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Microwave-Assisted Solid Extraction from Natural Matrices

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Abstract

The extraction of secondary metabolites from plants, and natural sources in general, is a cornerstone in medicinal chemistry and required the development of sustainable extraction techniques. Microwave-Assisted Solid Extraction (MASE) is a promising extractive methodology being more effective than traditional extraction techniques. It offers higher and faster extraction performance ability with less solvent consumption and protection toward thermolabile constituents. For these reasons, MASE resulted in a suitable extractive methodology in all aspects, including economical and practical, compared to traditional extraction techniques, especially over Soxhlet or solid–liquid extraction. In this chapter, a brief theoretical background about the use of microwave energy for extraction has been presented for better understanding. Then, the potential of MASE for the extraction of secondary metabolites from natural resources, for evaluating the plant productivity and for evaluating the quality of the natural matrices will be reviewed. The discussion is supported by reporting recent applicative examples of MASE applied to the extraction of the most representative chemical classes of secondary metabolites, with a special focus on some drugs or compounds of pharmaceutical and nutraceutical interest.

Keywords: MASE, plant material, natural matrices, secondary metabolite, bioactive compounds, plant productivity

1. Introduction

Nature Aided Drug Discovery (NADD) represents the most ancient approach in finding new active compounds for fighting human diseases, and still today it plays a crucial role in drug discovery [1]. New chemical entities (NCE) from natural derivation represent a relevant slice among the drugs approved by Food and Drug Administration (FDA) and the European Medicines Agencies (EMA) for commercialization and administration on humans [2]. More than half the total anti-infective drugs approved in the last forty years resulted from a NADD approach and a similar trend can be observed for anticancer drugs, where 41% of them derived from natural sources and only 16% are classifiable as totally synthetic small molecules [2]. Moreover, the Global Herbal Medicine Market Size is expected to increase to USD 129 billion by 2023, according to Market Research Future [3].

The success of NADD finds its main reason in the wider and heterogeneous chemical space covered by natural products whether compared with synthetic derivatives. The 83% of the chemical scaffolds found in natural compounds are

unique and absent in synthetic NCE, due to the lack of commercially available syntheses or cumbersome and prohibitive synthetic procedures [4]. Thus, the screening of libraries of compounds derived from natural sources still remains a worthy procedure for the identification of new and unexplored NCE. Besides, marine sources or lichens are still almost uninvestigated and might therefore represent an inestimable treasure of new potential drugs [5–7].

The natural compounds of interest in NADD are secondary metabolites that are not directly involved in the essential functions of the cell cycle and duplication processes and are characterized by high structural variability. From a structural standpoint, they are classified into alkaloids, terpenoids, saponins, lignans, flavonoids, and tannins [8]. Secondary metabolites are produced in different amounts for vexillary functions or defensive responses to biotic or abiotic stress being involved in the system of plant defense [9]. For this reason, specific secondary metabolites may be considered as markers of the plant health and may be used to evaluate the quality of the selected natural matrix and the effects that the environmental factors have on it. The evaluation of their content in natural sources, as well as their recovery, require the exploitation of ad hoc extractive procedures since secondary metabolites are mainly present in the intracellular domain. Thus, appropriate extraction procedures involve the rupture of the cell wall and cellular membranes, thus favoring the passage of the secondary metabolites into the extraction solvent, from which they can be recovered.

Over the years different protocols and techniques have been developed for the extraction of metabolites from natural matrices, named Solid–Liquid Extraction (SLEs). SLEs are classified in conventional (or traditional) and non-conventional (or innovative) methodologies [10]. In conventional methodologies, SLE is performed by heating the natural source with conventional heating sources (i.e., flame, heating plate, or mantle) in the presence or not of solvent and with or without stirring. Examples of conventional methodologies are maceration (digestion, infusion, and decoction), percolation, and Soxhlet or steam distillation. Solvents, or generally mixtures of solvents, with wide grades of polarity such as methanol, ethanol, acetone, ethyl acetate, trichloromethane, hexane, *etc.* are employed. Nevertheless, conventional extraction methods usually require a large volume of solvents, long extraction time, and high temperature. Such harsh extraction conditions may lead to thermal or chemical degradation of the metabolites, thus resulting in a low yield of the final extract. Moreover, the upscaling at an industrial level would be impracticable, owing to energy consumption, technological inaccessibility, and environmental considerations [11]. Conversely, non-conventional methodologies exploit innovative chemical–physical principles and cutting-edge technologies to facilitate the extractive procedures and the recovery of the product of interest from the natural source. Varied energy sources and extractive principles may be exploited and, therefore, specific equipments are needed. Microwave-assisted Solid Extraction (MASE), ultrasound-assisted extraction (UAE), pressurized solvent extraction (PLE), and supercritical fluid extraction (SFE) are the most frequently non-conventional methodologies extensively used for NADD [11].

In this chapter, the potential of microwave-assisted irradiation for the extraction of secondary metabolites from plants, and natural resources in general, will be discussed, with a special focus on recent applicative examples of the most representative chemical classes.

2. Microwave-Assisted Solid Extraction (MASE)

The physical principles underlying MASE are completely different from those of conventional extraction because microwave irradiation can cause a more effective disruption of the cellular structures (cell walls and cellular membranes) thus

favoring the release of the cellular content and speeding up the extraction process. The interaction of microwave irradiation with the solvents, intracellular water, and ions causes an increase of the dipolar rotation and ionic conductivity of molecules with dipolar moments and ions, which results in a rise of the temperature inside the cell. The vaporization of the intracellular water causes the dehydration of the cellular wall and the reduction of its resistance. This, combined with the abrupt increment of the intracellular pressure, leads to the cell wall and membranes disruption, thus facilitating the passage of the secondary metabolites into the extractive solvent [12, 13].

This is the result of a synergistic combination of heat and mass gradient working in the same direction from the inside to the outside of the cell, as confirmed by Scanning Electron Microscopy (SEM) analysis (**Figure 1**) [14].

Monomodal and multimodal microwave systems also referred to as single- or multi-mode systems specifically designed for MASE are nowadays available. A single-mode system permits to focus the microwave radiation on a restricted zone where the sample is subjected to a much stronger electric field. Conversely, in a multimode system, the microwave radiation is randomly dispersed within the microwave cavity, where the sample is irradiated [15]. Both mono- and multi-modal microwave devices comprise a magnetron, which generates microwave radiation, a waveguide, which is used to propagate the microwave from the source to the microwave cavity, the applicator, where the sample is placed and a circulator that allows the microwave to move only in the forward direction. The main difference between the two systems relies on the applicator that in the case of a multimodal system is a closed cavity where the microwaves are randomly dispersed, whereas in the monomodal oven the extraction vessel is directly located in-line with the waveguide [15]. Both systems are effective in extracting metabolites from natural sources, and their use is usually related to the amount of natural matrix. Accordingly, the monomodal system is preferred when the amount of natural source to be treated is relatively low (milligram to gram scale), and vice versa. Microwave extraction may be performed using open or closed extraction vessel systems. The open vessel apparatus originated from a modification of domestic MW ovens. The extraction is conducted at ambient pressure and the vessel is directly connected to a condenser to avoid loss of solvent or volatile components. An evolution of the open vessel apparatus is the Focused Microwave-Assisted Soxhlet (FMASE) which combines the classical Soxhlet extraction (SE) technique with MW irradiation. In the closed vessel system the entire extraction vessel is located within the oven, thus allowing better control of the pressure and temperature during the extraction [16].

The closed-vessel system presents several advantages: i) a higher temperature than open vessel systems can be reached because of the increased pressure inside the vessel raises the boiling point of the solvents used, thus decreasing the time needed for the extraction; ii) the loss of volatile substances is completely avoided because they are confined within the vessel; iii) a low amount of solvent is required because no evaporation occurs and there is little or no risk of airborne contamination thus

Figure 1.
Microwave heating effect on cells.

preventing the oxidation of air-sensitive metabolites; iv) the fumes are contained within the vessel, reducing the hazard of the extractive procedure.

In a closed vessel system high pressure can be reached. The high pressure stimulates various phenomena according to LeChatelier's principle, such as the transition of phase from one form to another, change in reaction dynamics, change in molecular structure, *etc.* hence resulting in the enhancement of extraction efficiency. Accordingly, working under high pressure causes alterations in the structure of some constituents of the cells such as lipids, proteins, enzymes, and outer cell membranes, thus damaging the plant wall and internal structure of the cell and reducing the mass transfer resistance. In this way, the secondary metabolites are released, leaving behind other cellular components. The High-Pressure MASE (HPMAE) is considered a newly emerging technique. It is a time-efficient, convenient, eco-friendly, safe, and energy-efficient extraction method when compared to the traditional or conventional methods of extraction [17].

Nevertheless, the use of closed-vessel systems presents some drawbacks: the amount of samples that can be processed is limited; the addition of reagents or solvents during operation is forbidden and the vessel must be cooled down before it can be opened to avoid loss of volatile constituents [16]. For processing a large amount of material, an open-vessel system is more appropriate. It allows the possibility to add reagents and to refill the solvent during the extraction, and to remove the excess of solvent during the extraction procedure. Moreover, the extraction of thermolabile metabolites is allowed since it usually reaches low temperatures relative to closed-vessel systems. On the other hand, the open-vessel systems ensure less reproducible results compared to the closed-vessel systems and the extraction in open-vessel could require a longer time to obtain results comparable to those achieved in closed-vessel [16].

The microwave energy may be applied also to extraction in solvent-free conditions (Solvent Free Microwave Extraction-SFME) [12]. In this case, the plant material is directly placed into the microwave reactor, without the addition of any solvent. The heating of the water contained by the plant material distends the natural matrices and causes the rupture of the cells releasing their content. This process has been successfully applied for the extraction of the essential oils. After MW heating, the volatile components co-evaporate with the in-situ water contained in the natural matrix; the vapors condense outside the microwave oven into a collector where they separate between essential oils and the aqueous phase. The latter is brought back into the vessel to refresh the amount of water in the sample, whereas the essential oil is collected apart [12].

3. Procedure set-up

The performance of the MASE process is strictly related to the operative conditions employed. Several parameters, such as solvent, solvent-drug ratio, temperature, time, pressure, microwave power, water content, and characteristic of the matrix must be optimized in setting up a MASE procedure. Each of these parameters should not be considered alone, but they are all linked together and the comprehension of the effects and influences of these factors is pivotal for MASE efficiency. A brief discussion about the role of these parameters in the design of a MASE protocol is herein reported.

3.1 Solvent

The selection of the solvent plays a crucial role in MASE, as well as in other conventional extraction processes, and several solvent parameters (solubility of

the analyte, penetration, interaction with the matrix, dielectric constant, and mass transfer kinetic process) should be considered to perform the right choice. *In primis*, the solvent should assure the highest solubility of the analyte of interest while excluding undesired matrix components. However, conversely to the traditional extractive procedures, the chosen solvent must be able to absorb the microwave radiation and to convert it into heat. This depends on the dielectric constant and the dielectric loss of the solvent. Thus, in microwave application, solvents are usually classified in low, medium, or high absorbance whether they absorb at 300 W (i.e. carbon chlorides, 1,4-dioxane, tetrahydrofuran, diethyl ether, ethyl acetate, pyridine, toluene, chlorobenzene, xylenes, and hexane), 200 W (i.e. water, DMF, NMP, butanol, acetonitrile, HMPA, ketones, o-dichlorobenzene, 1,2-dichloroethane, 2-methoxy ethanol, acetic acid, and trifluoroacetic acid) or 100 W (i.e. DMSO, ethanol, methanol, propanol, nitrobenzene, formic acid, ethylene glycol), respectively. For low absorbing solvents, the heating rate can be increased by mixing with solvents with a higher dielectric constant or by adding salts to the mixture [18]. Recently, increasing interest in ionic liquids as MASE solvents or modifiers has been observed. Ionic liquids are organic salts with low melting points, and they are usually liquid at room temperature. They are characterized by extremely low vapor pressure, high stability and can solubilize both polar and non-polar metabolites. Moreover, they have the advantage to be eco-friendly solvents, although their extensive use is limited due to the high commercial costs. Another emerging eco-friendly alternative is the use of deep eutectic solvents. They possess physical and chemical properties similar to the ionic liquids, but they show better biodegradability, toxicity profiles, and solubility properties. Lastly, the content of water in the sample should be taken into account since it influences the heating rate and facilitates the transport of the analytes into the solvent at higher rates [19].

As stated in the previous paragraph, a Solvent-Free Microwave Extraction (SFME) is also possible. This procedure foresees the direct irradiation with MW of the plant material, fresh or rehydrated. A crucial role is played by the content of water in the sample, because it is the heating of such water to cause the rupture of the cells of the plant material, favoring the release of the content. This process is mainly applied for the extraction of volatile compounds such as essential oil. The oil evaporates by azeotropic distillation with the water contained in the sample. After cooling outside of the microwave reactor the oil separates from the water and can be collected through a modified Clevenger apparatus. Water is refluxed back into the microwave oven to allow the continuous extraction of the oil from the sample [12].

3.2 Liquid-Solid (L/S) ratio

Another important parameter to consider in the set-up of a MASE protocol is the ratio between the amount of sample and the volume of solvent. The latter should be enough to cover the sample during the entire process, especially when the matrix swells during the extraction process. Although in conventional extraction higher is the volume of solvent, higher is the yield of the extract, in MASE larger volume of solvent may result in more energy and time to heat the suspension and in a lower yield due to a non-uniform distribution and exposure to microwave. Usually, an L/S from 10:1 to 20:1 (mL/mg) is found to be the right ratio in many extractive processes reported in the literature [20].

Strictly correlated to the L/S ratio is the stirring rate since it affects the mass transfer process in MASE. However, the significance of this parameter is rarely explored. By stirring, the mass transfer barrier created by the concentrated compounds in a localized region due to insufficient solvent can also be minimized resulting in better extraction yield. In other words, agitation accelerates the

extraction speed by accelerating the desorption and dissolution of compounds bound to the sample matrix [17].

3.3 Time

Extraction times in MASE lasts from a few minutes up to half an hour, and this represents an advantage for the extraction of thermal or oxygen labile compounds since it avoids the degradation of the compounds of interest [21]. The extraction yield is directly proportional to the extraction time, although it has been observed that this increment is very small for an extremely long time. Moreover, for longer extraction time overheating may occur, especially with high absorbent solvents, thus exposing thermolabile compounds to degradation. Whether longer extraction time is required, consecutive and shorter extraction cycles are preferable. The solvent can be collected after each extraction cycle and a fresh solvent could be added to the residue to guarantee the exhaustion of the matrix. This discontinuous procedure has been applied for the extraction of several secondary metabolites from plant material, allowing an enhanced yield and low decomposition of thermolabile compounds [22].

3.4 Temperature and microwave power

Temperature and MW power are strictly correlated. Power is the driving force of the process since it provides the energy necessary to excite the dipolar moments and the ionic conduction of the constituent of the sample, resulting in a proportional increase of the temperature and promoting the destruction of the natural matrix. Thus, the power of the microwave irradiation has to be carefully dosed in function of the amount of the sample, the solvent employed, the extraction time required, and the chemical stability of the secondary metabolites of interest [23]. Increasing the power results in an improved extraction yield and shorter extraction time. However, this result is true until the reaching of an optimal temperature beyond which a decrease in yield is observed, mainly due to the thermal stability of the target metabolite [24].

Accordingly, the temperature is a key parameter to enhance the efficacy of MASE and to avoid at the same time the degradation of the sample. Thus, the choice of the extraction temperature is strictly related to the properties of the solvent, the chemical stability of the metabolites of interest, and the microwave system used. In particular, at high temperatures the viscosity and the surface tension of the solvent diminish; moreover, the capability to solubilize the analytes, and to wet and penetrate the matrix increase, thus resulting in improved extractive efficacy. Also, when operating in a close-vessel, there is the advantage of heating the solvent above its boiling point, thus leading to a more performing extraction [25].

To conclude, the development of a proper MASE methodology must consider at least four variables: solvent, liquid/solid ratio, temperature, and time. To speed up the set-up of the procedure, the Design of Experiment (DoE) approach may be applied. This is a systematic statistic-based tool to assess the best experimental conditions both in the academic and industrial fields [26]. Thanks to this approach, all the variables and their interactions can be evaluated while doing the minimum number of runs.

Over the years, microwave-assisted extraction has been successfully applied to extract diverse classes of secondary metabolites (i.e. polyphenols, flavonoids, coumarins, terpenoids, cannabinoids, and alkaloids) from natural sources, for evaluating the plant productivity, for extracting bioactive compounds both for drug discovery or for commercial purposes.

Here below, studies of plant productivity based on MASE methodology and specific cases of extraction of natural compounds of pharmaceutical and nutraceutical interest will be discussed, with a special focus on resveratrol, terpenoids, and cannabinoids.

4. MASE procedures successfully applied to secondary metabolites extraction

4.1 Evaluation of plant productivity

Numerous applications report about the use of the MW to assist the extraction of organic and organometallic compounds from various matrices (soils, sediments, water samples, botanicals), with special emphasis on environmental applications [27, 28]. Extraction of natural matrices is essential to compare their productivity under different stress conditions [9], harvesting time [29], and places [30]. MASE offers the possibility of performing multiple extractions and therefore, it is suitable for the rapid screening of a numerous set of samples to evaluate the productivity of organisms.

An example is a work performed by Martino et al., regarding the MASE of *Melilotus officinalis*, harvested in different environmental situations, to compare the amount of coumarin and related compounds, and to find the best condition for its cultivation [22]. The Authors developed a rapid, reliable, and reproducible method of extraction from *M. officinalis* inflorescence of coumarin (**Figure 2**), melilotic acid, and o-coumaric acid, considered as productivity markers of the plants. A comparison of different extraction techniques evidenced that MASE is the best procedure in terms of both yields and extraction time [22]. The optimal extraction conditions consisted of two successive irradiations of 5 min each at 50°C, with a cooling step in between, and it resulted suitable for application to large sets of samples [22].

Another example is the setup of a fast and reproducible extraction methodology of vitexin and its isomer isovitexin from *Crataegus monogyna* (**Figure 2**) for evaluating the plant productivity and determining the best ecological conditions for hawthorn cultivation in northern Italy (Lombardy). These metabolites have a high pharmaceutical value due to their anti-hyperalgesic and neuroprotective effects and their activity against oxidative stress, cancer, and inflammation [31].

Within this context, Martino et al. set up a MASE procedure that can be applied for quantitative extraction of both metabolites from *C. monogyna* in just 3 minutes [50% aqueous methanol (v/v), 120°C, 120 W], bringing advantages both in terms of time (3 min *vs* 6 hours) and solvent consumption (0.05 vs 0.10 g/mL) over standard extraction methods [30]. The developed MASE protocol combined with isocratic

Figure 2.
Secondary metabolites extracted via MASE approach and considered as markers of the plant productivity.

HPLC analysis is suitable for the rapid screening of plant materials collected in different environmental conditions, and to determine the best ecological conditions for its cultivation. To extract vitexin and isovitexin from *Crotalaria sessiliflora*, Tang et al. exploited a microwave-assisted cloud-point extraction (MACPE). MACPE combines cloud-point extraction (CPE) with MAE. This has emerged as a technique to extract and separate bioactive compounds from medicinal plants [32]. Of note, using MACPE, hydrophobic compounds present in the aqueous phase can be favorably extracted into the hydrophobic core of micelles [33]. Applying MACPE, vitexin and isovitexin have been obtained in high yields and short times [34].

MASE can also be applied to study the effect of micronutrients or pollutants on secondary metabolites production. Amri et al. investigated the impact of soil copper (II) concentrations on nutrient uptake and the antioxidant system of *Marrubium vulgare*. Owing to waste deposition and agricultural practices, copper (II) tends to accumulate in high and toxic concentrations, leading to an alteration of the vital physiological or biochemical functions of the plants. As it is the case of *M. vulgare*, these effects may have a great impact on human health, since such a plant is used worldwide for its medicinal properties. To perform the study, the Authors selected marrubiin (**Figure 2**) as a reference compound, since it is the main secondary metabolite produced by *M. vulgare* leaves. A MASE protocol was developed for the easy extraction of marrubiin. This procedure allowed to evaluate the quality of a wide range of samples of white horehound. To optimize the process, the Authors used the statistical DoE approach. DoE findings indicated that the highest extraction efficiency of marrubiin with high repeatability was obtained using 100% ethanol at 120°C for 15 min, with significant benefits in terms of extraction times and environmental impact, given that ethanol is completely biodegradable. The MASE methodology may be applied for the characterization of *M. vulgare* herbal drug samples, thus evaluating their exposure to abiotic stress, revealing their phytochemical status, and facilitating the identification of raw materials obtained from a plant grown under stress conditions.

To sum up, MASE procedures is a versatile technique suitable for the evaluation of the plant productivity, and to assess the quality of vegetal matrices, since it is fast, reproducible, suitable for extraction of a large number of samples and requires a low amount of natural matrix.

4.2 Extraction of secondary metabolites for drug discovery or commercial purposes

4.2.1 Alkaloids

Alkaloids are a well-known class of secondary metabolites characterized by basic nitrogen. Over the years, many active alkaloids have been extracted via MW irradiation, e.g. ephedrine alkaloids, cocaine, and ergot alkaloids [35–37]. Unfortunately, results obtained for many of them have been comparable or worst if compared with the traditional method [38]. Nevertheless, microwaves have also spurred the discovery of new active alkaloids at the early stage of drug discovery. MASE protocols can be exploited to extract different alkaloids (examples are reported in **Figure 3**) from different botanicals like tuberous roots, leaves, and seeds [37, 39, 40].

As an example of MASE applied to the extraction of alkaloids, Pan et al. obtained a good recovery of caffeine and polyphenols from the leaves of green tea (*Thea sinensis* L.). MASE provided high extraction and selectivity, required a short time, and less labour-intensive, thus resulting in an efficient method in comparison with the conventional extraction procedures [41].

Figure 3.
Examples of alkaloids extracted via the MASE approaches.

Xiong et al. developed an efficient MASE protocol, within a drug discovery process, for the isolation of bioactive alkaloids (e.g. liensinine, isoliensinine, neferine, dauricine, nuciferine, **Figure 3**) from *Lotus plumule*. The optimal extraction conditions required a 65% aqueous methanol as a solvent and irradiation at 200 W for 260 seconds [42]. Another interesting example, reported by Zhou et al., is the microwave-assisted aqueous two-phase extraction, useful for rapid and simultaneous extraction and separation of alkaloids. This technique was applied to *Radix Sophorae tonkinensis*. The optimum conditions were summarized as follows: ethanol/ Na_2HPO_4 as the extraction solvent, 100 mesh as particle size, 1:75 of S/L ratio, irradiating at 90°C for 5 min [43]. Matrine, sophocarpine, oxymatrine, oxysophocarpine, 5 α -hydroxysophocarpine, sophoranol, cytisine, N-methylcytisine, and sophoridine were efficiently extracted.

Recently, Belwal et al. reported an optimized MASE protocol, defined by multicomponent analysis, for the extraction of berberine (**Figure 3**) and polyphenols from diverse species of *Berberis*. The medical properties of berberine (anti-diabetic, hepato-protectant, anti-arthritis, antioxidants, anti-microbial, neuro-protective, and hypo-lipidemic activity) are widely recognized, and it is used in pharmaceuticals and nutraceuticals preparation. In this study, multi-component analysis (MCA) has been used to extract berberine and polyphenols from *B. jaeschkeana* roots under microwave-assisted extraction (MAE) conditions. All the variables, above described, were considered under 42 experiments and the results of the model showed significant model fitness. Under optimized MAE condition, (i.e. 100% methanol, pH 2.0, 598 W, 2 min of irradiation time), the berberine and palmatine (**Figure 3**) contents were recorded in 4.6% and 2.0%, respectively. Under the optimized condition, the yield of alkaloids was found closer to the models' predicted value [34].

Regarding the alkaloids employed as drugs, or of interest for the toxicological use and/or abuse, few extractive procedures by MASE are reported in the literature. As an example, Brachet et al. extracted cocaine and benzoylecgonine from

the leaves of *Erythroxylum coca* by MASE. Different solvents, particle size, time, and power were evaluated. Since MeOH is a high absorbing microwave solvent, and cocaine is highly soluble in it, it was found to be the best extraction solvent [36]. Interestingly, MASE found application in the forensic field as a rapid and cleanup-free method for the extraction and quantification of drugs of abuse and the respective metabolites from human fluids and tissues. Fernandez et al. reported the simultaneous extraction of cocaine, benzoylecgonine, cocaethylene, morphine, 6-monoacetylmorphine, and codeine from human urine [44], hair [45], and vitreous humor samples [46]. The MASE procedure reduces the extraction time, avoids the cleanup steps, and allows a quantitative recovery of the drugs.

4.2.2 Stilbene-based polyphenolic compounds

Stilbene-based polyphenolic compounds, i.e. resveratrol, pterostilbene, and piceatannol, are of particular interest from a medicinal chemistry standpoint, having multiple pharmacological activities (**Figure 4**).

In particular, *trans*-resveratrol (3, 5, 4'-trihydroxystilbene) became popular as a result of an attempt to explain the "French paradox" [47]. Resveratrol and other polyphenolic-stilbene derivatives showed a wide range of beneficial physiological properties. They possess antibacterial, anti-inflammatory, hypolipidemic, cardiovascular-hepatoprotective, and anticancer activities [48–50]. In particular, the hypolipidemic and cardiovascular protective activity derived from the agonistic activity against PPAR α and PPAR γ receptors [51, 52]. For all this benefit, resveratrol has attracted the attention of the scientific community and pharmaceutical and nutraceutical industries. Indeed, several drugs and dietary supplements containing resveratrol are commercially available.

Even though resveratrol is produced naturally in plants, the extraction of resveratrol in commercial quantities is a problem, because of its low concentration, multiple steps of isolation and purification, unfriendly environmental issues, and seasonal occurrence [53]. Moreover, the preparation of resveratrol by synthesis is difficult owing to the formation of many unwanted side products [54, 55]. Only recently, the production of resveratrol in heterologous engineered microorganisms

Figure 4.
Chemical structure of polyphenolic-stilbene based secondary metabolites.

has been proposed [56]. Thus, resveratrol is still being extracted from wild *Polygonum cuspidatum*'s root (Japanese knotweed), grape skins and seed, and the domestic giant knotweed of China, which is the world's largest producer [53, 56–58]. Garcia-Ayuso *et al.*, in late 1998, found that by applying the MW irradiation to SE, the last of the extraction was drastically reduced from 8 hours to 60 minutes with comparable results to SE in yield. MASE was further optimized by testing solvents and times on bark extraction and compared to SE on the same tree sample. The results suggested that microwave extraction may be more efficient than SE.

The extraction of resveratrol by MASE from different plant materials (i.e. *Arachis repens* and grape seeds) has also been investigated. To exhaustively extract resveratrol from *A. repens*, commonly known as peanut grass, three different methodologies (conventional maceration, ultrasound-assisted extractions, and MASE) have been compared. Although sonication resulted more effectively in the extraction of resveratrol compared to MASE and maceration, MASE showed to be an excellent choice since it extracted high yields in a reduced time [59].

In another study, Dang *et al.* combined the aqueous two-phase extraction technique (ATPE) with MASE for the extraction of the total polyphenol content, including resveratrol, from grape seeds [60]. Microwave-assisted ATPE (MAATPE) required lower solvent concentration and less time compared with other methods such as refluxing solvent or SE. A higher level of resveratrol was obtained with MAATPE, in contrast to ATPE. The Authors also compared the effectiveness of three solvents (water, water: ethanol (1:1) and ethanol) and three extraction methods, including MASE and ultrasound-assisted extraction (UAE) and the conventional SE. MASE provided a better extraction with water and ethanol (1:1) obtaining extracts very rich in polyphenolic substances, including stilbenes.

Lastly, MASE has successfully applied also for the extraction of other polyphenolic-stilbene based compounds such as pterostilbene, mainly found in blueberries and in *Pterocarpus marsupium* heartwood, and ϵ -viniferin, found in *Vitis coignetiae*, a wild grapevine (**Figure 4**). Kim *et al.* reported the MASE of pterostilbene, and other derivatives, from *Vitis coignetiae*, using 80% ethanol at 90 W for 15 min, resulting in a stilbenoids overall yield of 0.13%, with pterostilbene the most representative compound in the extract [61]. An optimized protocol (70–150 W for 8–18 min, using 30–50% ethanol) was further developed for the extraction of viniferin from the same drug [48]. Recently, Pinero *et al.* disclosed a new process for recovering stilbenes from woody vine by-products such as grape stem and cane samples. MASE was carried out under different extraction conditions. The best results were achieved from grape stems, using 80% ethanol in water as an extraction solvent, a temperature of 125°C, an irradiation power of 750 W for 5 min [49].

4.2.3 Terpenoids

4.2.3.1 Artemisinin and paclitaxel

Terpenes and isoprenoids, in general, gained much attention for their physiological functions (i.e., hormones, aliphatic membrane anchors, maintaining membrane structure), ecological roles (i.e., defense compounds, insect/animal attractants), and extensive pharmaceutical applications such as flavors, fragrances, and medicines.

In particular, artemisinin and paclitaxel represented two milestones in the fight against malaria and cancer, respectively. Artemisinin (**Figure 5**) is a sesquiterpene lactone isolated from *Artemisia annua* and it is a first-class drug for the treatment of drug-resistant malaria. The conventional artemisinin extraction procedure requires room temperature, heat-reflux, or SE. Hao *et al.*, in 2002 reported a first attempt to extract artemisinin from *Artemisia annua* by MASE. Several solvents were explored,

Figure 5.
Chemical structures of artemisinin and paclitaxel.

such as ethanol, trichloromethane, cyclohexane, n-hexane, petroleum ether, and two in-house oils. Compared with SE, supercritical CO₂ extraction, and normal stirring extraction, MASE of artemisinin from *Artemisia annua* considerably reduced the processing time to 12 minutes and resulted in a 92.1% extraction rate (compared to several hours and 60% extraction yield obtained with Soxhlet) [50]. Later, Liu et al. applied the MASE for the isolation and quantification of artemisinin in comparison with the traditional protocols. MASE confirmed shorter extraction time necessity, reduced solvent consumption, and higher recovery of artemisinin than conventional procedures. The best extraction solvent was petroleum ether–acetone (4:1 v/v), because of the high solubility of artemisinin and adequate microwave energy absorption, at 50°C. The highest yield of artemisinin achieved was 0.55% in 30 minutes among all the extractive methods used [62].

Recently, Misra et al. developed a rapid and reliable MASE and HPTLC protocol for the analysis of artemisinin. The optimized MASE conditions required 100 mg of dried and grinded drug with a size of 14 mesh dispersed into 10 mL of toluene. The irradiation of the sample at 160 W for 120 seconds led to the extraction of 0.816% of the content of artemisinin.

Paclitaxel (**Figure 5**) is a member of the taxane class, and it is one of the most important anticancer drugs approved for human use against ovarian, breast, and pulmonary cancer.

Although the total synthesis of paclitaxel has been reported, its application for the commercial production of this drug is impracticable. Thus, paclitaxel is still produced by extraction from taxol biomass. The most commonly used methods for the extraction of paclitaxel require the use of methanol at ambient temperature, although other protocols requiring refluxing methanol, 1:1 methanol–chloroform at ambient temperature, and percolation using ethanol or 95% ethanol–water at ambient temperature have been reported. However, these methods require a long time (12–24 h) for a complete extraction. Incorvia-Mattina et al. reported for the first time in 1997 the use of MASE to optimize the efficiency of the extraction of paclitaxel. The effects of the biomass, solvent ratio and water content on taxane recovery were also determined. Under appropriate MASE conditions an extract equivalent to the one obtained by conventional extraction methods was produced [63].

Talebi et al. investigated the use of MASE to extract paclitaxel from the needles of *Taxus baccata* L. The extraction parameters were investigated resulting in 90% aq. MeOH as a solvent, a temperature of 95°C, 7 min of extraction time, and a closed-vessel system as the best performing extractive conditions [64].

Recently, another study for the extraction of paclitaxel from biomass through MASE and based on kinetic and thermodynamic analysis has been carried out. The

majority of paclitaxel was recovered from the biomass (~99%) within 6 min in a single cycle of microwave-assisted extraction at microwave powers of 50–150 W and temperatures of 30–45°C [64].

4.2.4 Phytocannabinoids

Cannabis sativa L. has always been considered a controversial plant due to its use as both medicine and illicit drug. Nevertheless, Cannabis is a good source of nutrients, fibers, and natural compounds thus, its industrial and pharmaceutical use is undoubtful. Cannabis produces a peculiar class of natural compounds, namely phytocannabinoids. The two most important and renowned phytocannabinoids are the cannabidiol derivatives (i.e. CBD, CBDV, CBDB, and CBDP) [65, 66] and the tetrahydrocannabinol derivatives (i.e. Δ^9 -THC, Δ^9 -THCV, Δ^9 -THCB, and Δ^9 -THCP) [66, 67] reported in **Figure 6**. Δ^9 -THC is responsible for the recreational use of hemp and therefore its use is banished or tightly regulated by national governments.

CBD-like derivatives are non-psychoactive compounds but with other recognized pharmacological properties such as anti-inflammatory, antioxidant, and anticonvulsant. As an example, Epidiolex, a CBD-based anticonvulsant drug, has been approved in 2018 by Food and Drug Administration for the treatment of seizures associated with Lennox–Gastaut syndrome (LGS), Dravet syndrome, or tuberous sclerosis complex (TSC) in patients 1 year of age and older.

The discovery of a plethora of pharmacological activities ascribed to CBD and other minor phytocannabinoids has increased attention from both scientists and industries for medical, nutraceutical, and cosmetic applications of these cannabinoids.

Several synthetic procedures have been developed and optimized for the industrial preparations of phytocannabinoids and in particular of CBD. However, this process suffers from several drawbacks such as the cost of the starting materials, reagents and solvents, the formation of by-products with consequent cumbersome purification procedures, and the difficulty to control the stereochemistry, the isomerism of the terpenic double bond, and the easy interconversion of CBD into THC in the synthetic conditions.

Thus, the extraction and purification of phytocannabinoids from *C. sativa* remain the preferred procedure for its cost-effectiveness. Besides, tight monitoring of the chemical consistency of the extracts results therefore mandatory in producing consistent and reliable medical cannabis preparations for human uses. Recently, Nahar et al. reviewed all the procedures adopted at the present for the extraction of naturally occurring phytocannabinoids [68].

Figure 6.
 Chemical structures of CBD-like and THC-like major phytocannabinoids present in *C. sativa*.

Focusing on MASE, Lewis-Bakekr et al. investigated the potential to directly extract and decarboxylate dried Cannabis material with the microwave reactor [69]. Dried plant material, suspended in ethanol, was subjected to heating with stirring in a microwave reactor at 150° C. Extraction yield for the concentrated resin was in the range of 19.6–24.4% and it resulted directly proportional to the heating time and dependent on the cultivar employed in the process. Interestingly, a complete decarboxylation of the phytocannabinoids was achieved in one step following this process and no acid forms of phytocannabinoids such as Δ^9 -THCA and CBDA were detected in the resulting extract. Thus, MASE proves to be a worthy method for extraction and decarboxylation of phytocannabinoids due to the possibility to apply controlled temperatures and shorter extraction times. Moreover, this procedure ensures a more consistent and reproducible *Cannabis* extract with consequent reproducible efficacy of the therapeutic results. Kore et al. investigated and optimized the MASE process applied to *C. sativa* resulting in a patent application where they disclosed an improved method for extracting and decarboxylating cannabinoids from cannabis plant material, before, during, or after extraction [70]. MASE was compared to or used in tandem with other extraction strategies such as ultrasound extraction, SE, and supercritical fluid extraction.

The effect of time and temperature was investigated first. Extraction and decarboxylation of phytocannabinoid resulted in time and temperature dependence. To obtain 100% decarboxylation, the temperature must be sustained over a period without the burning of the cannabis material or the boiling/evaporation of the solvent. Because the solvent of choice is ethanol (b.p. 78°C at 1 atm), to reach a higher boiling temperature (i.e. 100–170°C) the extraction process must be carried out in a sealed vessel and under pressure. 170°C was the highest operative temperature achieved since higher temperatures (>180°C) resulted in the microwave run abortion due to the high pressure reached within the vial.

The extraction of cannabis by MASE at 100°C, 130°C, 150°C, and 170°C for 10 minutes resulted in a 23–25% yield of extract.

Interestingly, it appeared that the addition of a second step, such as SFE, after the MASE did not change the cannabinoid profile in the extract. Thus, MASE alone can perform an almost complete extraction of the cannabinoids from the cannabis plant material. Besides, the extraction and conversion of THCA and CBDA into THC and CBD was better at a temperature above 130°C, than at 100°C.

MASE was compared with the effectiveness of the commonly employed extractive procedure, namely maceration in ethanol, SE, and SFE. The conventional extractive procedures resulted in a low concentration of Δ^9 -THC, THCA, and CBD, whereas the addition of the microwave step resulted in a significant increase in the concentration of CBD and THC. As expected, no THCA was detected.

To sum up, a worth general procedure for the extraction and decarboxylation of CBD and THC from cannabis plant material can be thus resumed: i) the drug is weighed and macerated in a mortar; ii) the grinded drug is charged in a microwave vial along with a stir bar; iii) the drug is submerged with ethanol and the vial is sealed; iv) the vial is irradiated with MW using the following conditions [a) Pre-stirring = 30 sec; b) run time = 10 min; c) temperature = 150°C; d) absorption = Normal]; v) the suspension is filtered, and the filtrate concentrated; iv) residual plant material may be subjected (but not necessarily) to SFE.

Drinic et al. extended these studies over other polyphenols and flavonoids as well as phytocannabinoids [71]. In particular, the effects of different extraction parameters, namely ethanol concentration, extraction time and solid/liquid ratio on extraction yield, total phenol content, total flavonoid content, antioxidant activity, reductive capacity, CBD content, and THC content were investigated. For MASE,

a domestic microwave oven and a round-bottom flask connected with a condenser were used. The solid drug was mixed with the solvent (30, 50, or 70% v/v ethanol) in the selected solid/liquid ratio (S/L = 5, 10, or 15). The extraction was performed irradiating at a potency of 580 W without agitation and for a total extraction time of 10, 20, or 30 min. The results of each extraction were analyzed using response surface methodology. The influence of the three process parameters was investigated on total polyphenols yield, total flavonoids yield, antioxidant activity, and reductive capacity as well. The optimal conditions for the highest CBD content and lowest THC content resulted in 47% ethanol concentration, 10 minutes of extraction time, and an S/L ratio of 5. The model was successfully validated by preparing the Cannabis extract under the calculated conditions.

Alongside the pharmaceutical uses of Cannabis extracts, hemp seeds are widely employed to produce hemp oil. However, the content of Δ^9 -THC in the processed hemp seed oils must be under the limits imposed by the jurisdictions of each State. Indeed, although the hemp seeds produce negligible amounts of THC, their outer surface can be contaminated with the enriched in the phytocannabinoids resin secreted by the seeds' bracts. The presence of Δ^9 -THC in the final hemp seed products had led to intoxication symptoms in the final consumers. Thus, nowadays the content of THC in hemp products is tightly regulated. Yang et al. investigated the effectiveness of various chemical procedures for the extraction of Δ^9 -THC from three brands of hemp seeds and how the extractive methods could influence their commercialization [72]. Four extraction methods were employed, namely, i) microwave extraction, ii) sonication, iii) SE and iv) SFE. As already investigated by Kore et al., the extraction was performed in ethanol at 150°C with stirring, obtaining a complete conversion of CBDA and THCA into the corresponding neutral form. Hemp seeds were macerated in a mortar, transferred into a microwave vessel, and suspended in ethanol. The suspension was irradiated at 150°C with stirring for 20 min in a closed vessel. The yield of the resin (27–38%) achieved was comparable to the other three extraction procedures. In contrast, SE provided higher yields of Δ^9 -THC and CBD than the other procedures, resulting in a more robust and appropriate extraction methodology for the testing of hemp seed products. Since the same solvent was used in all the compared extractions, the differences in the number of phytocannabinoids can be attributed to the extraction methods themselves. The results suggest that prolonged heating and solvent cycling in extracting phytocannabinoids from lipid-rich materials such as hemp seeds is mandatory.

5. Conclusion

MASE has rapidly risen during the latest decades as a method for the extraction of secondary metabolites or compounds of pharmaceutical and nutraceutical interest. The use of microwave can generate peculiar, and otherwise impossible to reach extraction mechanisms. As a result, a reduction of the extraction time, improvement of the extraction efficiency, high reproducibility, and robustness of the procedure can be achieved. An increase of the sample throughput is in addition possible, thus it can be considered as the elective technique when a high number of samples have to be processed specially during the first stage of the NADD process, and for evaluating the quality of the natural matrices [9, 29]. For these reasons, MASE has proven to be effective in all aspects, including economical and practical, compared to traditional extraction techniques, especially over SE. Conversely, in MASE the development of the method must be carefully assessed, and all the variables and factors described above must be thoroughly considered to provide some extraction selectivity. Hence,

DoE, response surface methodology, and other statistical approaches are of great help to quickly determine the best conditions to achieve the highest yield of the metabolite of interest from the natural source. However, in the past year, the application of MASE in scalable industrial processes has always encountered several limitations due to the presence of some technological barriers, mainly related to the design of safe instrumentation. Thanks to the technological progress witnessed in recent years, the first industrial-scale ovens finally became commercially available [73, 74].

Food, pharmaceutical, and nutraceutical industries would be benefited from this emerging technology of MASE, which is an excellent substitute for traditional methods such as SE, and other environmentally benign technologies. The promise to be the technique that can respond to the necessities in this field will make MASE the extraction method of choice for the next years.

Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Medina-Franco JL. Discovery and Development of Lead Compounds from Natural Sources Using Computational Approaches. In: Evidence-Based Validation of Herbal Medicine. Elsevier; 2015. p. 455-475.
- [2] Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod. 2020 Mar;83(3):770-803.
- [3] Herbal Medicine Market Value to Surpass USD 129 Billion Revenue Mark by 2023 at 5.88% CAGR, Predicts Market Research Future. Market Research Future GlobeNewswire. 2019.
- [4] Hert J, Irwin JJ, Laggner C, Keiser MJ, Shoichet BK. Quantifying Biogenic Bias in Screening Libraries. Nat Chem Biol. 2009;5(7):479-83.
- [5] Popovici V, Bucur LA, Schröder V, Gherghel D, Mihai CT, Caraiane A, et al. Evaluation of the Cytotoxic Activity of the *Usnea barbata* (L.) F. H. Wigg Dry Extract. Molecules. 2020 Apr;25(8):1865.
- [6] Freysdottir J, Omarsdottir S, Ingólfssdóttir K, Víkingsson A, Ólafsdóttir ES. In vitro and in vivo immunomodulating effects of traditionally prepared extract and purified compounds from *Cetraria islandica*. Int Immunopharmacol. 2008;
- [7] McCauley EP, Piña IC, Thompson AD, Bashir K, Weinberg M, Kurz SL, et al. Highlights of marine natural products having parallel scaffolds found from marine-derived bacteria, sponges, and tunicates. J Antibiot (Tokyo). 2020 Aug;73(8):504-25.
- [8] Verpoorte R, van der Heijden R, Memelink J. Engineering the plant cell factory for secondary metabolite production. Transgenic Res. 2000;9(4-5):323-43; discussion 321.
- [9] Martino E, Della Volpe S, Cavalloro V, Amri B, Kaab LBB, Marrubini G, et al. The use of a microwave-assisted solvent extraction coupled with HPLC-UV/PAD to assess the quality of *Marrubium vulgare* L. (white horehound) herbal raw material. Phytochem Anal. 2019;30(4).
- [10] Borges A, José H, Homem V, Simões M. Comparison of Techniques and Solvents on the Antimicrobial and Antioxidant Potential of Extracts from *Acacia dealbata* and *Olea europaea*. Antibiotics. 2020 Jan;9(2):48.
- [11] Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018 Dec;13(1):20.
- [12] Li Y, Fabiano-Tixier AS, Vian MA, Chemat F. Solvent-free microwave extraction of bioactive compounds provides a tool for green analytical chemistry. Trends Anal Chem. 2013;47:1-11.
- [13] Vinatoru M, Mason TJ, Calinescu I. Trends in Analytical Chemistry Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. Trends Anal Chem. 2017;97:159-78.
- [14] Xue H, Xu H, Wang X, Shen L, Liu H, Liu C, et al. Effects of Microwave Power on Extraction Kinetic of Anthocyanin from Blueberry Powder considering Absorption of Microwave Energy. 2018;2018.
- [15] Vats T, Mishra A. Sustainable Syntheses with Microwave Irradiation. Encycl Inorg Bioinorg Chem. 2016;
- [16] Marco B, Agnese C, Giuseppe T. Quality Preservation and Cost

Effectiveness in the Extraction of Nutraceutically-Relevant Fractions from Microbial and Vegetal Matrices. In: Scientific, Health and Social Aspects of the Food Industry. InTech; 2012.

[17] Chan C-H, Yusoff R, Ngoh G-C, Kung FW-L. Microwave-assisted extractions of active ingredients from plants. *J Chromatogr A*. 2011 Sep;1218(37):6213-25.

[18] Ibrahim NA, Zaini MAA. Solvent selection in microwave assisted extraction of castor oil. *Chem Eng Trans*. 2017;56:865-70.

[19] Xu W, Chu K, Li H, Zhang Y, Zheng H, Chen R, et al. Ionic Liquid-Based Microwave-Assisted Extraction of Flavonoids from *Bauhinia championii* (Benth.) Benth. *Molecules*. 2012 Dec;17(12):14323-35.

[20] Du F-Y, Xiao X-H, Xu P-P, Li G-K. Ionic liquid-based microwave-assisted extraction and HPLC analysis of dehydrocavidine in *corydalis saxicola* Bunting. *Acta Chromatogr [Internet]*. 2010 Sep;22(3):459-71. Available from: <https://akjournals.com/doi/10.1556/achrom.22.2010.3.9>

[21] Vigani B, Rossi S, Gentile M, Sandri G, Bonferoni MC, Cavalloro V, et al. Development of a mucoadhesive and an in situ gelling formulation based on κ -carrageenan for application on oral mucosa and esophagus walls. II. Loading of a bioactive hydroalcoholic extract. *Mar Drugs*. 2019;17(3).

[22] Martino E, Ramaiola I, Urbano M, Bracco F, Collina S. Microwave-assisted extraction of coumarin and related compounds from *Melilotus officinalis* (L.) Pallas as an alternative to Soxhlet and ultrasound-assisted extraction. *J Chromatogr A*. 2006 Sep;1125(2):147-51.

[23] Xue H, Xu H, Wang X, Shen L, Liu H, Liu C, et al. Effects of Microwave

Power on Extraction Kinetic of Anthocyanin from Blueberry Powder considering Absorption of Microwave Energy. *J Food Qual*. 2018;2018:1-13.

[24] Routray W, Orsat V. Microwave-Assisted Extraction of Flavonoids: A Review. *Food Bioprocess Technol*. 2012 Feb;5(2):409-24.

[25] Pimentel-Moral S, Borrás-Linares I, Lozano-Sánchez J, Arráez-Román D, Martínez-Férez A, Segura-Carretero A. Microwave-assisted extraction for *Hibiscus sabdariffa* bioactive compounds. *J Pharm Biomed Anal*. 2018 Jul;156:313-22.

[26] N. Politis S, Colombo P, Colombo G, M. Rekkas D. Design of experiments (DoE) in pharmaceutical development. *Drug Dev Ind Pharm [Internet]*. 2017 Jun 3;43(6):889-901. Available from: <https://www.tandfonline.com/doi/full/10.1080/03639045.2017.1291672>

[27] Camel V. Microwave-assisted solvent extraction of environmental samples. *TrAC Trends Anal Chem [Internet]*. 2000 Apr;19(4):229-48. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0165993699001855>

[28] Sanchez-Prado L, Garcia-Jares C, Dagnac T, Llompарт M. Microwave-assisted extraction of emerging pollutants in environmental and biological samples before chromatographic determination. *TrAC Trends Anal Chem [Internet]*. 2015 Sep;71:119-43. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0165993615001442>

[29] Granata MU, Bracco F, Catoni R, Cavalloro V, Martino E. Secondary metabolites profile and physiological leaf traits in wild and cultivated *Corylus avellana* under different nutritional status. *Nat Prod Res*. 2019 Oct;1-8.

[30] Martino E, Collina S, Rossi D, Bazzoni D, Gaggeri R, Bracco F, et al.

Influence of the extraction mode on the yield of hyperoside, vitexin and vitexin-2''- O -rhamnoside from *Crataegus monogyna* Jacq. (hawthorn). *Phytochem Anal.* 2008 Nov;19(6):534-40.

[31] He M, Min J-W, Kong W-L, He X-H, Li J-X, Peng B-W. A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia* [Internet]. 2016 Dec;115:74-85. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0367326X16304488>

[32] Tang X, Zhu D, Huai W, Zhang W, Fu C, Xie X, et al. Simultaneous extraction and separation of flavonoids and alkaloids from *Crotalaria sessiliflora* L. by microwave-assisted cloud-point extraction. *Sep Purif Technol* [Internet]. 2017 Mar;175:266-73. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1383586616307110>

[33] Madej K. Microwave-assisted and cloud-point extraction in determination of drugs and other bioactive compounds. *TrAC Trends Anal Chem.* 2009 Apr;28(4):436-46.

[34] Belwal T, Pandey A, Bhatt ID, Rawal RS. Optimized microwave assisted extraction (MAE) of alkaloids and polyphenols from *Berberis* roots using multiple-component analysis. *Sci Rep* [Internet]. 2020 Dec 22;10(1):917. Available from: <http://www.nature.com/articles/s41598-020-57585-8>

[35] Pellati F, Benvenuti S. Determination of ephedrine alkaloids in *Ephedra* natural products using HPLC on a pentafluorophenylpropyl stationary phase. *J Pharm Biomed Anal.* 2008 Sep;48(2):254-63.

[36] Brachet A, Christen P, Veuthey J-L. Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. *Phytochem Anal.* 2002 May;13(3):162-9.

[37] Nowak J, Woźniakiewicz M, Klepacki P, Sowa A, Kościelniak P. Identification and determination of ergot alkaloids in Morning Glory cultivars. *Anal Bioanal Chem.* 2016 May;408(12):3093-102.

[38] Petruczynik A. Analysis of alkaloids from different chemical groups by different liquid chromatography methods. *Open Chem* [Internet]. 2012 Jun 1;10(3):802-35. Available from: <https://www.degruyter.com/view/journals/chem/10/3/article-p802.xml>

[39] Xie D-T, Wang Y-Q, Kang Y, Hu Q-F, Su N-Y, Huang J-M, et al. Microwave-assisted extraction of bioactive alkaloids from *Stephania sinica*. *Sep Purif Technol.* 2014 Jun;130:173-81.

[40] Ganesapillai M, Singh A, Subba Rao B, De D, Juneja U. EXTRACTION OF ALKALOIDS FROM MICROWAVE DRIED *ADATHODA VASICA* LEAVES - A COMPARATIVE STUDY. *Int J Pharma Bio Sci.* 2015;6(1):121-39.

[41] Pan X, Niu G, Liu H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem Eng Process Process Intensif* [Internet]. 2003 Feb;42(2):129-33. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0255270102000375>

[42] Xiong W, Chen X, Lv G, Hu D, Zhao J, Li S. Optimization of microwave-assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology. *J Pharm Anal.* 2016 Dec;6(6):382-8.

[43] Zhou S, Wu X, Huang Y, Xie X, Lin Y, Fan H, et al. Microwave-assisted aqueous two-phase extraction of alkaloids from *Radix Sophorae Tonkinensis* with an ethanol/Na₂HPO₄ system: Process optimization, composition identification and quantification analysis. *Ind Crops Prod.* 2018 Oct;122:316-28.

- [44] Fernández P, Lago M, Lorenzo RA, Carro AM, Bermejo AM, Tabernero MJ. Microwave assisted extraction of drugs of abuse from human urine. *J Appl Toxicol* [Internet]. 2007 Jul;27(4):373-9. Available from: <http://doi.wiley.com/10.1002/jat.1216>
- [45] Fernández P, Lago M, Lorenzo RA, Carro AM, Bermejo AM, Tabernero MJ. Optimization of a rapid microwave-assisted extraction method for the simultaneous determination of opiates, cocaine and their metabolites in human hair. *J Chromatogr B* [Internet]. 2009 Jun;877(18-19):1743-50. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1570023209003055>
- [46] Fernández P, Seoane S, Vázquez C, Bermejo AM, Carro AM, Lorenzo RA. A rapid analytical method based on microwave-assisted extraction for the determination of drugs of abuse in vitreous humor. *Anal Bioanal Chem* [Internet]. 2011 Oct 7;401(7):2177-86. Available from: <http://link.springer.com/10.1007/s00216-011-5279-6>
- [47] Rimando AM, Kalt W, Magee JB, Dewey J, Ballington JR. Resveratrol, Pterostilbene, and Piceatannol in Vaccinium Berries. *J Agric Food Chem*. 2004 Jul;52(15):4713-9.
- [48] Kim H-K, Do J-R, Lim T-S, Akram K, Yoon S-R, Kwon J-H. Optimisation of microwave-assisted extraction for functional properties of Vitis coignetiae extract by response surface methodology. *J Sci Food Agric*. 2012 Jun;92(8):1780-5.
- [49] Piñeiro Z, Marrufo-Curtido A, Vela C, Palma M. Microwave-assisted extraction of stilbenes from woody vine material. *Food Bioprod Process*. 2017 May;103:18-26.
- [50] Hao J, Han W, Huang S, Xue B, Deng X. Microwave-assisted extraction of artemisinin from *Artemisia annua* L. *Sep Purif Technol*. 2002 Sep;28(3):191-6.
- [51] Giampietro L, Angelo AD, Giancristofaro A, Ammazalorso A, Filippis B De, Matteo M Di, et al. Effect of Stilbene and Chalcone Scaffolds Incorporation in Clofibric Acid on PPAR Agonistic Activity. *Med Chem (Los Angeles)*. 2014;10:59-65.
- [52] De Filippis B, Linciano P, Ammazalorso A, Di Giovanni C, Fantacuzzi M, Giampietro L, et al. Structural development studies of PPARs ligands based on tyrosine scaffold. *Eur J Med Chem*. 2015;89:817-25.
- [53] Mei Y-Z, Liu R-X, Wang D-P, Wang X, Dai C-C. Biocatalysis and biotransformation of resveratrol in microorganisms. *Biotechnol Lett*. 2015 Jan;37(1):9-18.
- [54] Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angew Chemie Int Ed*. 2011 Jan;50(3):586-621.
- [55] Saraswati S. Velu, Noel F. Thomas, Jean-Frederic F. Weber. Strategies and Methods for the Syntheses of Natural Oligomeric Stilbenoids and Analogues. *Curr Org Chem*. 2012 Apr;16(5):605-62.
- [56] Donnez D, Jeandet P, Clément C, Courot E. Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends Biotechnol*. 2009 Dec;27(12):706-13.
- [57] Nopo-Olazabal C, Hubstenberger J, Nopo-Olazabal L, Medina-Bolivar F. Antioxidant Activity of Selected Stilbenoids and Their Bioproduction in Hairy Root Cultures of Muscadine Grape (*Vitis rotundifolia* Michx.). *J Agric Food Chem*. 2013 Dec;61(48):11744-58.

- [58] Almagro L, Belchí-Navarro S, Sabater-Jara AB, Vera-Urbina JC, Sellés-Marchart S, Bru R, et al. Bioproduction of trans-Resveratrol from Grapevine Cell Cultures. In: Natural Products. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 1683-713.
- [59] Garcia L, Garcia R, Pacheco G, Sutili F, Souza R De, Mansur E, et al. Optimized Extraction of Resveratrol from *Arachis repens* Handro by Ultrasound and Microwave: A Correlation Study with the Antioxidant Properties and Phenol Contents. *Sci World J*. 2016;2016:1-10.
- [60] Dang Y-Y, Zhang H, Xiu Z-L. Microwave-assisted aqueous two-phase extraction of phenolics from grape (*Vitis vinifera*) seed. *J Chem Technol Biotechnol*. 2014 Oct;89(10):1576-81.
- [61] Kim J-S, Ha T-Y, Ahn J, Kim H-K, Kim S. Pterostilbene from *Vitis coignetiae* protect H₂O₂-induced inhibition of gap junctional intercellular communication in rat liver cell line. *Food Chem Toxicol*. 2009 Feb;47(2):404-9.
- [62] Liu C-Z, Zhou H-Y, Zhao Y. An effective method for fast determination of artemisinin in *Artemisia annua* L. by high performance liquid chromatography with evaporative light scattering detection. *Anal Chim Acta*. 2007 Jan;581(2):298-302.
- [63] Mattina MJI, Berger WAI, Denson CL. Microwave-Assisted Extraction of Taxanes from Taxus Biomass. *J Agric Food Chem*. 1997 Dec;45(12):4691-6.
- [64] Lee S-H, Kim J-H. Kinetic and thermodynamic characteristics of microwave-assisted extraction for the recovery of paclitaxel from *Taxus chinensis*. *Process Biochem*. 2019 Jan;76:187-93.
- [65] Citti C, Linciano P, Forni F, Vandelli MA, Gigli G, Laganà A, et al. Analysis of impurities of cannabidiol from hemp. Isolation, characterization and synthesis of cannabidibutol, the novel cannabidiol butyl analog. *J Pharm Biomed Anal*. 2019;175.
- [66] Linciano P, Citti C, Luongo L, Belardo C, Maione S, Vandelli MA, et al. Isolation of a High-Affinity Cannabinoid for the Human CB1 Receptor from a Medicinal *Cannabis sativa* Variety: Δ^9 -Tetrahydrocannabutol, the Butyl Homologue of Δ^9 -Tetrahydrocannabinol. *J Nat Prod*. 2020;83(1):88-98.
- [67] Citti C, Linciano P, Russo F, Luongo L, Iannotta M, Maione S, et al. A novel phytocannabinoid isolated from *Cannabis sativa* L. with an in vivo cannabimimetic activity higher than Δ^9 -tetrahydrocannabinol: Δ^9 -Tetrahydrocannabiphorol. *Sci Rep*. 2019;9(1):1-13.
- [68] Nahar L, Uddin SJ, Alam MA, Sarker SD. Extraction of naturally occurring cannabinoids: an update. *Phytochem Anal*. 2020 Sep;pca.2987.
- [69] Lewis-Bakker MM, Yang Y, Vyawahare R, Kotra LP. Extractions of Medical Cannabis Cultivars and the Role of Decarboxylation in Optimal Receptor Responses. *Cannabis Cannabinoid Res*. 2019 Sep;4(3):183-94.
- [70] Kotra LP, Lewis MM, Wasilewski E, Grover H. Decarboxylated Cannabis Resins, Uses Thereof and Methods of Making Same. WO/2018/000094, 2018.
- [71] Drinić Z, Vladić J, Koren A, Zeremski T, Stojanov N, Kiproviski B, et al. Microwave-assisted extraction of cannabinoids and antioxidants from *Cannabis sativa* aerial parts and process modeling. *J Chem Technol Biotechnol*. 2020 Mar;95(3):831-9.

[72] Yang Y, Lewis MM, Bello AM, Wasilewski E, Clarke HA, Kotra LP. Cannabis sativa (Hemp) Seeds, Δ^9 -Tetrahydrocannabinol, and Potential Overdose. Cannabis Cannabinoid Res. 2017 Jan;2(1):274-81.

[73] Li Y, Radoiu M, Fabiano-Tixier A-S, Chemat F. From Laboratory to Industry: Scale-Up, Quality, and Safety Consideration for Microwave-Assisted Extraction. In 2012. p. 207-29.

[74] Périno S, Pierson JT, Ruiz K, Cravotto G, Chemat F. Laboratory to pilot scale: Microwave extraction for polyphenols lettuce. Food Chem. 2016;204:108-14.