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# Green technology: Economically and environmentally innovative methods for extraction of medicinal & aromatic plants (MAP) in Egypt

**Khaled A. Shams*, Nahla S. Abdel-Azim, Ibrahim A. Saleh, Mohamed-Elamir F. Hegazy, Mostafa M. El-Missiry and Faiza M. Hammouda**

*National Research Centre, 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt*

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**ABSTRACT**

*Extraction process 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. Yet the extraction step remains often a neglected area, which over the years has received much less attention and research. Traditional methods of extraction and processing of herbs and medicinal plants such as solid liquid extraction (Soxhlet), steam distillation or cold press are still used in Egypt. These methods of extraction lack selectivity, give lower yields and because it uses large volume of organic solvents it present safety concern and environmental risk. Several new extraction techniques for improving efficiency and selectivity are now replacing the old methods of extraction. However, recently exports of medicinal and aromatic plants products from Egypt to other countries are becoming more and more restricted due to the presence of unacceptable levels of contaminants and occasionally the occurrences of heavy metals and pesticides that attributed to the drawbacks of traditional extraction methods. The fact that one single plant can contain several secondary metabolites makes the need for the development of high performance and rapid extraction methods an absolute necessity. 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), Accelerated Solvent Extraction (ASE), Subcritical Water Extraction (SWE) and Ultrasound Assisted Extraction (USE) have drawn significant research attention in the last decade. In this review, we discussed the principles, affecting factors, advantages and disadvantages of traditional and innovative extraction techniques. We also suggested some ideas to establish these innovative technologies for extraction in Egypt.*

**Keywords:** Innovative Green Techniques, Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SCFE), Accelerated Solvent Extraction (ASE), Subcritical Water Extraction (SWE), Ultrasound Assisted Extraction (USE).

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## INTRODUCTION

Medicinal plants have been used as a source of remedies since ancient times. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases. The healing of sick persons was carried out by priest doctors who prescribed and prepared medicaments. The first recorded prescriptions were found in Ancient Egyptian tombs. The writing on the temple walls and in the papyri revealed that Ancient Egyptians used many herbal drugs for the same purposes as they are used today**[1-4]**.

Medicinal plants and natural herb production and export from Egypt have been a major business and important source of income for many years. Among others; chamomile, fennel, anise, basil, peppermint and many other essential and fixed oils crops that are cultivated in Egypt have a very good reputation in the international markets. However, recent exports of these products from Egypt to other countries are becoming more and more restricted due

to the presence of unacceptable levels of contaminants and occasionally the occurrences of heavy metals and pesticides. Traditional methods of extraction and processing of herbs and medicinal plants such as solid liquid extraction (Soxhlet), steam distillation or cold press are still used in Egypt. These methods of extraction lack selectivity, give lower yields and because it uses large volume of organic solvents it present safety concern and environmental risk. Several new extraction techniques for improving efficiency and selectivity are now replacing the old methods of extraction **[5-7]**.

Plants have been the source of potential therapeutic agents ever since mankind has evolved. Although several active phytoconstituents and high activity profile drugs have been discovered from plants but the quality and safety related problems of herbal drugs have still been a challenge for researchers. The major reasons for these drawbacks are the lack of high performance, reliable extraction techniques and methodologies for establishing the purity and standard for herbal medicines **[8, 9]**. Due to these factors, the herbal medicines have still to find their way in order to be accepted in global market. In research related to discovery of new active phytoconstituents, extraction is one of the important steps as it is the starting point for the isolation and purification procedures. An individual plant may consist of several active phytoconstituents existing in abundance along with certain constituents of low activity profile. Thus, there arises a need for the development of extraction and analysis techniques with high performance [**10**]. There has been a need for better and newer extraction techniques, in the herbal drug industry so that the extraction time and the cost of solvent consumption is decreased **[11,12]**.

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and to improve the mass transfer. Usually the traditional techniques require longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents **[13]**. Novel extraction methods including microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), accelerated solvent extraction (ASE), Subcritical Water Extraction (SWE) and ultrasound assisted extraction (USE) have drawn significant research attention in the last decade **[14]**. If these techniques are explored scientifically, they can provide an efficient extraction technology for ensuring the quality of herbal medicines worldwide. For the past 126 years, Soxhlet extraction has been the most used among all other conventional techniques **[13]**. 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, and 24 hours or more, which results in consumption of considerable time and heat energy [**15**]. 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 [**16,17**]. 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 **[13,18]**.

**1. An Overview of Extraction Techniques for Medicinal and Aromatic Plants:**

Some of the major constraints in sustainable industrial exploitation of medicinal and aromatic plants (MAPs) are due to the fact that the developing countries including Egypt have poor agricultural practices for MAPs, unscientific and indiscriminate gathering practices from the wild, poor postharvest and post-gathering practices leading to poor quality raw material, lack of research for the development of high-yielding varieties of MAPs, poor propagation methods, inefficient processing techniques, poor quality control procedures, lack of research on process and product development, difficulty in marketing, non-availability of trained personnel, lack of facilities and tools to fabricate equipment locally, and finally lack of access to the latest technologies and market information **[19]**. This calls for co-operation and coordination among various institutes and organizations of the region, in order to develop MAPs for sustainable commercial exploitation. The process of extracting MAPs determines how efficiently we add value to MAP bioresources. In case of essential oils, the extraction process affects the physical as well as internal composition. External appearance, at times, can result in rejection of the batch even if the analytical results are within acceptable limits. Furthermore, essential oils are evaluated internationally for their olfactory properties by experienced perfumers and these olfactory qualities supersede analytical results. Variations in the chemical constituents of the extracts of medicinal plants may result by using non-standardized procedures of extraction. Efforts should be made to produce batches with quality as consistent as possible (within the narrowest possible range) **[8, 20]**.

**2. Parameters for Selecting an Appropriate Extraction Method:**

**i)** Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated.

**ii)** Use the right plant part and, for quality control purposes, record the age of plant and the time, season and place of collection.

**iii)** Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Hot or cold blowing air flow for drying is generally preferred. If a crude drug with high moisture content is to be used for extraction, suitable weight corrections should be incorporated.
**iv)** Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.
**v)** Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.
**vi)** Nature of constituents:
**a**) If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. Likewise, for plants with the active constituents like glycosides and hence a polar solvent like aqueous methanol may be used.
**b**) If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. For thermostable constituents, Soxhlet extraction (if nonaqueous solvents are used) and decoction (if water is the menstruum) is useful.
**c**) Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g. flavonoids and phenyl propanoids.
**d**) In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.
**e**) Standardization of time of extraction is important, as:

- Insufficient time means incomplete extraction.
- If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.

**f**) The number of extractions required for complete extraction is as important as the duration of each extraction.
**vii)** The quality of water or menstruum used should be specified and controlled.
**viii)** Concentration and drying procedures should ensure the safety and stability of the active constituents. Drying under reduced pressure (e.g. using a Rotavapor) is widely used. Lyophilization, although expensive, is increasingly employed.
**ix)** The design and material of fabrication of the extractor are also to be taken into consideration.
**x)** Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documented to monitor the quality of different batches of the extracts **[10,13,20]**.

## I. Conventional Solvent Extraction of Medicinal Plants:

Classic techniques for solvent extraction of active constituents from medicinal plant matrices are based on the choice of solvent coupled with the use of heat or agitation. Existing classic techniques used to obtain active constituents from plants include: Soxhlet, hydrodistillation and maceration with an alcohol-water mixture or other organic solvents. Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for, in limited fields of application, the extraction of thermolabile compounds **[21,22]**.

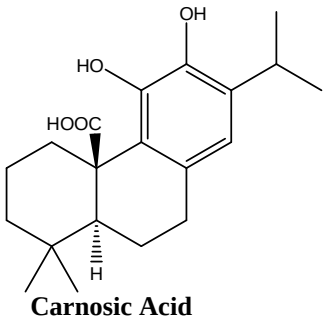
### 1. Advantages and Disadvantages of Soxhlet Extraction:

**- Advantages:**
1. The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix.
2. Maintaining a relatively high extraction temperature with heat from the distillation flask.
3. No filtration of the extract is required.

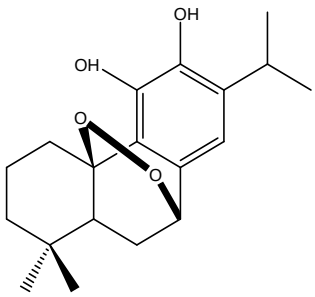
**- Disadvantages:**
1. Agitation is not possible in the Soxhlet device.
2. The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time **[23]**.

Worldwide, most of solvent extraction units are based on Soxhlet principle with recycling of solvents. Basic equipment for a solvent extraction unit **(Fig. 1)** consists of a drug holder-extractor, a solvent storage vessel, a reboiler kettle, a condenser, a breather system (to minimize solvent loss) and supporting structures like a boiler, a refrigerated chilling unit and a vacuum unit **[24]**.

A notable example is the extraction of carnosol from Labiatae herbs. Carnosol was thought to be the main active principle as it had been extracted and isolated by conventional extraction techniques. The true active compound, carnosic acid, was eventually isolated by **SCFE** and carnosol was found to be an artifact resulting from the oxidation of carnosic acid during the extraction by the conventional techniques **[25].**




**Carnosic Acid**




**Carnosol**

## 2. Extraction of Aromatic Plants

The types of volatile isolates that are obtained commercially from aromatic plants are essential oils, concretes, absolutes, pomades and resinoids. Essential oils are isolated from plant material by distillation whereas other volatile isolates are obtained by solvent extraction.

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. The world production and consumption of essential oils and perfumes are

**(Fig. 1) Soxhlet Extraction of Plant**

increasing very fast. Production technology is an essential element to improve the overall yield and quality of essential oil. The traditional technologies pertaining to essential oil processing are of great significance and are still being used in many parts of the globe. Water distillation, water and steam distillation, steam distillation, and maceration and are the most traditional and commonly used methods **[26, 27]**.

Maceration is adaptable when oil yield from distillation is poor. Distillation methods are good for powdered almonds, rose petals and rose blossoms, whereas solvent extraction is suitable for expensive, delicate and thermally unstable materials like jasmine, tuberose, and hyacinth. Water distillation is the most favored method of production of citronella oil from plant material **[28]**.

**Drawbacks of Conventional Methods of Essential Oils Extraction:**

1. Common distillation techniques are capable of isolating volatile chemicals having boiling points within a relatively narrow range. For this reason, losses of both very light and heavy fractions are often unavoidable during the distillation of natural aromatic materials.
2. During distillation, chemicals contained in natural materials may undergo thermal reactions and artefacts are often formed. Therefore, distilled oil contains both natural compounds as well as their thermal decomposition products. As water and steam distillation processes are universally accepted such oils are considered as natural pure oils. The oil distilled may not therefore represent the true odour of the aromatic material from an olfactory point of view.
3. Distillation does not separate closely boiling substances regardless of differences in their chemistry.
4. Distillation is inappropriate if the recovery of trace amounts from a bulk mixture is desired even when the boiling points are markedly different.
5. Distillation is quite inefficient in recovering aroma chemicals from dilute aqueous media, such as hydrosols and fruit juices.

**3. Modern (Non-traditional) Methods of Extraction of Essential Oils**

Traditional methods of extraction of essential oils have been discussed and these are the methods most widely used on commercial scale. However, with technological advancement, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as the production of costly essential oils in a natural state without any alteration of their thermosensitive components or the extraction of essential oils for micro-analysis. These techniques are as follows:

- Headspace trapping techniques
- Static headspace technique
- Vacuum headspace technique
- Dynamic headspace technique
- Solid phase micro-extraction (SPME)
- Supercritical fluid extraction (SFE)
- Phytosol (phytol) extraction
- Protoplast technique
- Simultaneous distillation extraction (SDE)
- Microwave distillation
- Controlled instantaneous decomposition (CID)
- Thermomicrodistillation
- Microdistillation
- Molecular spinning band distillation
- Membrane extraction

**II. Innovative Extraction Techniques:**

Several new extraction techniques for improving efficiency and selectivity are now replacing the traditional methods of extraction. These novel extraction methods include **Accelerated Solvent Extraction (ASE), Subcritical Water Extraction (SWE), Supercritical Fluid Extraction (SCFE)**, **Microwave Assisted Extraction (MAE)** and **Ultrasound Assisted Extraction (UAE)**.

Developing an alternative rapid, sensitive, safe, energy conserving extraction technique is highly desirable. We need simplicity in application of new technique with no-risk or danger, no need to toxic and / or radioactive chemical input, cheaper and with less input – low process cost, flexibility and functional usage, adaptation to different field easily, automation and validation.

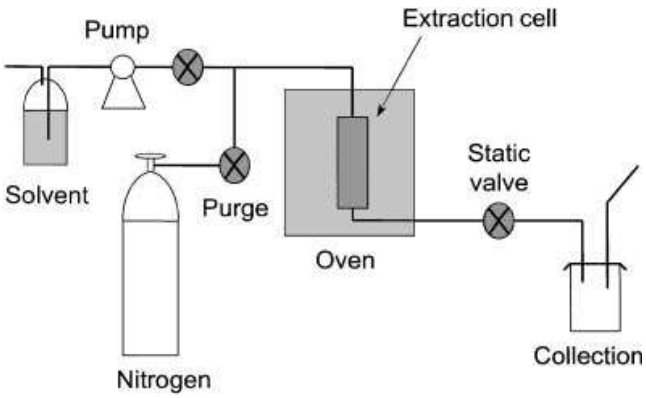
**1. Accelerated Solvent Extraction (ASE):**

Accelerated solvent extraction (**ASE**) or sometime called pressurized solvent extraction (**PSE**) or pressurized liquid extraction (**PLE**) is a technique which has been developed as an alternative to current extraction methods such as Soxhlet, maceration, percolation or reflux, offering advantages with respect to extraction time, solvent consumption, extraction yields and reproducibility **(Fig 2,3,4)**. It uses organic solvents at elevated pressure and temperature in order to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus enabling safe and rapid extractions [**29, 30, 31, 32**]. Furthermore, high pressure forces the solvent into the matrix pores and hence, should facilitate extraction of analytes. High temperatures decrease the viscosity of the liquid solvent, allowing a better penetration of the matrix and weakened solute matrix interactions. Also, elevated temperatures enhance diffusivity of the solvent resulting in increased extraction speed. Solvents can be selected based on the polarity of the analyte and compatibility with any post-extraction processing steps and quantification equipment. In addition, **ASE** technology is automated.

For rapid and efficient extraction of analytes from solid matrices such as plant materials, **extraction temperature** is an important experimental factor, because elevated temperatures could lead to significant improvements in the capacity of extraction solvents to dissolve the analytes, in the rates of mass transport, and in the effectiveness of sample wetting and matrix penetration, all of which lead to overall improvement in the extraction and desorption of analytes from the surface and active sites of solid sample matrices. To achieve all these advantages, **elevated pressure** is required to maintain the extraction solvents as in liquid state at high temperatures (usually above their boiling points) [**33, 34**]. In addition to extraction temperature, **the choice of extraction solvent** is another important factor. Most **ASE** applications reported in the literature employed the organic solvents commonly used in conventional techniques, e.g. methanol, in which many organic compounds are very soluble. **ASE** was used for the extraction of paclitaxel (commonly known as taxol, which has anticancer activity), from the bark of ***Taxuscus pidata*** and showed that use of water alone as the extraction solvent is a viable alternative. It was found that use of hot water as the extraction solvent under atmospheric or higher pressure conditions was very efficient for extracting many phytochemicals **[35]**. The use of **ASE** with subcritical water as the extractant for the extraction and analysis of analytes from medicinal plants might have interesting potential, because water is inexpensive, non-toxic, and environmentally friendly.

**(Fig. 2) Accelerated Solvent Extraction Up to 24 samples (Phytochemistry Dept., NRC)**

**(Fig. 3) Accelerated Solvent Extraction (single-cell system)**




**(Fig. 4) Scheme of an ASE apparatus**

Also, by simply increasing the temperature at constant pressure, the relative permittivity of water can be reduced, so that analytes with a wide range of polarity can be extracted.

Most research publications before 2000 on **ASE** were associated with environmental analysis but since then, use of PLE for extraction of botanicals with medicinal properties has increased considerably. Also, an in-depth review of **ASE** applications in food and biological samples was published **[33]**.

**ASE** has been reported to be more efficient than other extraction methods by consuming less solvent and allowing faster extraction (**Table 1**).

**(Table 1) Advantages & Dis-advantages of Using Accelerated Solvent Extraction Technique**

| Advantages | Dis-advantages |
|---|---|
| **1.** Better extraction kinetics due to increased operating temperature.<br>**2.** Processing parameters can be altered to extract different components or to increase selectivity.<br>**3.** Better method precision and reproducibility compared to conventional methods [**40,42**].<br>**4.** Inert atmosphere (nitrogen) reduces oxidation risk of compound compared to conventional extraction processes.<br>**5.** Method development is relatively simple compared to SFE.<br>**6.** Shorter extraction time and reduced solvent consumption.<br>**7.** Possibility for automation | **1.** Extraction is performed at elevated temperature, thus thermal degradation is a cause of concern, especially for thermolabile compounds in the extracts.<br>**2.** Selectivity is mainly affected by varying the solvent type. Extraction tends to be exhaustive leading to nonselective extractions.<br>**3.** Post-extraction clean up step is still necessary. |

**Factors affecting (ASE)**

- **The effect of pressure:**

The elevated pressure serves to maintain the solvent in the liquid phase during extraction and to ensure that the solvent remains in contact with the sample. Some researchers reported that pressure had little effect on the extraction process [**36, 29, 37**]. However, others suggested that high pressure might increase extraction efficiency by forcing the solvent into the matrix pores demonstrated that the pressure effect was matrix-dependent [**31, 38**, **39]**. In their investigations, **ASE** carried out on tea leaves at temperature of 70 and 100 °C resulted in lower caffeine yields when the pressure was increased above 100 bars. The elevated pressure might have caused compression of the soft tea matrix, reducing the efficiency of transport of the compound out of the matrix and penetration of the solvent into all areas of the matrix.

- **The effect of temperature:**

In **ASE**, extraction is usually carried out above the boiling point of the solvent to improve the extraction kinetics by the disruption of matrix-analyte interactions (i.e. van der Waals forces, hydrogen bonding, dipole interactions), increased molecular motion of solvent molecules and enhanced compound solubility in the extraction solvent as a result of the elevated temperature [**30; 10; 31; 40; 32; 41**]. Increasing the temperature of extraction at elevated pressure also reduces the dielectric constant of the organic solvent used [**41**]. At elevated pressure, the dielectric constant of water would decrease, rendering it less polar and thus more solubilizing towards organic compounds. Studies replacing organic solvents with water, i.e. pressurized hot water extraction (**PHWE**) have also been performed [**40, 42, 43, 44, 45, 46, 47**]. It has also been argued that the temperature effects were more important for solvents that modified the matrix but less significant for those governing the extraction of analytes [**45**].

- **The effect of static extraction time:**

During the static phase of extraction, the diffusion of compounds from the matrix into the solvent occurs without the outflow of solvent from the extraction vessel. At the end of the extraction process, the extract is collected by rapidly flushing the extraction cell with fresh solvent and an inert gas, nitrogen. An increase in the static extraction time generally increases the extraction yield until equilibrium is reached, increasing the static extraction time further does not result in further improvement on compound recovery [**29**].

- **The effect of flush volume:**

The flush volume, typically 40 – 60 % of the cell volume, is the amount of fresh solvent that is used to flush the sample at the end of the extraction process [**32**]. This serves to discharge the previous volume of solvent used in the extraction into the collection vial. While it was reported increased yields of active compounds from *Angelica sinensis* with reduced flush volumes [**48**], flush volume was reported to have no significant effect on extraction yields of Cortex Dictam [**37**]. The effect of flush volume on PLE was not as widely studied as that of other process variables.

- **The effect of vessel void volume:**

Reduction of vessel void volume with an inert packing material ensures better solvent-matrix contact, reduces analyte oxidation due to presence of air, and reduces solvent consumption [**32**]. Diatomateous earth is commonly used especially if the material to be extracted exists as a fine powder [**48**]. Neutral glass has also been used as a dispersant to reduce the volume of solvent used for extraction [**39,49,50**].

**2. Subcritical water extraction (SWE):**

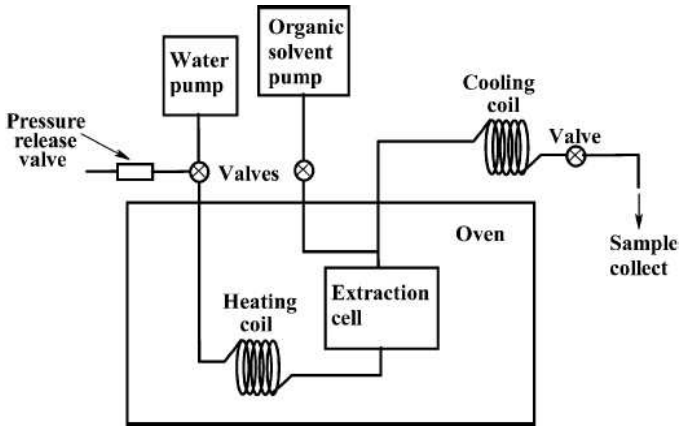
**Principles and instrumentation:**

Subcritical water extraction (**SWE**), i.e., extraction using hot water under pressure, has recently emerged as a useful tool to replace the traditional extraction methods. **SWE** is an environmentally-clean technique that, in addition, provides higher extraction yields to extract solid samples [**51**].

SWE is carried out using hot water (from 100 $^{\circ}$C until 374 $^{\circ}$C, the latter being the water critical temperature) under high pressure (usually up to 10 bar) enough to maintain water in the liquid state. The most important factor to take into account in this type of extraction procedures is the **dielectric constant (ε)**. This parameter can be modulated easily, within a wide range of values, by only tuning the extraction temperature. Water at room temperature is a very polar solvent, with a dielectric constant close to 80. However, this level can be significantly decreased to values close to 27 when water is heated up to 250 $^{\circ}$C, while maintaining its liquid state applying pressure. This dielectric constant value is similar to that of ethanol being therefore appropriate to solubilize less-polar compounds [**52]**.

Basically, the experimental set-up needed to use this technique is quite simple. The instrumentation consists on a water reservoir coupled to a high pressure pump to introduce the solvent into the system, an oven, where the extraction cell is placed and where the extraction takes place, and a restrictor to maintain the pressure along the extraction line. Extracts are collected in a collector vial placed at the end of the extraction system. Additionally, the system can be equipped with a coolant device for a fine control of the temperature. Also the instrumentation can include a nitrogen circuit to purge the system once the extraction is completed **(Fig. 5)**.

To the best of our knowledge, up to now there is no specific device commercially available for **SWE**. That is, all published results were obtained using home-made devices by adapting a gas chromatographic oven [**53, 54, 55**], designing a new type of oven [**56**], in an **SCFE** system [**57,58**] or in a commercially available **ASE** device [**59, 60**].




**(Fig. 5) Schematic diagram of the basic SWE equipment**

Although this technique has been mainly used in batch processes, there is a work published about the on-line coupling of a **SWE** system to **HPLC** equipment via a solid phase trapping [**61**].

**Extraction from plants using SWE:**
Subcritical water extraction has been widely applied to extract different compounds from several vegetable matrices.

- **Extraction of antioxidant compounds of rosemary by SWE :**

One of the most deeply studied materials has been rosemary (***Rosmarinus officinalis L.***). The extraction of antioxidant compounds of rosemary by **SWE** testing a wide range of temperatures was studied. Also in this work, an exhaustive characterization of the extracts obtained was carried out. Several temperatures, from 25 to 200 °C were tested to study the extraction selectivity towards antioxidant compound [**62**].

There was a clear effect of **water temperature** on the extraction yield, increasing at higher extraction temperatures. It was found that the most polar compound (i.e. **rosmanol**) was the main compound extracted at low temperatures ( 25°C). When the extraction was performed at 200 °C, a decrease on the capability of water to dissolve the most polar compounds was observed, while a high concentration of other kind of compounds, as carnosic acid of medium-lower polarity, was obtained. Besides, the possibility to obtain antioxidant extracts comparable to those achieved using supercritical carbon dioxide extraction was demonstrated.

In addition to antioxidants from rosemary, the **SWE** of aroma compounds has been also studied for this plant [**63**] and other plants as savory (***Satureja hortensis***) and peppermint (***Mentha piperita***) [**64**].

- **Extraction of essential oil from coriander (*Coriandrum sativum* L.) seeds:**

The application of **SWE** in the extraction of essential oil from coriander (***Coriandrum sativum* L.**) seeds was studied. Ground coriander seeds (3-4 g) were subjected to SWE with water for an extraction time of 15 min under several extraction conditions (pressures of 870 and 1000 psi and temperatures of 65, 100 and 150 °C). The **SWE** method was compared with hydrodistillation performed by treating 10 g of ground coriander seeds with 100 mL of water for 3 hours. Compounds were removed from the aqueous extract with hexane and determined by gas chromatography mass spectrometry (GC-MSD). It was found that the efficiency (g oil/g of coriander) of SWE was higher than that provided by hydrodistillation with reduced extraction time. The major compounds found were linalool, isoborneol, citronellyl butyrate and geraniol. **SWE** method has the possibility of manipulating the composition of the oil by varying the temperature and adjusting the pressure [**65**].

Some studies have been conducted to compare **SWE** to traditional extraction methods (such as Soxhlet extraction). Thus, clove (*Syzygium aromaticum*) extractions were performed by Clifford *et al.* [**66**] who demonstrated that the amount of eugenol and eugenyl acetate recovered using subcritical water at 150 ºC was similar to those achieved using Soxhlet extraction and hydrodistillation. These compounds are well known to possess antioxidant properties similar to other natural compounds such as α-tocopherol [**67**].

Interestingly, in general **the use of subcritical water extraction provides a number of advantages over traditional extraction techniques (i.e. hydrodistillation, organic solvents solid-liquid extraction). These are, mainly, low extraction times, higher quality of the extracts (mostly for essential oils), lower costs of extractant agent, environmentally cleaner technique, and better and adjustable selectivity that can be easily changed by tuning the extraction temperature [68].**

These advantages have been verified on several plants as laurel [**69**], fennel [**70],** oregano [**71**] and kava [**72**], among others. **Ozel *et al.*** studied the extraction of essential oil from *Thymbra spicata* considering the influence of several factors such as temperature (100, 125, 150 and 175 °C), pressure (20, 60 and 90 bar) and flow rate (1, 2 and 3 ml/min) for dynamic extractions. In this work, it was proved that the best extraction yields (3.7 %) were obtained at 150 °C and 60 bar using a flow rate of 2 ml/min for 30 minutes. Later, an analysis of the extracts, by means of a two-dimensional gas chromatography-TOF-Mass spectrometry demonstrates the type of compounds selectively extracted under the mentioned conditions. Moreover, the essential oils of *Timbra spicata* were found to inhibit mycelial growth of the several fungi species [**73**].

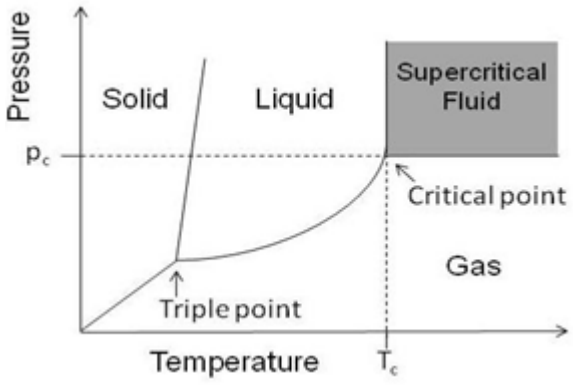
Subcritical water under pressure, and held at temperatures between 110-160°C was utilized for the extraction of anthocyanin-based pigments from fruit berries (both wet and dry), such as elderberry, raspberry, bilberry, chokeberry; and their associated stems, skins, and pomaces [**74**].

**(Fig. 6) Lab. Scale $CO_2$ Extractor**

**Supercritical Fluid Extraction (SCFE):**

A supercritical fluid is an element or compound above its critical pressure and temperature [**75**]. In this state, it is compressible and possesses both the properties of a gas and a liquid, providing the supercritical fluid with improved solvating power and has an edge over conventional liquids.The main attraction of SCFE **(Fig. 6)** is the use of carbon dioxide (**$CO_2$**) as the solvent. Unlike most organic solvents, **$CO_2$** is not as harmful environmentally and has been described as a "green solvent". It is an inexpensive, relatively inert and non-inflammable gas of low toxicity, with easily achievable supercritical conditions (Tc =31.3°C, Pc = 73.8 bar) **(Fig. 7, 8)**. Supercritical **$CO_2$** has virtually no surface tension, thereby allowing better penetration into botanical matrices compared to liquid solvents. An added advantage is the possibility of conducting solvent less extraction with **SCFE**, as **$CO_2$** would simply depressurize, subsequently depositing the extract into the collection vessel. However, **$CO_2$** is a nonpolar fluid and has no permanent dipole moment. Thus, there is limited ability to dissolve polar or high molecular weight compounds [**76; 77; 78; 79**]. **(Table 2)**.


**(Fig.7) Advantages of $CO_2$**




**(Fig. 8) Phase Diagram of $CO_2$, as Tc is the Critical Temperature (31.3 °C)**

**Extracting Solvent and Pc is the Critical Pressure (73.8 Bar)**

The extraction of natural products with supercritical fluids, especially with carbon dioxide, has found numerous large-scale applications in the food and perfume industries including, for example, the decaffeination of coffee beans, extraction of bitter principles from hops, extraction of essential and pungent principles from spices, natural food colorants, and extraction of essential oils for perfumery [**75**]. Numerous applications of analytical **SCFE** to various herbs and natural products classes have been published [**80, 81**]. Despite these reports, the number of industrial-scale applications of **SCFE** in medicinal plant extraction has remained very small. First of all, the lipophilic nature of supercritical carbon dioxide has been a major limiting factor. Many natural products classes which are of importance as pharmacologically active substances in medicinal plants, such as phenolics, alkaloids and glycosidic compounds, are poorly soluble in carbon dioxide and hence not extractable. **SCFE** protocols for the extraction of selected medicinal plants are under development. Addition of polar co-solvents (modifiers) to the supercritical solvent is known to increase significantly the solubility of polar compounds. Typically, modifiers are selected to interact strongly with the compounds of interest. The most widely used modifiers are methanol and ethanol, which undergo dipole–dipole interactions and hydrogen-bonding with polar functional groups. The density and dielectric constant, and hence the solvating power, of supercritical carbon dioxide depends on its pressure and temperature [**75, 81**]. Increase in the pressure enhances the solubility of solutes such that **SCFE** at very high pressure could represent an additional possibility to increase the solvating power of the extraction fluid. Most **SCFE** applications have been carried out in the pressure range between the critical pressure of carbon dioxide and ca. 300 bar [**81**]. Few examples of the extraction of natural products at higher pressures have been published, and the scope of these applications is very limited. The current scarcity of high-pressure **SCFE** applications is mainly due to technical limitations of most analytical and pilot-scale **SCFE** equipment. Recently, laboratory and pilot-scale SFE instruments operating at pressures well above 500 bars have become available. Therefore, the potential and limitations of natural product extraction by combining very high pressures (300 bars) and a pharmaceutically acceptable polar modifier (ethanol) was explored in a more systematic manner.

**(Table 2) The Advantages and Disadvantages of the SCFE Process**

| Advantages | Dis-advantages |
|---|---|
| **1.** Enhanced extraction efficiency.<br>**2.** Tunability of the solvent strength.<br>**3.** Low organic solvent consumption.<br>**4.** Preservation of bioactive properties and organoleptic properties of the extracts<br>**5.** In-line integration with sample preparations and detection methods.<br>**6.** High capital investment. | **1.** High capital investment.<br>**2.** Large number of variables to optimize<br>**3.** Strong dependence on matrix analyte interactions.<br>**4.** Difficulties in scale up and technology transfer.<br>**5.** Difficulty in implementing continuous extraction processes<br>**6.** Difficulty in extracting more polar compounds. |

**Factors Affecting SCFE:**

Sample preparation, sample size, particle size, homogeneity of sample, water/moisture content, drying agent (type, ratio), vessel volume and packing density.

**Extraction Parameters**

Supercritical fluid (extractant), pressure, temperature, flow rate, modifier (type, amount), extraction time and restrictor temperature.

**Trapping:**

Collection technique, liquid trapping solvent or solid trapping agent used, trap size or solvent volume, collection temperature, trap elution solvent, trap elution volume and temperature and flow rate.

**The Effect of Modifier**

A polar modifier is usually added to improve the solubility of the more polar analyte in the supercritical fluid or to competitively displace the analyte from the matrix active sites [**82, 83**]. Polar modifiers ,such as water or methanol, have the added advantage of inducing swelling of the matrix thus improving accessibility to more remote matrix interior sites [**84, 85**].

The addition of a modifier is reported to increase the yield of the analyte of interest by up to three-fold [**86,87**]. The most common modifier used is methanol which has excellent hydrogen bond donating and accepting properties and is thus useful for extracting polar analytes, although ethanol is sometimes used **[88-91]**. Methanol-modified $CO_2$ also led to the recovery of an additional flavanone from Osage orange tree (***Maclura pomifera***) root bark **[88]**. This additional flavanone was absent in the extracts obtained by the conventional solvent extraction methods.

In some cases, water **[85, 91, 92, 93]** as well as natural plant oils [**91,94**] were used as modifiers. The addition of a modifier will, however, add to the complexity of the **SCFE** system.

First and foremost, the critical conditions of the system will change with the use of a modifier and the extraction conditions must be modified to ensure a homogeneous single phase of the extractant fluid. In **SC-$CO_2$** extraction, it is recommended that temperature should be increased to around 70-80 °C with the addition of 10 % v/v modifier, although lower temperatures have been used in practice **[95]**.

Secondly, there may be poorer selectivity for the analyte due to increased co-extraction of unwanted substances [**96**]. Thirdly, the modifier may condense on the solid-phase trappings, reducing the transfer efficiency of analyte from **$CO_2$** to the solid phase **[95]**. Hence, a modifier should only be used when increasing the SF density fails to extract the compound of interest. Even so, poor yields may still result despite the use of high extraction pressures and modifiers. Yields of polyphenolic and glycosidic compounds from hawthorne, marigold and chamomile were shown to remain low even at a high extraction pressure of 689 bar, with 20 % ethanol in **$CO_2$ [97]**, and hypericin yields remained low despite addition of ethanol as a modifier **[98]**.

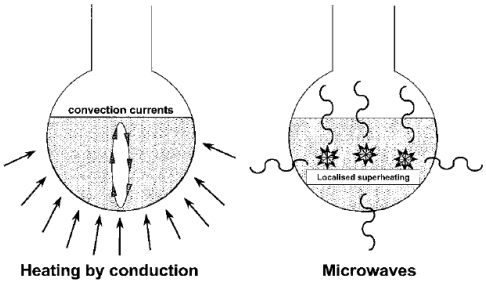
**3. Microwave assisted extraction (MAE):**

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz and positioned between the X- ray and infra-red rays in the electromagnetic spectrum **[99]**. 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 (Fig.9)**, 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.

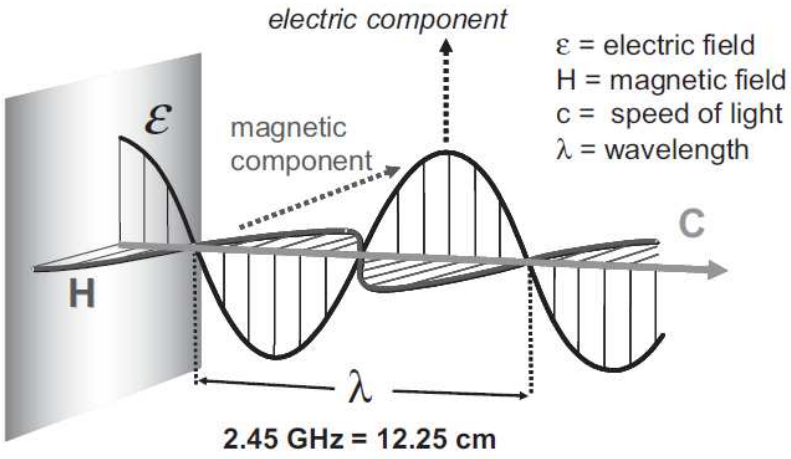
This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet **[8]**. The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenons: ionic conduction and dipole rotation **(Fig. 10)**. Both of them are responsible for heating of substances **[100,101]**, which in most cases occurs simultaneously **[99]**. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field.


**(Fig. 9) Microwave assisted extraction (MAE) (Phytochemistry Dept., NRC)**

The two types of oscillating perpendicular fields that generate microwaves are the electric field and magnetic field **(Fig. 11)**. 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 **[18]**.




**(Fig. 10) The principle of heating using microwave**




**(Fig. 11) Electric and magnetic field components in microwaves**

Only dielectric material or solvents with permanent dipoles do get heated up under microwave. The efficiency with which different solvents heat up under microwave depends on the dissipation factor, which is the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules **[18]**. **(Table 3)** lists solvents that are commonly used in **MAE** with their physical microwave characteristics **[102]**.

(**Table 3**) **Physical Properties of Common Solvents**

| Solvent | b. p. (C°) | έ | ε̆ | Tan δ | Microwave absorbance |
|---|---|---|---|---|---|
| **Ethylene glycol** | 197 | 37.0 | 49.950 | 1.350 | very good |
| **Dimethyl sulfoxide** | 189 | 45.0 | 37.125 | 0.825 | good |
| **Ethanol** | 78 | 24.3 | 22.866 | 0.941 | good |
| **Methanol** | 63 | 32.6 | 21.483 | 0.659 | good |
| **Water** | 100 | 80.4 | 9.889 | 0.123 | medium |
| **1-methyl-2-pyrrolidone** | 204 | 32.2 | 8.855 | 0.275 | medium |
| ***N,N*-dimethylformamide** | 154 | 37.7 | 6.070 | 0.161 | medium |
| **1,2-dichlorobenzene** | 180 | 9.9 | 2.772 | 0.280 | medium |
| **Acetonitrile** | 81 | 37.5 | 2.325 | 0.062 | medium |
| **Dichloromethane** | 40 | 9.1 | 0.382 | 0.042 | low |
| **Tetrahydrofuran** | 66 | 7.4 | 0.348 | 0.047 | low |
| **Toluene** | 110 | 2.4 | 0.096 | 0.040 | very low |

**Instrumentation:**

Microwave systems for extraction and laboratory use are available in two forms:

- Closed extraction vessels/Multi-mode microwave ovens.
- Focused microwave ovens.

The extraction in a closed extraction vessel/ Multi-mode microwave oven is brought about by controlled pressure and temperature. Whereas in focused microwave assisted Soxhlet or solvent extraction (**FMASE)**, as the name indicates, only the part of the extraction vessel containing the sample is focused for irradiation with microwave. Both the closed vessel type and the focused type are available commercially as multimode and single- mode or

focused systems. A multimode system allows random dispersion of microwave radiation within the microwave cavity, so every zone in the cavity and sample is irradiated evenly **[103]**.

**Extraction technique**

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 **[102]**. 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 phytoconstituents. This phenomenon can even be more intensified if the plant matrix is impregnated with solvents with higher heating efficiency under microwave. 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 **[104]**. 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. The effect of microwave energy is strongly dependent on the dielectric susceptibility of both the solvent and solid plant matrix. Most of the time, the sample is immersed in a single solvent or mixture of solvents that absorb microwave energy strongly. 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 was tested for thermo labile components to prevent their degradation **[18, 99, 102]**.

**Factors Affecting Microwave Extraction:**

- **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 **[99]**. Preferably the solvent should have a high selectivity towards the analyte of interest excluding unwanted matrix components. **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. The use of ethanol as an extracting solvent gave significantly higher yield than hexane 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. Both the efficacy and selectivity of **MAE** depend significantly on the dielectric constant of the extracting solvent mixture **[105, 106]**.

Adding 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.

Solvent free **MAE** (**SFMAE**) was also investigated 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 **[89,107]**. **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 **[102]**. 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 **[108,109]**.

- **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. 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 [110]**.

- **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 extraction efficiency. 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. 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 thermo labile constituents. Presoaking of the dried plant material in the extracting solvent prior to **MAE** improved the yield **[102]**.

- **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 **[99]**. This elevated temperature will result in improving 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. Temperature can be effectively controlled in open vessel system by proper combinations of extracting solvents which heat up differently.

**4. Ultrasound Assisted Extraction (UAE):**

The mechanical effect of ultrasound accelerates the release of organic compounds contained within the plant body due to cell wall disruption, mass transfer intensification and easier access of the solvent to the cell content. Ultrasonic-assisted extraction is one of the important techniques for extracting the valuable compounds from the vegetal materials **[111]**, and it is quite adaptable on a small or large scale (i.e. on a laboratory or industry scale) **[112]**. The general ultrasonic devices are ultrasonic cleaning bath and ultrasonic probe system **(Fig. 12)**. The former is suitable to sample preparation for analytical purpose and the latter is efficient in large-scale extraction **[112-114]**.


**(Fig. 12) Ultrasonic Probe System (Phytochemistry Dept., NRC)**


**(Fig. 13) Ultrasonic Cleaning Bath (Phytochemistry Dept., NRC)**

**Ultrasound Assisted Extraction synonyms**
Sonication-assisted extraction or Ultrasonication assisted extraction

- **Direct Ultrasonic Application**

Direct application is achieved through ultrasonic probes, which are immersed into sample, performing ultrasonication directly over the solution without any barrier other than the solution itself (**Fig. 12)**

- **Indirect Ultrasonic Application**

Indirect application is performed, generally, using an ultrasonication bath, the ultrasonic wave needs first to cross the liquid inside the ultrasonic device and then to cross the wall of the sample container (**Fig. 13**).

**Factors affecting extraction efficiency:**
Factors affecting extraction efficiency were examined either individually or in combination. These factors include: (1) the nature of the tissue being extracted and the location of the components to be extracted with respect to tissue structures, (2) pretreatment of the tissue prior to extraction, (3) the nature of the components being extracted, (4) the effects of ultrasonics primarily involve superficial tissue disruption, (5) increasing surface mass transfer[(115,116)], (6) intraparticle diffusion, (7) loading of the extraction chamber with substrate, (8) increased yield of extracted components and (9) increased rate of extraction, particularly early in the extraction cycle enabling major reduction in extraction time and higher processing throughput **[117,118]**.

Living tissues where the desired components are localized in surface glands can be stimulated to release the components by relatively mild ultrasonic stressing **[119]**. In tissues where the desired components are located within cells, pre-ultrasound treatment by size reduction to maximise surface area is critical for achieving rapid and complete extraction[(120,112,115)]. Where pre-hydration is necessary to achieve extraction, ultrasound effectively accelerates the hydration process **[112]**. Ultrasound induced cavitation bubbles present hydrophobic surfaces within the extraction liquid thereby increasing the net hydrophobic character of the extraction medium. Thus it is possible to extract polar components into otherwise hydrophilic aqueous extraction media, reducing the need for generally undesirable hydrophobic or strongly polar extraction media. The disruption of tissue surface structures is revealed with microscopic examination **[112,121,122,115]**.

Ultrasonic equipment engineering is such that it is commercially viable and scalable to consider industrial-scale ultrasonic aided extraction. Potential exists for applying **UAE** for enhancement of aqueous extraction and also where organic solvents can be replaced with generally safe solvents. UAE can also provide the opportunity for enhanced extraction of heat sensitive bioactive and food components at lower processing temperatures. There is also a potential for achieving simultaneous extraction and encapsulation of extracted components to provide protection through the use of ultrasonic. **Compared with other extraction techniques such as microwave-assisted extraction, the ultrasonic device is cheaper and its operation is much easier [102,123]**.

**UAE** uses acoustic cavitation to cause disruption of cell walls, reduction of particle size, and enhancement of contact between the solvent and the target compounds **[124]**.

The ultrasonic field enables generation, locally, of micro-cavitations in the liquid surrounding the plant material. The effects are two fold: **mechanical disruption** of the cell's wall releasing its content and **local heating** of the

liquid, increasing the extract diffusion. The kinetic energy is introduced in the whole volume following the collapse of cavitation bubbles at or near walls or interfaces thus improving the mass transfer across the solid-liquid interface. The mechanical effects of ultrasounds induce a greater penetration of solvent into cellular membranes walls, facilitating the release of contents of the cells and improve mass transfer **[125]**.

**Instrumentation for Ultrasound-Assisted Extraction:**
In **UAE** only a small portion of the ultrasound spectrum is used, namely power ultrasound. Power ultrasound, having frequencies between 20 kHz and 100 MHz, are now well known to have significant effects on the rate of various physical and chemical processes such as cleaning, degassing, solubilization, homogenization, emulsification, sieving, filtration, and crystallization. Power ultrasound involves the mechanical and chemical effects of cavitation. When a liquid is irradiated by ultrasound, microbubbles form, grow, and oscillate extremely quickly, and eventually collapse powerfully if the acoustic pressure is high enough. These collapses, occurring near a solid surface, generate microjets and shock waves that result in cleaning, erosion, and fragmentation of the surface. Micro discharges due to high electrical fields generated by deformation and fragmentation of the bubbles and the formation of radicals could be responsible for the observed chemical effects **[126]**.

The two ultrasound apparatus most commonly used for extraction are the **ultrasonic cleaning bath** and the more **powerful probe system**. For small extraction volumes, an ultrasound horn with the tip submerged in the fluid can be sufficient. Large volumes of fluids have to be sonicated in an ultrasound bath or in continuous or in continuous or recycled-flow sonoreactors **[126]**. Although most of the research effort in UAE has concentrated on ultrasound itself, some studies have also examined the coupling between ultrasound and other techniques. When combined with supercritical fluid extraction, **UAE** enhances mass transfer of the species of interest from the solid phase to the extraction solvent **[127]**.

**UAE** of bioactive compounds is increasingly efficient at directly transferring knowledge into technology for commercial development. This novel process can extract analytes under a concentrated form (low volumes of solvent) and free from any contaminants or artifacts. **The new systems developed so far clearly demonstrated the advantages of UAE in terms of yield, selectivity, operating time, energy input, and even preservation of thermolabile compounds [126]**.

**5. Enhancing Values of Medicinal Plant Extracts by Nanotechnology**:
Milling, grinding or pulverization objective is to produce a suitable sized product for extraction. In extraction processes, milling increases the surface area to volume ratio for improved solvent contact during the extraction process. Raw material factors influencing the extraction process include particle size and size distribution, as well as particle shape and bed packing properties of the material.

The mechanical breakdown of the tough cellulosic plant wall material which forms a barrier against solvent extraction, also improves extractability from the botanical material. It was also reported that fine milling of the botanical matrix may lead to degradation of sensitive compounds as well as loss of volatile components during the extraction process.
A high proportion of oversize particles would result in under-extraction or inefficient extraction processes. A large particle size distribution was reported to result in lower yields and poor reproducibility.

**Nanotechnology** can simply be defined as the technology at the scale of one billionth of a metre **[129]**. Materials exhibit unique properties at nanoscale of 1 to 100 nanometre (nm). The changes in properties are due to increase in surface area and dominance of quantum effects which is associated with very small sizes and large surface area to volume ratio **[130]**. The unique features of nanoparticles have raised great expectations in the field of drug delivery because of their potential to improve clinical efficacy of problematic drug compounds **[131]**. Besides, nanoparticulate drug delivery systems offer exceptional flexibility as a wide variety of active agents including hydrophilic and hydrophobic drugs, proteins, vaccines, and biological macromolecules may be delivered via numerous routes of administration **[132]**. Some of the challenges of most drug delivery systems include poor bioavailability, *in vivo* stability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, side effects and plasma fluctuations of drugs which either fall below the minimum effective concentrations or exceed the safe therapeutic concentrations. However, nanotechnology in drug delivery is an approach designed to overcome these challenges due to the development and fabrication of nanostructures at submicron scale and nanoscale which are mainly polymeric and have multiple advantages **[129]**. Nanotechnology is strategic in developing drug delivery systems which can expand drug markets. Nanotechnology can be applied to reformulate existing drugs thereby extending products' lives, enhance their performance, improve their acceptability by increasing effectiveness, as well as increase safety and patient adherence, and ultimately reduce health care costs

**[133, 134]**. Nanotechnology may also enhance the performance of drugs that are unable to pass clinical trial phases **[134]**.

**1. Enhanced Antioxidant Bioactivity of *Salvia miltiorrhiza* (Danshen) Products Prepared Using Nanotechnology:**

The antioxidant activities of the medicinal plant ***Salvia miltiorrhiza*** (**Danshen**) prepared using nanotechnology or traditional grinding methods were compared using three biological assays. It was found that the nanotechnology preparation had stronger antioxidant bioactivities. Complementary quantitative analysis of four active constituents, salvianolic acid B, cryptotanshinone, tanshinone I and tanshinone IIA, by HPLC revealed only marked differences for salvianolic acid B. The results indicate that the polar active constituent in the nanotechnology samples was released faster compared to the traditionally powdered samples **[135]**.

**2. Survey of herbal drugs formulated in nanodelivery systems:**

**1. Curcumin** is a natural diphenolic compound derived from timeric *Curcuma longa* and proven to be a modulator of intracellular signaling oathways that control cancer cell growth, inflammation, invasion and apoptosis, revealing its anticancer potential **[136]**. Free cucumin was shown not to be cytotoxic to normal cells, including hepatocytes, mammary epithelial cells etc. Curcumin also, suppresses growth of several bacteria like *Streptococcus, Staphylococcus, Lactobacillus, etc.* Several types of NP have been found to be suitable for the encapsulation or loading of curcumin to improve its effects in cancer therapeutics. The characteristics of these curcumin nanoformulations can be tailored according to the specific requirement for inducing cellular death by various mechanisms **[136]**. A simple way to improve the bioavailability of curcumin, protect it from degradation and metabolism, and increase its targeting capacity toward cancer tumors is to formulate it in NP, such as polymer NPs, polymeric micelles, liposome/phospholipid, nano-/microemulsions, nanogels, solid lipid nanoparticles, polymer conjugates, self assembliesand so on, in order to deliver an active form of curcumin to tumors **[137]**. Safe toxicological profiles of the various curcumin nanoormulations and their efficacy in the cell line models highlight their potential for evaluation in *in vivo* models. Human trials need to be conducted to establish their effectiveness in clinical applications as an improved therapeutic modality for cancer treatment **[136]**.

**2. Quercitin** (**QU**) is a well known flavonoid distributed ubiquitously in fruits, vegetables and herbs or related products, e.g., apples, onions **[138]**. QU has been extensively investigated for its pharmacological effects that include antitumor, anti-inflammatory, antioxidant and hepatoprotective activities **[139]**. Various techniques have been used to increase the solubility of **QU** including complexation with cyclodextrin and liposome **[140]**. Nanoparticles are particularly useful in drug delivery for water-insoluble compounds, because their size (less than 1000 nm) can increase the absorption and the bioavailability of the delivered drug. A novel quercitin nanoparticle system (**QUEN**) was prepared by simple nanoprecipitation **[139]**. The QUEN were successfully developed by the nanoprecipitation technique. Increased antioxidant activities of QU from QUEN were correlated with the improvements of physicochemical characterization and dissolution property. Thus **QUEN** may be applied in clinical setting and warrant further studies **[139]**.

**3. Silymarin**, silybin is a main biologiucally active component in silymarin, which is an antihepatotoxic polyphenolic substance isolated from the milk thistle plant named silybum marianum, and has been widely used as a therapeutic agent for a variety of acute and chronic liver diseases**(141)**. However, the therapeutic effect of silybin is discounted by its extremely poor aqueous solubility, which results in poor oral absorption and bioavailability. To solve this problem, srveral approaches such as formation of silybin-phospholipid complex, silymarin solid dispersions, silymarin encapsulated liposomes, silymarin self microemulsifying drug delivery system and so on have been employed to improve the dissolution rate of silybin or silymarin thus enhancing its bioavailability**(141)**. An optimized nanoemulsion formulation consisted of sefsol-218 oil, tween 80 surfactant, ethanol cosurfactant, having low particle size and viscosity showed the AUC and $C_{max}$ of the nanoemulsion after oral administration to be 4-fold and 6-fold higher than those of drug suspension of silymarin **[142]**.

**4. Naringenin** is a natural flavonoid aglycone of naringin and widely distributed in citrous fruits, tomatoes, cherries, grapefruit and cocoa. As a well known antioxidant compound, the bioactivity of NAR has been attributed to its structure activity relationship. The number of hydroxyl substitutions of NAR can donate hydrogen to ROS, allowing acquisition of stable structure, thus enabling scavenging of these free radicals. In addition, NAR has ben extensively investigated for its pharmacological activities, including antitumor, anti inflammatory and hepatoprotective effects. Although NAR possesses excellent free radical scavenging ability and pharmacological activities, clinical studies showed hampering of its activities owing to its extreme water insolubility. Novel drug delivery systems can greatly improve the performance of drugs in terms of efficacy, solubility and bioavailability. Particularly, nanoparticle system is an emerging highly promising technology in enhancing drug delivery. Novel NAR- loaded nanoparticles could enhance the drug's hepatoprotective, antioxidant and antiapoptotic effects in ameliorating $CCl_4$ toxicity-

triggered necrosis and apoptosis. The small particle size was an important factor for acquiring optimal in vivo efficacy. Nanoparticles could be used to improve bioavailability of NAR on oral administration. NAR merits further investigation for clinical application as in prophylaxis of chronic liver diseases **[143]**.

**(Table 5)** represents examples of some phyto-constituents extracted from medicinal and aromatic plants using innovative extraction methods.

**Some Applied Research Results Throughout project ID: 973 Funded by STDF:**
**1. Extraction and Evaluation of medicinal plants extracts (*Cynara scolymus* L.):**
Three Samples of 50 g of dry leaves of ***Cynara scolymus* L.** (Artichoke) were extracted by 3 different extraction methods for each *viz.* traditional, microwave and sonicator methods **[128]**.

Evaluation of the efficiency of the extraction methods was done with respect to the total yield of the obtained extracts as well as the standardization of the main active constituents. (**Table 4**) summarizes the obtained yield for the extracted plants.

**HPLC analysis of *Cynara scolymus* L. extracts:**
HPLC was applied to quantify letuoline-7-glucoside, chlorogenic acid and cynarin in the different ***Cynara scolymus* L.** extracts. Cynarine, chlorogenic acid and Luteolin- 7-glucoside appeared at Rt 12.579 min, 8.151 and 17.391min; respectively (**Table 4**).

**(Table 4) shows the total yield percent & percentages of cynarine, chlorogenic acid and Luteolin-7-glucoside in the different prepared extract**

| Extraction method | Total Yield % | Cynarin%, Rt 12.579 min | chlorogenic acid%, Rt 8.151 min | Luteolin-7-glucoside%, Rt17.391min |
|---|---|---|---|---|
| **Microwave** | 30% | 1.936% | 50.607% | 6.62% |
| **Sonication** | 18% | 2.123% | 50.761% | 5.76% |
| **Traditional** | 17% | 1.406% | 18.02% | 4.29% |

Microwave extraction technique seems to be economically promising, simple and efficient. The total extraction yield was 30% while that produced by traditional method was 18%. Ultrasonic assisted extraction yielded 17% of the total extract which is not significantly different than the traditional method extract. These experiments demonstrate that the use of microwave in *Cynara scolymus* L. extraction produced the highest yield.

**From this table we can conclude the following:**
- Both microwave-assisted extraction (**MAE**) and sonication assisted extraction have shown to enhance the extraction efficiency of the interested components. Microwave-assisted extraction gave the highest yield of extract as well as higher percentages of both cynarin and luteolin-7-glucoside than those detected in the extract prepared by traditional method.
- Despite ultrasonic assisted extraction gave a lower extraction yield than the traditional method, the percentage of both cynarin and luteolin-7-glucoside were higher in its extract than the traditional method. Chlorogenic acid; which is the most potent compound in Cynara scolymus preparations (BP 2009); was obtained in 3 folds by the new techniques.
- The utilization of **MAE** and **UAE** techniques has proven to be a much simpler and more effective than the conventional extraction methods for obtaining active compounds from *Cynara scolymus* L .

**In conclusion**, comparison of these extraction methods revealed that they produce extracts with similar qualitative characteristics, however high quantitative differences.

**(Table 5): Phyto-constituents extracted from medicinal and aromatic plants using innovative extraction methods**

| No. | Plant, Secondary metabolite | Extraction Method | Performance | Ref. |
|---|---|---|---|---|
| 1 | Anthraquinones from the roots of (*Morinda citrifolia*) | US 38.5 kHz bath | UAE reduced the time of extraction and increased extraction yield compared to silence maceration. | [144] |
| 2 | Artemisinin of *Artemisia annua* leaves | US 40 kHz bath | The concentration of artemisinin at an extraction time of 60 min shows a percentage increase of approximately 58% when ultrasound is applied at 25 °C compared to conventional maceration. | [145] |
| 3 | Vanillin of vanilla pods | US 22.4 kHz probe | The use of UAE increased significantly extraction efficiency compared to soxhlet extraction | [146] |
| 4 | acid from the roots of Glycyrrhizic *Glycyrrhizia glaubra* | Open vessel microwave extraction | MAE was about 10 times faster than conventional extraction methods including extraction at room temperature, Soxhlet, heat reflux and ultrasonic extraction. Optimal conditions (50–60% ethanol, ammonia concentrations of 1–2% for 4–5 min and solvent/solid ratios of 10:1(mL/g)). | [147] |
| 5 | Capsaicinoids from capsicum fruit | Closed extraction vessel system | Extraction using MAE gave double the yield of capsaicinoid that recovered using shaken flask and heat reflux extraction methods. Optimal conditions (acetone for 7 min and 30% power of 300 W) | [148] |
| 6 | Essential oils from orange peel | Open vessel microwave extraction | Microwave accelerated distillation (MAD) offers shorter extraction times and better yields (0.42% against 0.39% for hydrodistilation). Optimal conditions ( solvent free extraction for 30 min at 100 °C and 1000 W power) | [149] |
| 7 | Essential Oil of *Foeniculum vulgare* Mill (Fennel) Fruits | Hydrodistillation, supercritical fluid extraction and microwave assisted techniques | Both MAE and SFE enhanced the extraction efficiency of the interested components. MAE gave the highest yield of oil as well as higher percentage of Fenchone (28%), whereas SFE gave the highest percentage of anethol (72%). | [150] |
| 8 | Isoflavones from soybeans | PLE | PLE increased the extraction efficiency of isoflavones when compared to shaking, vortexing, sonication, stirring and Soxhlet | [151] |
| 9 | Catechin and epicatechin form grape seeds | PLE | Compared to magnetic stirring and ultrasound-assisted extraction, PLE was a better methodology in terms of recovery, reproducibility and time efficiency, PLE optimum conditions ( extraction using methanol for 10 min at 130 °C) | [152] |
| 10 | Total Extraction Yield and Silymarin Content of *Silybum marianum* L. Seeds. | Effect of particle size (nano-particle) | Optimization of milling parameters was found to be a crucial step in determining the extracted silymarin contents. As expected, a smaller particle size has led to higher extraction yield. | [153] |
| 11 | Silymarin content of *Silybum marianum* seeds | UAE | UAE using 20 kHz probe and 40 kHz bath increased the extraction yield of silymarin when compared to conventional maceration | [154] |
| 12 | Effect of Extraction Method on Lipid Contents of Three Medicinal Plants of Apiaceae" | UAE, supercritical fluid extraction and Percolation, | Both UAE and SFE enhanced the extraction efficiency of the fatty acid of Celery, Parsley and Fennel. UAE gave the highest percentage yield. | [155] |
| 13 | Enhanced Extraction Yield of Glycyrrhizic Acid from *Glycyrrhiza glabra* L. | MAE, UAE and percolation | the highest glycyrrhizic acid content was obtained in sample prepared by ultrasound extraction | [156] |

## DISCUSSION

The need for development of existing methods of extraction and the introduction of the new technique in Egypt will give rise to the discovery of new biologically active compounds from phyto-pharmaceutical sources. More research is needed to improve the understanding of extraction mechanisms, remove technical barriers, improve the design and scale up of the novel extraction systems for their better industrial applications through:

1. **Establishing novel technology** for the extraction of medicinal and aromatic plants in Egypt (Transfer of Technology) needs:

- Carrying comparative studies to assess the new techniques results against the conventional ones. These studies include quantitative and qualitative studies using the recent techniques for analysis e.g. High Performance liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), UV spectroscopic determinations.
- Establishing standard operating procedures for extraction, new methods for herbal products standardization and establishing quality assurance and quality control protocols according to the obtained results for assuring that existing Egyptian essential oils and medicinal plant extracts meets specifications and requirements
- Organizing workshops, seminars, training courses for research institutes, universities and emphasizing the attendance and contribution of the industrial sector interested in this technology.

- Value addition of the medicinal plants can be achieved either directly by improving the quality of the plant material or indirectly by quality assurance of the plant material or the semi-processing of the material to a value added product (extraction process) through Introducing novel and innovative techniques for the extraction of medicinal and aromatic plants to Egypt and Improving selectivity and efficiency of extracting the Egyptian medicinal and aromatic plants.

**2. Optimizing Extraction Techniques:**
In all of the proposed methods of extraction there are several factors that influence the selectivity and the efficiency of the procedure.

These factors include the nature of solvent, temperature, time, power, pressure, matrix nature, and water contents of the extracted materials. In addition to these factors severely interacting with one another, a statistical optimization strategy needs to be adopted for determination of the optimum operating conditions. An orthogonal L9 array design can be used for the extraction optimization of **Taguchi approach [157]**. 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 **[158]**. 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.

**3. Exploring the application of the different extraction techniques at semi pilot and pilot scales:**
Egypt will have the opportunity to be a pioneer and a focal point for this new technology in the region (Middle East and Africa) and enlarge the industrial and social impact by transferring basic research to applied one through different channels including:

- Scaling up and pilot experiments for model verification.
- National cooperation between interested and involved sectors for production of valuable outcomes.
- Establishing a National Research and Development Network for Egyptian Medicinal and Aromatic Plants with the aim of boosting the research performance, application and maximizing the utilization of research results nationally as well as internationally.
- Spreading of this new technology among research institutes, universities and to the industrial sector (Pharmaceutical & Food industries) through training courses, workshops and seminars .
- Exploring and introducing new extraction techniques for preparation of Pharmaceutical raw materials to the Egyptian pharmaceutical market.
- Decreasing hazards due to introducing the environmentally friendly green techniques.
- Continuous ongoing training process for the young generation for using new technologies for extraction of medicinal and aromatic plants abroad to get an extensive experience directly related to the issues addressed. They handle many basic research with special emphasis on introducing, emerging green technology for extraction of medicinal and aromatic plants Building up the scientific capacity of the young Egyptian scientists for different disciplines concerning extraction process of medicinal and aromatic plants, with collaboration with the international experts.

**4. Applying Nanotechnology:**

- The unique features of nanoparticles have raised great expectations in the field of drug delivery because of their potential to improve clinical efficacy of problematic drug compounds **[131]**.
- Nanotechnology is strategic in developing drug delivery systems which can expand drug markets.
- Some of the challenges of most drug delivery systems include poor bioavailability, in vivo stability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, side effects and plasma fluctuations of drugs which either fall below the minimum effective concentrations or exceed the safe therapeutic concentrations. However, nanotechnology in drug delivery is an approach designed to overcome these challenges due to the development and fabrication of nanostructures at submicron scale and nanoscale which are mainly polymeric and have multiple advantages **[129]**.
- Nanotechnology can be applied to reformulate existing drugs thereby extending products' lives, enhance their performance, improve their acceptability by increasing effectiveness, as well as increase safety and patient adherence, and ultimately reduce health care costs **[133,134]**. Nanotechnology may also enhance the performance of drugs that are unable to pass clinical trial phases **[134]**.

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## REFERENCES

[1] Lise Manniche. An Ancient Egyptian Herbal. Published by British Museum Press, ISBN 0-7141-1704-8. **1999**.
[2] Kamal Prasad Acharya and Mohan Acharya, *Journal of Medicinal Plants Research* **2010**; 4(2): 235-239.
[3] Rajadurai, M.; Vidhya, V.G.; Ramya, M. and Anusha Bhaskar, *Ethno-Med*, **2009**; 3(1): 39-41.
[4] Pande, VV; Shastri, KV; Khadse, CD; Tekade, AR; Tankar, AN and Jain, BB, *International Journal of Green Pharmacy*, **2008**; 2(2): 69:71.
[5] Anderson ML, Burney DP, *J AOAC Int*., **1998**; 81:105.
[6] Picker L. *Anal Chem*. **1999**; 71:2929.
[7] Lixi X. S. D., Feng J. A., Huang Y. J., *J. Pract. Chin. Med.*, **2008**; 191: 806–808.
[8] Huie C.W., *Anal. Bioanal. Chem.*, **2002**; 373: 23-30.
[9] Vivekananda Mandal, Yogesh Mohan, S. Hemalatha, *Pharmacognosy Reviews*, **2007**; 1(1): 7:18.
[10] Smith R.M., *J. Chromatogr. A.*, **2003**; 1000: 3-27.
[11] Nyiredy, S., *J. Chromatogr B.*, **2004**; 812: 35-51.
[12] Romanik, G.; Gilgenast, E.; Przyjazny, A.; Namiesnik, J. and M. Kaminski., *J. Biochim. Biophys. Methods*, **2007**; Article in press.
[13] Luque de Castro M.D. and Garcia-Ayuso L.E., *Anal.Chim.Acta*, **1998**; 369: 1-10.
[14] Letellier, M; . Budzinski, H.; Charrier, L.; Capes, S. and Dorthe, A.M., *J. Anal. Chem.*, **1999**; 364: 228-37.
[15] Pastot, E. Vazquez, R. Ciscar and M. De la Guardia, *Anal.Chim.Acta*, **1997**; 344: 241-49.
[16] Sanghi, R. and Kannamkumarath, S.S., *J. Anal. Chem.*, **2004**; 59: 1032-1036.
[17] Luque-Garcia, J.L. and M.D. Luque de Castro, *Talanta*, **2004**; 64: 571-577.
[18] Zuloaga, O.; Etxebarria, N.; Fernandez, L.A. and Madariaga, J.M., *Talanta*, **1999**; 50: 345-357.
[19] Handa SS, Rakesh DD and Vasisht K., Compendium of Medicinal and Aromatic Plants, Asia. **2006**; Vol. II, ICS-UNIDO, Trieste, Italy.
[20] Upasana Ghosh, MA Badhul Haq, Somnath Chakraborty, *International Journal of Chemical and Analytical Science*, **2011**; 2(9): 1153-1158.
[21] Soxhlet, F., *Polytechnisches J.*, (Dingler's) **1879**; 232: 461.
[22] William B. Jensen, *Journal of Chemical Education*, **2007**; 84(12): 1913.
[23] Ribereau - Gayon P. Plant Phenolic. Heywood V.H., Ed.: Hafner Publishing Co. New York. **1978**.
[24] Laurence M. Harwood, Christopher J. Moody, Experimental organic chemistry: Principles and Practice. (Illustrated edition). pp. 122–125. ISBN 978-0-632-02017-1. **2002**.
[25] Nguyen, T. T., Dale, L. C., von Bergmann, K. & Croghan, I. T. *Mayo Clin. Proc.* **1999**; 74:1198-1206.
[26] Ana Cristina Atti-Santos, Marcelo Rossato, Luciana Atti Serafini1, Eduardo Cassel and Patrick Moyna, *Brazilian Archives of Biology and Technology*, **2005**; 48(1):155-160.
[27] Miguel, G.; Cruz, C.; Faleiro, M.L.; Simões, M.T.F.; Figueiredo, A.C.; Barroso, J.G. & Pedro, L.G., *Natural Product Research*. **2011**; 25(5): 526-541.
[28] Baser, K.H.C. and Buchbauer, G. (**2010**). "Handbook of Essential Oils: Science, Technology and Applications". CRC Press, Boca Raton, London, New York. ISBN 978-1-4200-6315-8.
[29] Lou Xianwen, Hans-Gerd Janssen, and Carel A. Cramers, *Anal. Chem.* **1997**; 69: 1598-1603.
[30] Benthin B, Danz H and Hamburger M., *J. Chromatography A.*, **1999**; 837: 211-219.
[31] Kaufmann B and Christen P, *Phytochem. Anal.*, **2002**; 13: 105–113.
[32] Luthria, D.L., Vinjamoori, D. and Noel, K. Effect of moisture content, grinding, and extraction technologies on assay of crude fat. p. 101-118. In: D.L. Luthria (ed.), Oil Extraction and Analysis: Critical Issues and Comparative Studies, AOCS Press, Champaign, Ill. **2004**.
[33] Carabias-Martinez, R., E. Rodriguez-Gonzalo, P. Revilla-Ruiz and J. Hernandez-Mendez, *J. Chromatogr. A.*, **2005**; 1089: 1-17.
[34] Mendiola, J.A., M. Herrero, A. Cifuentes, and E. Ibanez, *Journal of Chromatography A.*, **2007**; 1152: 234–246.
[35] Kawamura, F., Y. Kikuchi, T. Ohiro, and M. Yatagai, *J. Nat. Prod.*, **1999**; 62: 244–247.
[36] Richter, B.E., B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, and C. Pohl., *Analytical Chemistry*, **1996**; 68: 1033–1039.
[37] Jiang Y., Li S.P., Chang H.T., Wang Y.T., Tu P.F., *Journal of Chromatography A.*, **2006**; 1108: 268–272.
[38] Rostango, M. A., Palma, M. and Barroso, C. G., *Analytica chimica Acta*, **2004**; 522 (2): 169-177.
[39] Dawidowicz AL, Wianowska D., *J Pharm Biomed Anal.*, **2005a**; 37(5): 1155-9.
[40] Ong E.S. and Len S.M., *Analytica Chimica Acta*, **2003**; 482, 81–89.

[41] Pitipanapong J, Chitprasert S, Goto M, Jiratchariyakul W, Mitsuru SM, Shotipruk A., *Sep. Purif. Technol.*, **2007**; 52: 416-422.
[42] Ong, E.-S., Woo, S.-O. and Yong, Y.-L., *J. Chromatogr. A.*, **2000**; 313: 57-64.
[43] Pongnaravane B, Goto M, Sasaki M, Anekpankul T, Pavasant P, Pavasant P, Shotipruk A., *J Supercritical Fluids*, **2006**; 37:390-6.
[44] Hartonen, K.; Parshintsev, J.; Sandberg, K.; Bergelin, E.; Nisula, L. & Riekkola, M.L., *Talanta*, **2007**; 74(1): 32 ,38-ISSN 0039-9140.
[45] Nieto, A., F. Borrull, E. Pocurull, and R.M. Marcé., *TrAC Trends in Analytical Chemistry*, **2010**; 29: 752–764.
[46] Fernandez-Gonzalez V., Muniategui-Lorenzo S., Lopez-Mahia P., Prada-Rodriguez D., *Journal of Chromatography A.*, **2008**; 1207: 136–145.
[47] Teo, C.C.; Tan, S.N.; Yong, J.W.H.; Hew, C.S.; and Ong, E.S., *Journal of Chromatography A.*, **2010**; 1217: 2484–2494.
[48] Li P., Li S.P., Lao S.C., Fu C.M., Kan K.K.W., Wang Y.T., *Journal of Pharmaceutical and Biomedical* Analysis, **2006**; 40: 1073–1079.
[49] Dawidowicz AL, Wianowska D., *J Pharm Biomed Anal.*, **2005b**; 37(5):1161-5.
[50] Waksmundzka-Hajnos, M.; Petruczynik, A.; Dragan, A.; Wianowska, D.; Dawidowicz, A.L., *Phytochem. Anal.* **2004**; 15: 313-319.
[51] Luque de Castro, M.D., Jiménez-Carmona, M.M. and Fernȷndez-Pérez, V., *TRAC, Trends in Analytical Chemistry*, **1999**; 19: 708-716.
[52] Miller, D.J. & Hawthorne, S.B., *Journal of Chemical Engineering Data*, **2000**; 45: 315-318.
[53] Hawthorne S.B.; Grabanski, C.B.; Martin, E.; Miller, D.J., *J. Chromatogr. A.*, **2000**; 892, 421.
[54] Lou, X.; Miller, D.J.; Hawthorne, S.B., *Anal. Chem.*, **2000**; 72, 481.
[55] Hageman, K.J.; Mazeas, L.; Grabanski, C.B.; Miller, D.J.; Hawthorne, S.B., *Anal. Chem.*, **1996**; 68, 3892.
[56] Jime´nez-Carmona, M.M.; Manclu´s, J.J.; Montoya, A.; Luque de Castro, M.D., *J. Chromatogr. A*, **1997**; 785, 329.
[57] Hawthorne, S.B.; Yang, Y.; Miller, D.J., *Anal. Chem.*, **1994**; 66, 2912.
[58] Hartonen, K.; Inkala, K.; Kangas, M.; Riekkola, M.L., *J. Chromatogr. A.*, **1997**; 785, 219.
[59] Wennrich, L.; Popp, P.; Mo¨der, M., *Anal. Chem.*, **2000**; 72, 546.
[60] Wennrich, L.; Popp, P.; Breuste, J., *Chromatographia Suppl.*, **2001**; 53, 380.
[61] Li, B., Yang, Y., Gan, Y., Eaton, C.D., He, P. & Jones, A.D., *Journal of Chromatography A.*, **2000**; 873: 175-184.
[62] Ibanez, E.; Kuvatov **,**A.; Senorans, F.J.; Cavero, S.; Reglero, G. & Hawthorne, S.B., *Journal of Agricultural and Food Chemistry*, **2003**; 51: 375-382.
[63] Basile, A.; Jiménez-Carmona, M.M. and Clifford, A.A., *Journal of Agricultural and Food Chemistry*, **1998**; 46: 5205-5209.
[64] Kuvatova, A.; Lagadec, A.J.M.; Miller, D.J. & Hawthorne, S.B., *Flavour and Fragrance Journal*, **2001a**; 16: 64-73.
[65] Norashikin Saim, Rozita Osman, Wan Azriza Hirmi Md Yasin, Rossuriati Dol Hamid, *The Malaysian Journal of Analytical Sciences*, **2008**; 12(1): 22.
[66] Clifford, A.A.; Basile, A. & Al-Saidi, S.H., *Fresenius Journal of Analytical Chemistry*, 1999; 364: 635-637.
[67] Lee, K.G. & Shibamoto, T., *Food Chemistry*, **2001**; 74: 443-448.
[68] Herrero, M.; Jaime, L.; Martinz-lvarez, P.J.; Cifuentes, A. and Ibanez E., *Journal of Agricultural and Food Chemistry*, **2006**; 54: 5597–5603.
[69] Fernandez-Pérez, V.; Jiménez-Carmona, M.M & Luque de Castro, M.D. *Analyst.* **2000**; 125:481-485.
[70] Gamiz-Garcia, L. & Luque de Castro, M.D., *Talanta*, **2000**; 51: 1179-1185.
[71] Ayala, R. S. and Luquede Castro, M. D., *Food Chemistry*, **2001**; 75: 109–113.
[72] Kuvatova **,**A.; Miller, D.J. & Hawthorne, S.B., *Journal of. Chromatography A.*, **2001b**; 923: 187-194.
[73] Ozel, M.Z.; Gogus, F. & Lewis, A.C., *Food Chemistry*, **2003**; 82: 381-386.
[74] King, J.W.; Grabiel, R.D. and Wightman, J.D. Subcritical Water Extraction of Anthocyanins from Fruit Berry Substrates. In: Proc. Super Green 2002, K. Park (ed.), Suwon, South Korea, November 3-6, p. 61. **2002**.
[75] Taylor, L.T. Supercritical Fluid Extraction. John Wiley & Sons, Inc., New York, NY. **1996**.
[76] Wright, B. W.; Frye, S. R.; McMinn, D. G.; Smith, R. D., *Anal. Chem.*, **1987**; 59: 640-644.
[77] Jian Ya Ling, Guo Ying Zhang, Zhao Jie Cui, Chang Kai Zhang, *Journal of Chromatography A.*, **2007**; 1145: 123-127.
[78] Del Valle, J.M.; Rivera, O.; Mattea, M.; Ruetsch, L.; Daghero, J. and Flores, A., *J. Supercrit. Fluids*, **2004**; 31, 159 .
[79] Doane-Weideman, T. and Liecheski, P. B., Chapter 5. Analytical supercritical fluid extraction for food applications. In: Luthria, D. L. (Ed.), Oil Extraction and Analysis: Critical Issues and Comparative Studies, American Oil Chemists' Society ,Maryland **2004**; 69-99.
[80] Modey WK, Mulholland DA, Raynor MW., *Phytochem Anal*., **1996**; 7: 1–15.

[81] Kaiser CS, Rompp H, Schmidt PC, *Pharmazie*, **2001**; 56: 907–926 .
[82] Luque de Castro, M.D.; Valcarel, M.; Tena, M.T. Analytical Supercritical Fluid Extraction. Springer-Verlag, Berlin. **1994**.
[83] Jeong, Mijeong Lee and Chesney, David J., *The Journal of Supercritical Fluids*, **1999**; 16(1): 33–42.
[84] Langenfeld, J.J.; Hawthorne, S.B.; Miller, D.J.; Pawliszyn, J., *Analytical Chemistry*, **1994**; 66 (6): 909-916.
[85] Kim JW, Kim JD, Kim J, Oh SG, Lee YW. *J. Food Eng.* **2008**; 89: 303-309.
[86] Choi ES, Noh MJ, Yoo KP (**1998**). *J. Chem. Eng. Data*, 43(1): 6-8.
[87] Wang HC, Chen CR, Chang CJ. *Food Chem.* **2001**; 72: 505.
[88] Da Costa CT, Margolis SA, Benner BAJ and Horton D., *J. Chromatogr. A*., **1999**; 831: 167-178.
[89] Wang, Z.; Ding, L.; Li, T.; Zhou, X.; Wang, L.; Zhang, H.; Liu, L.; Li, Y.; Liu, Z.; Wang, H.; Zeng, H. and He, H., *J. Chromatogr. A*., **2006a**; 102: 111-117.
[90] Sanchez-Vicente Y. *et al., J. Supercrit. Fluids*, **2009**; 49:167-173.
[91] Shi, J. *et al., Sep. Purif. Technol.*, **2009**; 66: 322-328.
[92] Brunner, G., *J. Food Eng.*, **2005**; 67: 21-33.
[93] Iheozor-Ejiofor P. and Dey ES., *J. Supercrit. Fluids*, **2009**; 50: 29-32.
[94] Ciurlia, L.; Bleve, M.; Rescio, L., *J. Supercrit. Fluid*, **2009**; 49(3): 338-344.
[95] Hawthorne, S.B. and King, J.W. Principles and Practice of Analytical SFE in Practical Supercritical Fluid Chromatography and Extraction. M. Caude and D. Thiebaut (Eds.), Harwood Academic, Chur, Switzerland. **1999**; 219-282.
[96] Kohler, Marcel; Haerdi, Werner; Chisten, Philippe and Veuthey, Jean-Luc., *Journal of Chromatography A*., **1997**; 785(1-2): 353-360.
[97] Hamburger *et al., Phytochemical Analysis*, **2004**; 15: 46-54.
[98] Catchpole *et al., J Supercritical Fluids*, **2002**; 22:129-138.
[99] Letellier M. and Budzinski H., *Analysis*, **1999**; 27: 259-71.
[100] Smith, B.L. and Carpentier, M.H. The Microwave Engineering Handbook, Microwave Technology Series. Chapman and Hall, London. **1993**; 1-3.
[101] Bethe, H.A., *Phys. Rev.*, **1947**; 72: 339-341.
[102] Wang L. and Weller C.L., *Trends Food Sci. Technol.*, **2006**; 17: 300-12.
[103] Moen, R.D., Nolan, T.W. and Provost, L.P. Improving Quality Through Planned Experimentation. McGraw-Hill, Inc., New York, ISBN 0-07-042673-2. **1991**.
[104] Latha C., *Biotechnol. Lett.*, **2006**; 9243: 10529.
[105] Kiss, G.A.C; Forgacs, E.; Serati, T.C.; Mota, T.; Morais, H. and Ramos, A., *J. Chromatogr. A*., **2000**; 889: 41-49 .
[106] Talebi, M.; Ghassempour, A.; Talebpour, Z.; Rassouli, A. and Dolatyari, L., *J. Sep. Sci*., **2004**; 27: 1130-36.
[107] Wang, Z.; Wang, L.; Li, T.; Zhon, X.; Ding, L.; Yu, Y.; Yu, A. and Zhang, H., *Anal. Bioanal. Chem.*, **2006b**; 386: 1863-68.
[108] Li, H.; Chen, B.; Zhang, Z. and Yao, S., *Talanta*, **2004**; 63: 659-65.
[109] Wang, S.; Chan, F.; Wu, J.; Wang, Z.; Liao, X. and Ha, X., *J. Food Eng.*, **2007** (Article in press).
[110] Shu, Youn Yuen; Wang, Shu Shing; Mylaine Tardif, Yaping Huang, *Journal of Chromatography A*., **2003**; 1008: 1–12.
[111] Vilkhu, K., Mawson, R., Simons, L., & Bates, D., *Innovative Food Science and Emerging Technologies*, **2008**; 9: 161−169.
[112] Vinatoru, M., *Ultrasonics Sonochemistry*, **2001**; 8: 303−313
[113] Wu, J., Lin, L., & Chau, F., *Ultrasonics Sonochemistry*, **2001**; 8: 347−352.
[114] Luque-Garciá, J. L., & Luque de Castro, M. D., *Trends in Analytical Chemistry*, **2003**; 22: 41−47.
[115] Balachandran, S., Kentish, E., Mawson, R., & Ashokkumar, M., *Ultrasonics Sonochemistry*, **2006**; 13: 471−479.
[116] Jian-Bing, J., Xiang-hong, L., Mei-qiang, C., & Zhi-chao, X., *Ultrasonics Sonochemistry*, **2006**; 13: 455−462.
[117] Moulton, J., & Wang, C., *Journal of Food Science*, **1982**; 47: 1127−1129.
[118] Caili, F., Haijun, T., Quanhong, L., Tongyi, C., & Wenjuan, D., *Ultrasonics Sonochemistry*, **2006**; 13: 511−516.
[119] Toma, M., Vinatoru, M., Paniwnyk, L., & Mason, T., *Ultrasonics Sonochemistry*, **2001**; 8: 137−142.
[120] Riera, E., Golás, Y., Blanco, A., Gallego, A., Blasco, M., & Mulet, A., *Ultrasonics Sonochemistry*, **2004**; 11: 241−244.
[121] Chemat, S., Lagha, A., AitAmar, H., Bartels, V., & Chemat, F., *Flavour and Fragrance Journal*, **2004**; 19: 188−195.
[122] Haizhou, L., Pordesimo, L., &Weiss, J., *Food Research International*, **2004**; 37: 731−738.
[123] Chen, L., Jin, H., Ding, L., Zhang, H., Li, J., Qu, C., *et al., Separation and Purification Technology*, **2008**; 59:50−57.
[124] Ying, Z., X. Han, and J. Li, *Food Chemistry*, **2011**; 127: 1273–1279.

[125]Keil, F. J. Modeling of Process Intensification. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. **2007**.
[126]Ötles, S. Handbook of Food Analysis Instruments. 1st ed. Boca Raton: CRC Press Taylor & Francis Group. **2009**.
[127]Hu, A.J., S. Zhao, H. Liang, T.Q. Qiu, and G. Chen., *Ultrasonics Sonochemistry*, **2007**; 14: 219–224.
[128]Xianfeng Zhu, Hongxun Zhang, Raymond Lo, *J. Agric. Food Chem.*, **2004**; 4(52): 7272-7278.
[129]Ochekpe, NA, Olorunfemi, PO, Ngwuluka, NC, *Tropical Journal of Pharmaceutical research*, **2009**; 8 (3): 265-274.
[130]Williams, D., *Biomaterials*, **2008**; Doi: 10.1016 /J. biomaterials, 01.003.
[131]Bosselmann, S, Williams III, RO., *Drug Dev Ind Pharm*., **2012**; 38 (2): 158-170.
[132]Hans, ML, Lowman, AM., *Curr Opin Solid State Mat Sci*., **2002**; 6: 319-327.
[133]Sahoo, SK, Labhasetwar, V., *Drug Discovery Today*, **2003**; 8 (24): 1112-1120.
[134]Hughes, GA., *Nanomedicine: NBM*, **2005**; 1: 23-30.
[135]Je-Ruei Liua, Guo-Feng Chenb, Hui-Nung Shihb, Ping-Chung Kuob, *Phytomedicine*, **2008**; 15: 23–30.
[136]Yallapu, MM, Jaggi, M, Chauhan, SC., *Drug Disc Today*, **2012**; 17 (1/2): 71-80.
[137]Muqbil, I, *et al*., *Cancer*, **2011**; 3: 428-445.
[138]Hertog, MGL, Hollman, PCH, Venema, DP, *J Agric Food Chem*., **1992**; 40: 1591-1598.
[139]Wu, TH, Yen, FL, Lin, LT, Tsai, TR, Lin, CC, Cham, TM., *Int J Pharm*., **2008**; 346: 160-168.
[140]Yuan, ZP, Chen, LJ, Fan, LY, Tang, MH, Yang, GL, Yang, HS, DU, XB, Wang, GQ, Yao, WX, Zhao, QM, Ye, B, Wang, R, Diao, P, Zhang, W, Wu, HB, Zhao, X, Wei, YQ, *Clin Cancer Res*., **2006**; 12: 3193-3199.
[141]Zhang, ZB, Shen, ZG, Wang, JX, Zhang, HX, Zhao, H, Chen, JF, Yun, J, *Int J Pharm*., **2009**; 376: 116-122.
[142]Parveen, R, Baboota, S, Ali, J, Ahuja, A, Vasudev, SS, Ahmad, S, *Int J Pjarm*., **2011**; 413: 245-253.
[143]Yen, FL, Wu, TH, Lin, LT, Cham, TM, Lin, CC, *Pharm Res*., **2009**; 26 (4): 893-902.
[144]Hemwimol S, Pavasant P, Shotipruk A, *Ultrason. Sonochem*., **2006**; 13: 543-548.
[145]Briars R, Paniwnyk L., *Industrial Crops and Products*, **2013**; 42: 595-600.
[146]Jadhav D, Rekha BN, Gogate PR, Rathod VK, *Journal of Food Engineering*, **2009**; 93: 421-426.
[147]Pan X, Liu H, Jia G, Shu YY, *Biochemical Engineering Journal*, **2000**; 5: 173-177.
[148]Williams OJ, Raghavan GS, Orsat V, Dai J., *Journal of Food Biochemistry*, **2004**; 28: 113-122.
[149]Ferhat A, Meklati BY, Smadja J, Chemat F., *Journal of Chromatography A*., **2006**; 1112:121-126.
[150]Faiza M. Hammouda, Mahmoud A. Saleh, Nahla S. Abdel-Azim, Khaled A. Shams, Shams I. Ismail1, Abdelaaty A. Shahat and Ibrahim A. Saleh, *Afr J Tradit Complement Altern Med.,* **2014**; 11(2):378-380.
[151]Luthria DL, Biswas R, Natarajan S., *Food Chemistry*, **2007**; 105: 325-333.
[152]Piñeiro Z, Palma Ml, Barroso CG, *Journal of Chromatography A*., **2004**; 1026: 19–23.
[153]Ibrahim A. Saleh, Sherin A. Kamal, Khaled A. Shams, Nahla S. Abdel-Azim, Elsayed A. Aboutabl and Faiza M. Hammouda, *Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS)*, **2015**; 6(2), 803-809.
[154]Saleh I.A., Vinatoru M, Mason T.J, Abdel-Azim N.S., Aboutabl E.A., Hammouda F.M., *Res. J. Pharm. Biol. Chem. Sci.*, **2015**; 6: 709-717.
[155]Khaled A. Shams, Nahla S. Abdel-Azim, Wafaa A. Tawfik, Heba D. Hassanein, Mahmoud A. Saleh and Faiza M. Hammouda, *journal of chemical and pharmaceutical research*, **2015**; 7(4):1080-1088.
[156]Khaled A. Shams, Nahla S. Abdel-Azim, Sherin A Kamal, Husseiny A. Husseiny and Faiza M. Hammouda, *journal of chemical and pharmaceutical research*, **2015**; 7(4):725-728.
[157][Roy, R.K. Design of Experiments using Taguchi Approach: 16 Steps to Product and Process, (J. Willey and Sons,). ISBN: 0471361011. **2001**.
[158]Osborne, D.M. and R.L. Armacost, *Computers Ind. Engng*., **1996**; 31(1/2): 107-10.