukot Essent Fatty Acids. 81: 417-23.
50. Mitra, S. 2003. Sample Preparation Techniques In Analytical Chemistry. Wiley–IEEE. pp.142-173.
51. Mohameda, R.S., Mansoor, G.A. 2002. The use of supercritical fluid extraction technology in food processing. Food Technol Magazine. The World Markets Research Centre, London, UK.
52. Nair R, Kalariya T, Chanda S. 2005. Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol., 29: 41-47.
53. Orio L, Alexanderu L, Cravotto G, Mantegna S, Barge A. 2012. UAE, MAE, SFE-CO2 and classical methods for the extraction of Mitragyna speciosa leaves. Ultrason Sonochem. 19: 591-595.

54. Pan X, Niu G, Lio H. 2002. Comparision of microwave assisted extraction and conventional extraction techniques for the extraction of tanshinones from Saliva miltiorrhiza bunge. Biochem. Eng. J. 12: 71-77.
55. Pawliszyn, J. 1997. Solid Phase Micro Extraction: Theory and practice. Wiley-VCH, New York, pp.1-9. Proestos C, Komaitis M. 2007. Application of microwave assisted extraction to the fast extraction of plant phenolic compounds. LWT - Food Sci. Technol. doi:10.1016/j.lwt.2007.04.013.
56. Ramanuj K, Bachani P, Kothari V. 2012. In vitro antimicrobial activity of certain plant products/seed extracts against multidrug resistant Propionibacterium acnes, Malassezia furfur, and aflatoxin producing Aspergillus flavus. Res in Pharm. 2(3): 22-31.
57. Reverchon E, Marco I. 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit Fluids. 38: 146-166.
58. Sarker, S., Latif, Z., and Gray, A. 2006. Natural Products Isolation. 2nd ed., USA: Humana press. pp. 33-37.
59. Sasaki K, Honda W, Ohsawa S, Miyake Y, Kawashima Y. 1998. A study of microwave sterilizer for injection ampoules (no.4): Application to sterilization of thermally labile drug solutions. J. Pharm. Sci. Technol. 58: 125-135.
60. Singh N, Gupta M. 2007. Effect of ethanolic extract of Syzygium cumuni seed powder on pancreatic islets of alloxen diebetic rats. Indian J. Exp. Biol. 45: 861-867.
61. Smelcerovic A, Spiteller M, Zuehlk S. 2006. Comparison of methods for the exhaustive extraction of hypericins, flavonoids, and hyperforin from Hypericum perforatum L. J. Agric. Food Chem. 54: 2750-2753.
62. Sun Y, Liu Z, Wang J. 2011. Ultrasound-assisted extraction of five isoflavones from Iris tectorum Maxim. Sep. Purif. Technol. doi:10.1016/j.seppur.2011.01.017.
63. Taamalli A, Arraez RD, Ibanez E, Zarrouk M, Segura CA, Fernández GA. 2012. Optimization of Microwave-Assisted Extraction for the Characterization of Olive Leaf Phenolic Compounds by Using HPLC-ESI-TOF-MS/IT-MS2. J. Agric. Food Chem. 60:791-798. doi: 10.1021/jf204233u.
64. Thomas R, Tripathi R, Kamat SD, Kamat DV. 2012. Comparative study of phenolics and antioxidant activity of phytochemicals of T. chebula extracted using microwave and ultrasonication. Int. J. Pharm. Sci. Res. 3(1): 194-197.
65. Tonthubthimthong P, Chuaprasert S, Douglas P, Luewisutthichat W. 2001. Supercritical CO2 extraction of nimbin from neem seeds an experimental study. J. Food Eng. 47: 289-293.
66. Upadhyay R, Ramalakshmi K, Rao LJM. 2012. Microwave-assisted extraction of chlorogenic acids from green coffee beans. Food Chem. 130:184-188.
67. Wong SK, Lim YY, Chan EW. 2009. Antioxidant properties of Hibiscus species variation, altitudinal change costal influence and floral colour change. J. Tro. For. Sci. 21: 307-315.
68. Wu J, Lin L, Chau F. 2001. Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. Ultrasonics Sonochem. 8: 347-352.

69. Xia T, Shi S, Wan X. 2006. Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. J. Food Eng. 74: 557-560.

APPENDICES

Table: 1. Effect of Extraction Methodology on Extraction Efficiency

Plant material	Method	Solvent	Activity reported	Extraction efficiency (%)	Ref.
Syzygium cumini [seed]	MAE	Absolute Methanol	Antibacterial	16.4	Kothari, 2010
		50% Ethanol	Antibacterial	29	
	Cold Percolation	Absolute Methanol	Anti-inflammatory	10.36	Kumar *et al.*, 2008 b
			Anti-Diabetic	10.36	Kumar *et al.*, 2008 a
			CNS	-	Kumar *et al.*, 2008c
			Anti-Arthritic	10.38	
		Ethyl Acetate	CNS Activity Radio protective	1.81	Kumar *et al.*, 2008b; Jagetia *et al.*, 2005
	Soxhlet	50% Ethanol	Anti-Diabetic	20	Singh and Gupta, 2007
		Absolute Ethanol	-	0.9	

Table: 2. Type of Phytochemicals Extracted in Various Solvents (Houghton and Raman, 1998; Cowan, 1999)

Polarity	Solvent	Chemical class extracted
Low	Chloroform	Terpenoids, Flavonoids, Alkaloids, Aglycones
	Cyclohexane	Waxes, Fats
	Hexane	Waxes, Fats
	Dichloromethane	Terpenoids, Alkaloids, Aglycones
	Diethylether	Alkaloids, Aglycones,
	Ethylacetate	Alkaloids, Aglycones, Glycosides
	Acetone	Flavonols, Alkaloids, Aglycones
	Ethanol	Tannins, Polyphenols, Flavonol, Terpenoids, Sterols, Alkaloids, Polyacetylenes, Propolis.
Medium	Methanol	Saponins, Tannins, Phenones, Flavones, Sugars, Aminoacids, Anthocyanins, Terpenoids, Xanthoxyllines, Totarol, Quassinoids, Lactones, Polyphenols
High	Water	Sugar, Aminoacids, Saponins, Tannins, Lectins, Terpenoids, Anthocyanins, Starches, Polypeptides

Table: 3. Binary mixtures of solvents of differing polarity used for extraction

Method	**Solvent mixture**	**Reference**
MAE	Chloroform: Methanol	Kothari *et al.*, 2009; Kothari *et al.*, 2010
PMAE	Hexane: Acetone	
Soxhlet	Hexane: Acetone	Kothari *et al.*, 2010
Sonication	Dichloromethane: Acetone	

Table: 4. Properties of different solvents used for extraction of plant materials (Mendham *et al.*, 2005; Leonelli and Mason, 2010)

Solvent	**B.P.**	**Polarity index**	**Dielectric constant**	**Dissipation factor**
	(°C)			**(tan δ)**
Acetone	56.53	5.1	20.7	-
Chloroform	61.2	4.1	4.81	0.091
Chlorobenzene	132	2.7	2.71	-
Diethyl ether	35	2.8	4.34	-
Dichloromethane	40	3.1	9.1	-
Ethyl acetate	77	4.4	6.02	-
Ethylene glycol	182	6.9	37.7	-
Ethanol	78.5	4.3	24.6	0.941
Methanol	64.7	5.1	32.6	0.856
n-hexane	69	0.1	1.89	0.02
Petroleum ether	-	0.1	-	-
Water	100	10.2	80.4	9.889

Table: 5. Comparative account of various extraction methods (Ahuja and Diehl, 2006: Camel2001)

Method	Sample size (g)	Solvent volume (ml)	Operating temp (°C)	Suitability for thermolabile components	Suitability for volatile components	Time (h)	Possibility of solvent recovery	Automation level	Cost
Traditional Soxhlet	10-20	200-500	40-100	No	No	12-24	Yes	None	Low
Modern Soxhlet	10-20	50-100	40-100	No	No	1-4	Yes	Mostly	Moderate
Sonication	20-50	100-300	Ambient-40	Yes	Yes	0.5-1.0	No	None	Moderate
SFE	5-10	10-20	50-150	Yes	Yes	0.5-1.0	No	Fully	High
Pressurized fluid extraction	1-30	10-45	50-200	Yes	Yes	0.2-0.3	No	Fully	Moderate
Closed –vessel MAE	2-5	30	100-200	Yes	Yes	0.1-0.2	No	Mostly	High
Open-vessel MAE	2-10	20-30	Ambient	Mostly	No	0.1-0.2	No	Mostly	Low
Soxhwave	10-20	50-100	40-100	Yes	Yes	0.1-0.2	Yes	Mostly	Moderate