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Review Article

MICROWAVE ASSISTED EXTRACTION (MAE) OF ANTIOXIDANT CONSTITUENTS IN PLANT MATERIALS

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ABSTRACT
Antioxidant constituents in plant material act as radical scavengers and helps in converting free radicals to less reactive species. Free radical causes a change of the net charge of cells, modifies their osmotic pressure, induces swelling and causes death. A variety of free radical scavenging antioxidants is found in fruits, vegetables, herbs, tea etc. Microwave-Assisted Extraction (MAE) technique has been developed for the extraction of antioxidant constituents in plants. Several influential parameters of the MAE procedure such as ethanol concentration, solvent volume, and microwave power and extraction time are to be considered during extraction of plant phyto- constituents. MAE has been shown to be cost-effective when compared to other extraction methods. It surpasses supercritical fluid extraction technique because it is easy to operate at a cheaper cost. The main advantage of MAE over Ultrasonic Assisted Extraction and Soxhlet extraction is due to the fact that, it can be used to extract plant metabolites at a shorter time interval. This report reviews literature on, methods of phyto- constituents' extraction, operation and principle of microwave asisted extraction, extraction and isolation of phyto-constituents (antioxidants), factors to consider when using microwave assisted extraction method as well as advantages of MAE

KEY WORDS: Antioxidant, Free radicals, scavenging, phyto- constituents, microwave power

INTRODUCTION

Oxygen is absolutely essential for the life of aerobic organisms but it may become toxic if supplied at higher concentrations. The cellular damage by free radicals causes a change of the net charge of cells, as a result it modifies their osmotic pressure and induces swelling and finally causes their death. Free radicals act on mediators of inflammatory diseases and accelerate tissue damage. Moreover, cells lesions lead to an increase in the production of Oxygen Radical Species (ORS) which induces the consumption and the depletion of the endogenous chelating agents. Furthermore, free oxygen radicals play cardinal role in the etiology of several diseases like arthritis, cancer, atherosclerosis etc. The oxidative damage to DNA may play vital role in aging and the presence of intracellular oxygen can also to initiate a chain of inadvertent reaction at the cellular level and these reactions cause damage to critical cell biomolecules. (Cheeseman and Slater, 1993). To protect against oxygenated reactive species, the organism and living cells have developed several mechanisms including enzymes such as the superoxide dismutase, the catalase and the glutathione peroxidase, and also non-enzymatic homologues such as the glutathion, the ascorbic acid and l'α-tocopherol (Halliwell, 1995). The protective effect of plants secondary metabolites (flavonoids) as shown in Table 2 is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation (Halliwell, 1995). Flavonoids can prevent the damage caused by free radicals in several ways one of them is the direct trapping of the radicals. In this case, the flavonoids are oxidized by the radicals (R•) leading to less reactive and more stable species (Halliwell, 1995). Natural antioxidants (as shown in Table 1 & 2) present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects as indicated in Table 3. The increasing interest in the search for natural replacements for synthetic antioxidants has led to the antioxidant evaluation of a number of plants. However, the modernization of antioxidant products for sale has raised concerns in the area of safety and quality of these products. Additionally, the standardization and quality aspect of antioxidant products have become a problem. At present the quality and safety related problems seems to be overwhelming the potential genuine benefits connected to the use of antioxidant products and these problems may due to lack of high performance, reliable extraction, analytical techniques and methodologies for establishing a standard therapeutic functionality for antioxidant products. Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in the plants (Huie, 2002).Until now, the extraction step remains often a neglected area, which over the years has received much

less attention and research. An efficient or incomplete technique means considerable constraint on the analytical procedure (Romanik *et al.*, 2007). The traditional techniques of solvent extraction of plant materials is based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds. Moreover, the traditional technique requires longer extraction time and as a result causes thermal degradation for most of the phyto-constituents (Luque de Castro and Garcia-Ayuso, 1998). The fact that one single plant can contain several thousand secondary metabolites it necessitates the development of high performance and rapid extraction methods (Nyiredy, 2004). Soxhlet extraction method has been used for almost 126 years as the most predictable technique (Letellier *et al*., 1999). It used for the extraction and separation of phyto-constituents but it has a major shortcoming such as lengthy action time that can be 8, 16, 24 hours or more which results in consumption of considerable time and heat energy.

The lengthy time requirement makes it more labour-intensive and limits the number of samples that can be processed which may not be entertained from commercial aspects. It requires large amount of organic solvents as well as recovery step followed by evaporation to concentrate the extract which makes it a cumbersome process (Pastot, 1997). Several articles have been published on the application of microwave heating for the extraction of organic compounds and pesticide residue from environmental samples. Microwave has been useful for the extraction of plant materials. (Letellier *et al*., 1999; Pastot, 1997; Zuloaga, *et al*., 1999; Sanghi and Kannamkumarath, 2004). Microwave Assisted Extraction process is a method used to selectively extract target compounds from various raw materials. It uses energy of microwave radiation to heat solvents quickly and efficiently. Closed system extraction is performed at higher temperatures and leads to reduced extraction time. MAE is an innovative solvent-extraction technology which offers a better alternative to several thermal applications due to its efficient volumetric heat production and the fact that it has many advantages over conventional solid liquid extraction methods. Its application includes extraction of high-value compounds from natural sources, nutraceutical and functional food ingredients, and also pharma actives from biomass (Starmans and Nijhuis 1996).

In this piece of writing, the basis of microwave extraction technology and their advantages have been discussed. The application of microwave as an extraction tool for the extraction and isolation of phyto-constituents (antioxidants) have also been dealt with. Besides, this review also provides a short theoretical background of microwave extraction techniques and the basic principles of using microwave energy for extraction of phyto-constituents

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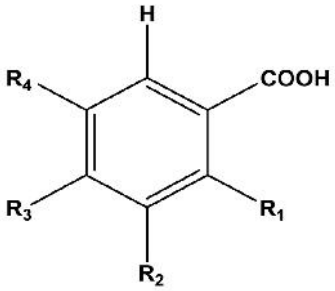
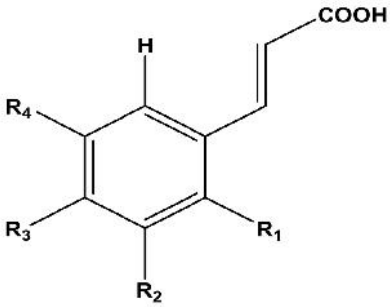


Hydroxycinnamic acid



Hydroxybenzoic Acids

Table-1

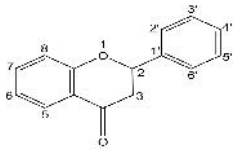
Name	R1	R2	R3	R4
Benzoic acid	H	H	H	H
p-Hydroxybenzoic acid	H	H	OH	H
Vanillic acid	H	OCH3	OH	H
Gallic Acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H
Syringic Acid	H	OCH3	OH	OCH3
Gentisic acid	OH	H	H	OH
Veratric acid	H	OCH3	OCH3	H
Salicyclic acid	OH	H	H	OH

Name	R1	R2	R3	R4
Cinnamic acid	H	H	H	H
o-coumaric acid	OH	H	H	H
m- coumaric acid	H	OH	H	H
p- coumaric acid	H	H	OH	H
Ferulic acid	H	OCH3	OH	HCH3
Sinapic acid	H	OCH3	OH	OCH3
Caffeic acid	H	OH	OH	H

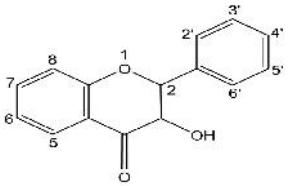
(Stalikas, 2007)

Table 2: Phyto-constituents mostly used as anti-oxidants

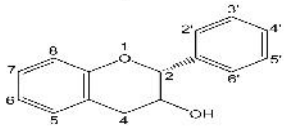
Flavones
- Apigenin
- Luteolin
- Chrysin



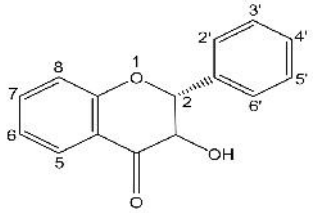
Flavonols
- Quercetin
- Kaempferol
- Galangin
- Fisetin
- Myricetin



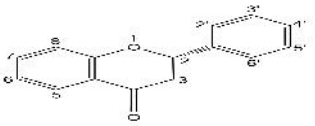
Flavan-3-ols
- (+)-Catechin
- (–)-Epicatechin
- (–)-Epigallocatechin



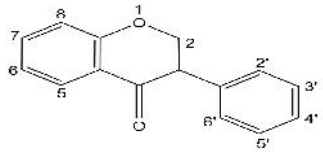
Flavanonol
- Taxifolin



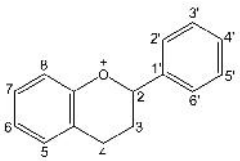
Flavanones
- Naringenin
- Naringin
- Hesperetin
- Hesperidin



Isoflavones
- Genistein
- Genistin
- Daidzein
- Daidzin
- Ononin



Anthocyanidins
- Cyanidin
- Cyanin
- Peonidin
- Delphinidin
- Pelargonidin
- Malvidin



(Stalikas, 2007)

TABLE 3: Functions of phyto- constituents

Classes of phenolics	Activity and therapeutic uses
Phenols, simple phenolic acids	Antiseptic, anti-inflammatory, antipyretic, enzyme inhibitor (cAMP phosphodiesterase, aldose reductase etc.) antibacterial, Antifungal, antioxidant (Koshihara et.al. 1984: Majieneet.al 2007; Blázovics (2007) Blázovics. (2009)
Coumarines	venous tonic, vascular protective, anti-psoriatic(Da Silva and Sobel, 2002)
Lignans and neolignans	antibacterial, antifungal, enzyme inhibitor (Bruneton, 1999: Ogata (2008)
Flavonoids	antioxidant, anti-inflammatory, hepatoprotective, anticancer, decrease capillary fragility and permeability, enzyme inhibitor (cAMP phosphodiesterase, histidine decarboxylase, proteinkinase etc.), estrogenic Ng et.al (2000): Blázovics (2009) Seelinger et.al., (2008)
Anthocyanins	decrease capillary fragility and permeability, antioxidant, antibacterial, vision improver (Ng et.al (2000): Mayer et.al., 2008 : Gosh and Konishi (2007)
Tannins	complexation with macromolecules and proteins, astringent, antioxidant, enzymatic inhibition (5-lipoxygenase) [Fernandez-Panchonet.al 2008:Llaudy et.al.(2004)
Quinones	antibacterial, antifungal, laxative Srinivas et.al., (2007) Bruneton,(1999): Evans, (2009)

Methods of phyto- constituent's extraction

According to Stalikas (2007) extraction procedure of plant phyto-constituents is categorized into different ways which includes pretreatment, extraction, isolation, and purification. The production of functional/ nutraceutical involves the additional step of encapsulation, which may involve simple drying or addition and mixing of different stabilizers followed by drying. It is important to note that, for the determination of flavonoid contents, extraction forms an important step.

Pretreatment steps consist of maceration, homogenization, grinding, milling, and drying, for example freeze drying to prevent degradation of the flavonoids. Drying helps to increase yield per unit weight of raw material, it increases storage life of the raw material, it decreases space requirement and in some cases ease processing. Maceration, grinding, milling, and homogenization increase the contact surface area between the solvent and the sample containing the solute. The solute being the flavonoid contained within the cellular structures of the biological samples. Pretreatment steps lead to the breakdown of cellular structures which enhances the yield of bioactive compounds. (Montedoro *et al*., 1992), mechanical stirring, and continuous rotary extraction are other methods which can be applied to increase molecular interactions (Stalikas, 2007).

The most appropriate method of extraction depends on the source and the type of flavonoid to be extracted. Some forms of flavonoids (aglycones) are reported to be more easily absorbed from the diet as a bioactive compound than the other forms of flavonoid glycosides (Stalikas, 2007). There have been numerous reviews and research on the advancement of different extraction techniques with comparative discussions between them (Camel, 2001; Dai *et al*., 2010; Kaufmann and Christen, 2002; Raynie, 2006; Stalikas, 2007). Solvent extraction is the most common method of extraction of flavonoids. Different types of solvent extraction methods are used currently, out of which hot water bath extraction and soxhlet extraction method are the most common for extraction of bioactive compounds. (De Rijke *et al*., 2006; (Kalia *et al*., 2008). For comparison of terms of yield, the soxhlet method is used as a standard in almost all cases (Fan *et al*., 2010; Macdonald *et al*., 2010). Methanol or acetone is used as the solvent for soxhlet extraction of flavonoids. Other solvent extraction method such as sonication assisted extraction is also used for the extraction of plant phyto-chemicals.

The use of ultrasound (sound waves which have frequencies higher than 20 kHz) can disrupt plant cell walls with subsequent increase in solvent penetration which helps in obtaining a higher extraction yield. Ultrasound-assisted extraction can be a technique of choice for thermo-labile components as the operating temperature can remain low during this process, thus maintaining extract quality. Ultrasounds have been applied in the case of extraction of phyto-chemicals from different sources such as medicinal plants like *Selaginella doederleinii* and *Citrus aurantium* (Yang *et al*., 2010; Wang and Weller 2006; Li *et al*., 2010).

Operation and Principle of Microwave Asisted Extraction

Microwaves are electromagnetic fields in the frequency range of 300 MHz to 300 GHz or between wavelengths of 1 cm and 1m. These electromagnetic waves are made up of two oscillating perpendicular fields: electrical field and magnetic field. Microwaves are used as information carriers or as energy vectors. The first application is the direct action of waves on material which is able to absorb a part of electromagnetic energy and to transform it into heat. The most commonly used frequency for commercial microwave instruments is 2450 MHz, which corresponds to energy output of 600- 700 Watts. (Kingston and Jassie, 1998). At this frequency, the electric field swings the orientation of water molecules 2.45 x 10^9 times every second and the chaos inherent to the system opposes the synchrony of the oscillation with that of the field. Thus creating an intense heat that can escalate as quickly as several degrees per second (estimated as 100 $^oC/S$ at 4.9 GHz) (Lew *et al*., 2002). The dielectric properties of

materials depend on two parameters. The first is 'ε ', the dielectric constant which describes the polarizability of the molecule in an electric field. The dielectric loss factor; ε '', measures the efficiency with which the absorbed microwave energy can be converted into heat. The ratio of the two terms is the dissipation factor, $\varepsilon'' / \varepsilon'$ ultrasonic extraction, $\delta = \varepsilon'' / \varepsilon' \ldots\ldots\ldots\ldots$eq.

The use of microwave irradiation is another way of increasing the efficiency of conventional extraction methods. Microwave assisted extraction consists of heating the solvent in contact with the sample by means of microwave energy. The process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of the ions, which enhance penetration of the solvent into the matrix, allowing dissolution of the components to be extracted. (Hudaib *et al*., 2003; Datta *et al*., 2005; Tang, 2005)

When choosing parameters for microwave extraction physical parameters like solubility, dielectric constant, and the dissipation factor (δ) must be considered. The first factor is to choose a solvent, with a high extracting power in which the target analyte is soluble. Usually higher dielectric constant gives higher degree of microwave absorption. Water has the highest dielectric constant and is the most common solvent. However, the dissipation factor is significantly lower than that of other solvents. Thus, the rate at which water absorbs microwave energy is higher than the rate at which the system can dissipate the heat. This phenomena account for the "superheating" effects, which occur when water is present in the matrix. Localized superheating can have positive or negative effects, depending on the matrix. In some cases it can increase the diffusivity of the analyte in the matrix. Moreover, the intense heating can cause degradation of the analyte and/or "explosion" of the solvent. To get maximum heat distributed through the matrix, it is good to choose a solvent that has a high dielectric constant as well as a high dissipation factor.

Extraction and isolation of phyto-constituents (antioxidants)

During MAE, a polar solvent with a high dielectric constant surrounds the matrix. Microwaves generated in a magnetron are applied in a pulsed fashion. The solvent molecules absorb the microwave energy and become polarized. When the microwave field is removed, thermally induced disorder is restored this process heats the bulk solution and may cause localized superheating effects (only in matrices that contain water). Thermal equilibrium is eventually established within the system because the heat is transferred from the bulk solution and the "pockets affected by superheating effects" via collisions so that the energy is distributed uniformly throughout the system. The final temperature of the extraction is proportional to the power (watts), time, and initial temperature; it is also inversely proportional to the heat capacity of the solvent, and the mass of sample in grams (Haswell and Kingston, 1997).

The heat produced by the interaction of the microwaves with the solvent subsequently increases the diffusivity of the solvent and expectantly that of the analyte. The solvent is then able to diffuse into the matrix and extract the phyto-constituents and finally diffuse out of the matrix and carry along the soluble components. The control factors that direct the extraction of an analyte from a matrix by MAE are the solubility of the analyte in the solvent, the mass transfer kinetics of the analyte from the matrix to the solution phase, and the strength of analyte/matrix interactions. For sample with a homogenous composition and limited porosity, the rate of extraction is determined by the diffusion of the analyte to the surface of the matrix particle. Higher temperatures and swelling of the matrix increase the rate of diffusion and promote faster extraction kinetics.

Factors to consider when using microwave assisted extraction method

There are a number of factors that one must consider when using microwave assisted extraction method such as the choice of solvent, microwave application time, microwave-power, uniqueness of the matrix; Effect of contact sample surface area, and effect of temperature the details of the factors are as follows:

The choice of solvent: The basic factor which affects an extraction process is the choice of appropriate solvent. In an experimental setup the choice of the solvent will be based upon the solubility of the desired analyte, the solvent-matrix interaction and the property of the solvent to absorb microwaves (Chen *et al*., 2008). The selected solvent should have a high selectivity towards the analyte of interest than the other matrix components and also a good compatibility with further chromatographic analytical steps. Solvents which are transparent to microwaves, do not heat up under microwave and those with good microwave absorbing capacity get heated up faster and enhances the extraction process. Hexane is an example of microwave transparent solvent whereas ethanol is an excellent microwave absorbing solvent. In order to get optimum extraction yields, researchers even use mixtures of high and low microwave absorbing solvents. As discussed earlier, we have known that inner glandular and vascular systems of the plant material have some moisture content. The rapid heating of these water molecules ruptures the cell walls and the phyto-constituents are released into the solvent environment. For extraction of volatile oils from several aromatic herbs Solvent free MAE (SFMAE) has been designed, where the moisture content within the plant matrix itself serves extraction and no solvent is used.

The matrix: The ratio of matrix to solvent also plays an important role in extraction (Luque-Garcia and Luque de Castro, 2003). The solvent volume should be sufficient enough to immerse the plant matrix completely in the solvent throughout the entire irradiation process. In conventional extraction methods, a higher ratio of solvent volume to solid matrix gives better extraction yields, whereas in case of MAE a higher solvent: ratio of solvent to matrix ratio may not give better yield due to non-uniform distribution and exposure to microwaves. Microwave

Application Time: Time period of heating is another important factor that influences the extraction process of MAE. The quantity of analyte extracted can be increased with an increase in the extraction time, but there is an associated risk of degradation of thermo labile components (Al-Harahsheh and Kingman, 2004). Varying time periods

are required for extraction of different matrices, but exposure of even few seconds have demonstrated to give excellent yields. The dielectric properties of the solvent influence irradiation time optimization. Longer exposure with microwave absorbing solvents like water, may risk the future of thermo labile constituents.

Microwave-power: The factors microwave power and irradiation times influence each other to a great extent. In order to optimize a MAE procedure a combination of low or moderate power with longer exposure is generally selected. Although with to the use of high power there is an associated risk of thermal degradation/deterioration there are reports which shows that varying of power from 500 W to 1000 W had no significant effects on the yield of flavonoids (Raner *et al*., 1993). Uniqueness of the matrix: The matrix characteristics like particle size and the nature of the material will affect the recoveries of the compounds to a considerable extent. The finer the particles size of the sample the larger the surface area the better the penetration of microwaves (Huie, 2002). Finer particle size of matrix, may sometimes pose a problem during separation of matrix from solvent after the extraction process. Additional step of centrifugation or filtration can serve as a remedy to this issue. If the samples are pretreated with microwave absorbing solvents, the solvent gets impregnated into the cells (Talebi, 2004). Some researchers used the technique of pre-leaching extraction, (soaking of matrix with solvent prior to irradiation) to get better yields. There was an increase in the yield of tashinones after pre-leaching for 2 min at room temperature (Pan *et al*., 2001).

When working with closed vessel system care must be taken for the microwave power and temperature used, as both are closely related to each other. The increase in temperature results in an improved extraction efficiency as desorption of analyte from active sites in the matrix increases. At higher temperatures surface tension and solvent viscosity decrease and solvents have higher capacity to solubilize the analytes by improving sample wetting and matrix penetration respectively. Effect of contact sample surface area; Increasing contact surface area of sample material increases, the extraction efficiency. This can be achieved by adhering strictly to the sample preparation steps which includes milling, grinding and homogenization. The aim is to increase the interaction of the biological cellular matrix with the solvent. This concept is useful when microwave extraction is used for flavonoids extraction. Kothari and Seshadri (2010) used finely ground seeds for the extraction of flavonoids from *Annona squamosa* and *Carica papaya* seeds. Effect of Temperature; High-temperature extraction can be profitable with the resulting increase in solubility. This is because higher temperature causes increased intermolecular interactions within the solvent, giving rise to higher molecular motion which increases the solubility.

The increasing temperature may also cause a cellular pressure build up which may cause cell rupture and opening of the cell matrix, and as a result, increased components availability to be extracted into the solution. Moreover, at high temperature, the solvent viscosity decreases, increasing its mobility and solubility, thus increasing the efficiency of extraction (Khajeh et al. 2009). However, in some cases, it has been observed that the extraction efficiency increases with the increase in temperature until an optimum temperature and solvent to solid ratio is obtained and then it starts decreasing with the further increase in temperature. This also varies with the type of compounds to be extracted as the degradation temperature of every compound is different. Higher temperature or prolonged application of high temperature, induced by prolonged application of high power levels can lead to degradation and subsequent decrease in yield and quality.

Advantages of MAE

The main advantages of microwave assisted extraction over the conventional extraction techniques is that it reduces solvent consumption, it has a shorter operational time, it possess moderately high recoveries, has a good reproducibility and minimal sample manipulation for extraction process (Garcia-Ayuso *et al*., 1999; Garcia-Ayuso *et al*., 2001; Proestos and Komaitis, 2008). Numerous biologically active compounds have been extracted by application of microwave-assisted extraction, such as extraction of taxanes from *Taxus brevifolia* needles (Mattina *et al*., 1999), extraction of azadiractine related limonoids from *Azadirachta indica* seed kernels (Dai *et al*., 1999), extraction of glycyrrhizic acid from *Glycyrrhizia glaubra* roots (Pan *et al*., 2000), extraction of tanshinones from *Salvia miltorrhiza bung* (Pan *et al*., 2002), extraction of artemisinin from *Artemisia annua* (Hao *et al*., 2002) and extraction of ginsenosides from *Panax ginseng* root (Shu *et al*., 2003). Besides, plant secondary metabolites such as phenolic acids, glycosides and flavonoids as well as total phenolics have been extracted from aromatic plants using microwave assisted extraction as shown in Table 4. According to Pan *et al.,* (2008) antioxidant activity of phenolic substances extracted from the peel of *Dimocarpus Longan* using microwave assisted extraction was better than that of Soxhlet extraction. Besides, microwave assisted extraction of curcumin from *Curcuma longa* was optimized using Taguchi L9 orthogonal test and it was evident that the technique showed a better results and a higher extraction yield with significant reduction in the extraction time, when compared to that of Soxhlet extraction, maceration and stirring extraction (Mandal *et al*., 2008). Microwave assisted extraction is relatively cost-effective when compared to accelerated solvent extraction (Kaufmann & Christen, 2002; Sticher, 2008). It also surpasses supercritical fluid extraction owing to its operation simplicity and low cost. The main advantage of microwave assisted extraction over ultrasonic-assisted extraction is its reduced extraction time.

TABLE 4: Total phenolic contents of spice extracts obtained with ultrasound and microwave assisted extraction

Sample	Total phenolic content (mg gallic acid/100 g)	
	UAE	MAE
Coriandrum sativum	41,812 ± 2,765	82,091 ± 8,432
Cinnamomum zeylanicum	506,597 ± 23,518	1679,201 ± 65,333
Cuminum cyminum,	290,296 ± 13,545	1159,542 ± 21,239
Crocus sativus	500,213 ± 34,745	2939,472 ± 24,610

(Monica *et al.,* 2010)

CONCLUSION

Extraction of phyto-constituents is aimed at the isolation and identification of naturally occurring substances such as phenolic acids and flavanoids which are known as antioxidants. Chemical analysis of extracts from plant material plays a central role in the development and modernization of functional foods. Majority of extraction procedures for the determination of plant antioxidants are developed in such a way that the final extracts are introduced into the Gas Chromatography (GC) or High Performance Liquid Chromatography-Mass Spectrometry/ Spectrometry (HPLC- MS/MS) .The use of MAE for the extraction of phyto-constituents from various plant extracts has showen that it is more suitable for extraction as compared to the other methods of extraction. MAE method provides high extraction efficiency in short time, and is not labor intensive.

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