Process Design for the Extraction of Bioactive Compounds from Several Mediterranean Medicinal Plants

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The objective of this study was the optimization of the extraction process of several medicinal plants of the Mediterranean flora, and their extracts' further evaluation regarding their potential biological activity. This work aimed to design and optimize the extraction method for recovering the targeted compounds from rosemary (Rosmarinus officinalis), St. John's wort (Hypericum perforatum) and chamomile (Matricaria recutita) towards the extraction yield and quality of the extracts.

Raw materials were collected, dried, and ground in desired particle sizes (200, 500, 1000 μm). Ethanol was used as solvent; and extraction was performed using conventional extraction methods (Soxhlet), as well as novel extraction techniques (Ultrasound Assisted Extraction (UAE), Microwave Assisted Extraction (MAE) and their combination (UAE-MAE)). The optimized parameters were Ultrasound and Microwave intensity, and extraction time. All extracts were evaluated towards their total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. TPC was determined by Folin–Ciocalteu method, while the antioxidant activity was estimated with the DPPH assay.

Optimum particle size was 500 μm for rosemary, and 1000 μm for both St. John's wort and chamomile. In general, the increase of extraction time leads to increase of efficiency. However, the extraction time is not an independent variable, since the ultrasound and microwave intensities affect the yield. In specific, the increment of intensity in both techniques, as well as their combination, increases the efficiency of the extraction and reduces the time. Moreover, it was observed that after a certain intensity value the yield remains constant or slightly decreases, due to degradation phenomena. Thus, the optimum ultrasound intensity for rosemary and chamomile was 450 W, while for St. John's wort was 700 W. Regarding microwave extraction parameters, for all tested plants the value of 200 W was selected as optimum, except for St. John's wort (200 W). The optimum set of values were also selected for UAE-MAE. Results also indicate that the extracts were rich in phenolic compounds, possessing also a remarkable antioxidant activity in all the organic solvent systems tested.

1. Introduction

In recent years, significant attempts are made to reduce the amount of synthetic enhancers that are added to food products. Apart from the need to replace the synthetic compounds that are used in the daily nutrition, with natural ones, consumers have recently increased interest to the consumption of functional foods that contain important bioactive compounds, which enhance their quality, nutritional and sensory characteristics (Nasri et al., 2014). Various plants and herbs constitute important natural sources significantly rich in numerous beneficial ingredients, such as antioxidants, colorants, essential oils, etc.

The Mediterranean region is considered as one of the richest areas worldwide in terms of its biodiversity, with regard to the wild flora (Papageorgiou and Vogiatzakis, 2006). Particularly, Greece has a long history of medicinal plants and herbs, with extensive literature and clinical applications covering thousands of years (Solomou et al., 2015).

Essential oils are usually isolated using distillation, while antioxidants are extracted by conventional extraction techniques (Soxhlet extraction), as well as novel extraction methods, such as ultrasound and microwave assisted extraction (Mustafa and Turner, 2011). The basic advantages of the novel extraction techniques are the improved preservation of some volatile compounds due to shorter extraction times and low thermal and hydrolytic effects, and the prevention of chemical changes under steam distillation condition (Lo Presti et al., 2005, Okoh et al., 2010). Microwave assisted extraction (MAE) is based on the combination of low microwave heating; and distillation is performed at atmospheric pressure conditions which appears to be particularly attractive for the isolation of herbal bioactive compounds (Chen and Spiro, 1994). Some advantages of this method compared to hydro distillation include high yield of isolated compounds, lower energy requirement and high purity of the extracted oil (Lucchesi et al., 2004).

Ultrasonic assisted extraction (UAE) seems to be a sustainable method to achieve reduction in extraction time with better quality of the extracted compounds (Wang and Wei, 2015). The use of ultrasound has been reported to increase the yield and rate of mass transfer in several solid–liquid extraction processes (Kaul et al., 2011). The maximum yield achieved with Soxhlet extraction is practically impossible to achieve in most industrial extraction applications based on mechanical agitation of raw materials with the solvent. The technique of mechanical solid-solvent extraction can be optimized by changing the particle size of the solids, as well as other parameters, such as solvent composition, solid-to-solvent ratio and extraction time, having as a key criterion the yield maximization.

The objective of the present study was to investigate the potential biological activity of the extracts of several herbs of the Mediterranean flora, by optimizing their extraction process towards their bioactive compounds (phenolics) content.

In particular, different values of microwave and ultrasound power, as well as experiments duration were examined while extraction temperature remained stable. The experiments were performed following the central composite design and parametric optimization of the extraction method, to recover the targeted compounds from rosemary (Rosmarinus officinalis), St. John's wort (Hypericum perforatum), and chamomile (Matricaria recutita) towards the extraction yield and quality of the extracts.

2. Materials & Methods

2.1 Materials

Rosemary (rosmarinus officinalis), St. John's wort (Hypericum perforatum), and chamomile (Matricaria recutita) dried leaves were purchased from Natural Food Additives G.P.. In order to obtain fractions of different particle sizes, a mill and sieves of different diameters were used. The particle sizes studied were 200 μm, 500 μm and 1000 μm. All reagents used for the experiments were of analytical grade.

2.2 Methods

The methods applied in order to extract the bioactive compounds of the selected medicinal plants were the following: Soxhlet extraction, and extraction by Ultrasound and/or Microwave Assistance (UAE/ MAE/ UAE-MAE). Soxhlet is a conventional, continuous method of extracting compounds from solid materials. By using the solvent reflux and siphon principle, the solid matter can be extracted by a pure solvent every time, leading to a high extraction efficiency. UAE uses high power, and low frequency sound waves to detach the solute of interest from the plant matrix. The sound waves that propagate into the solvent media result in alternating high/low pressure cycles, which produce cavitational bubbles. The energy generated from collapsing cavitational bubbles provides greater penetration of the solvent into the cellular material and improves mass transfer to and from interfaces (Knorr, 2003). Additionally, MAE is governed by two phenomena: ionic conduction and dipole rotation, which in most cases occur simultaneously in polar materials and solvents (Letellier and Budzinski, 1999, Hashim et al., 2020). This makes the temperature of the solvent to increase, enhancing solute solubility. It is clear that their combination is a novel, promising and efficient technique to extract the targeted bioactive compounds from the selected medicinal plants.

Some process parameters were selected and remained constant. Specifically, the mechanical stirring was maintained constant at 400 rpm and the maximum temperature was 45   for all herbs used. The solid-to-liquid ratio was 1:20 in all experiments performed.

Soxhlet extraction

Soxhlet extraction was applied in order to calculate the efficiency regarding the antioxidant capacity of each herb; as in Soxhlet extraction 100 % efficacy is considered to be achieved. Soxhlet extraction system includes a spherical flask placed in a heater mantle, attached to a horizontal extraction chamber and a condenser on top. The condenser is connected to a water supply and serves as a refrigerant.

The sample is added to a pressed-paper container and then placed in the special chamber of the extraction device. Table 1 presents the extraction conditions for each plant tested.

Table1. Soxhlet extraction protocol for the recovery of bioactive compounds

	Plant		
	Rosemary (Rosmarinus officinalis)	St. John's wort (Hypericum perforatum)	Chamomile (Matricaria recutita)
Extraction Solvents	Hexane – acetone system	Ethanol	Ethanol
Solid: Solvent ratio	1:20 w/v	1:20 w/v	1:20 w/v
Duration/ no siphons	4 h/ 10 siphons	5 h/ 13 siphons	5 h/ 13 siphons

Ultrasound Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE)

Ultrasound experiments were performed in the Ultrasonic microwave reaction system XO-SM50 (Nanjing Xianou Instruments Manufacture CO., LTD., China). The maximum intensity of ultrasound was 700 W, while the frequency was constant at 25 kHz. Extractions were performed using ethanol as solvent, and the particle sizes chosen resulted from the experimental design of the process for all the materials. The intensities of ultrasound were 0, 450 and 700 W (otherwise 0 %, 50 % and 78 % of the total intensity of the apparatus). The experiments were performed on three different microwave intensities, namely 0, 200 and 500 W, while the frequency was constant at 2450 MHz. The extraction duration was set to 6, 8 and 12 minutes. The experiments were executed in accordance to the central composite design and parametric optimization of the extraction method, for recovering the targeted compounds of rosemary (Rosmarinus officinalis), St. John's wort (Hypericum perforatum), and chamomile (Matricaria recutita) towards the extraction yield and the quality of the extracts.

In each experiment, standard values were set for certain parameters (agitation, maximum temperature, plant:solvent ratio):

a. Ultrasonic Microwave Reaction System:
   Stirrer: 400 rpm, Set Temperature: 45 ℃, plant:solvent ratio: 1:20

b. Ultrasonicator:
   On: 1 s (spraying duration), Off: 1 s (time between the spraying)

2.3 Properties

Total Phenolic Content

The total phenolic content (TPC) of the herbal extracts was determined by the Folin-Ciocalteu assay (Skotti et al., 2014) (Singleton et al., 1999) with some modifications. Thus 100 µL of each tested sample, 500 µL of Folin-Ciocalteu reagent and 7.9 mL of deionized water were transferred in a 10 mL flask and mixed thoroughly. After 30 min, 1.5 mL of 7.5 % Na_2CO_3 was added in the flask, mixed thoroughly again, and let standing in a heating bath (40 ^{o}C) for 30 min. Absorbance was measured by a spectrophotometer (Spectrometer 211 UV-M51, Bel Photonics) at 765 nm against a blank. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid, and expressed as gallic acid equivalents (GAE) in milligrams per gram of the initial dried raw material. All measurements were performed in triplicate.

DPPH

Antioxidant ability of all extracts was evaluated by an analytical method,1,1-diphenyl-2-picrylhydrazyl (DPPH), that measures the radical scavenging activity of antioxidants against free radicals (Surveswaran et al., 2007). The DPPH solution (3.9 mL; absorbance of 0.68 ± 0.005 at 515 nm) was added to 0.1 mL of the tested extracts. The mixture was shaken vigorously and left to stand for a 30 min incubation period at room temperature, and the absorbance was measured at 515 nm using methanol as blank. The radical scavenger activity was expressed in terms of the antioxidant amount necessary to decrease the initial DPPH absorbance by 50 % (IC50). The IC50 value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.
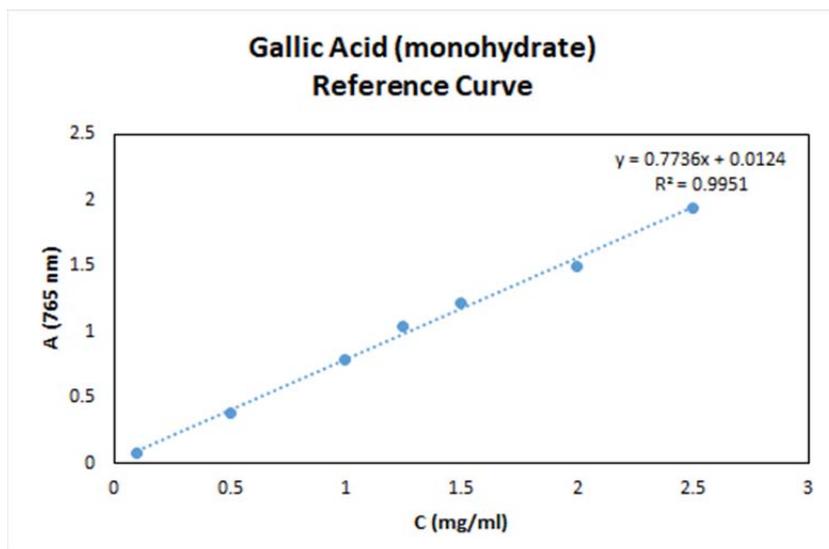
Figure 1. Reference curve of pure gallic acid (monohydrate).

3. Results and Discussion

It is known that the particle size of the powder of the herb, influences the yield of extraction. The smaller the particles; the higher the total mass transfer on the surface is, resulting in an improved yield (Zubairi et al., 2014). Although efficiency is the basic criterion for the process optimization, simplicity was also important regarding the optimum particle size selection for each herb. Ergo, in all cases where the yield was not significantly affected by the particle size, the larger value was selected since its production is easier and of lower cost. Therefore, the optimum particle size selected was 500 μm for rosemary, and 1000 μm for both St. John's wort and chamomile.

Extraction time is one of the most important parameters of the process, since it affects both cost and efficiency. It can also possibly influence the quality of the extract; since increased exposure to such conditions, influence the desired substances (Hong-yu et al.). The optimum extraction time in each case was selected using as a criterion the optimum yield and the most low-cost process. It should be noted that the extraction time in MW and US techniques, is not an independent variable, since the US and MW intensities affect the yield. The aforementioned intensities are the main parameters regarding the efficiency of the process (Cheng et al., 2011). The increase of ultrasound intensity increases the extraction efficiency and thereby reduces the extraction time. Nevertheless, it has been observed that after a certain intensity value the yield remains constant or slightly decreases (Cheng et al., 2011). Similarly, it would be expected that the increase of microwave intensity increases the yield. This happens until a threshold is reached. Over this value, phenomena of degradation of the desirable components of the extract occur, reducing the yield (Hong-yu et al.); (Cheng et al., 2011). At very high values of MW intensity, a significantly lower yield is observed compared to the US method (Monica et al., 2010). However, as already mentioned, the increase of the intensity in both techniques, as well as in their combination, increases the efficiency of extraction and reduces the time (Lou et al., 2010). Therefore, the choice of optimum conditions depends on a set of criteria.

Table 2 shows the optimum operating conditions of the US and MW extraction device for the selected plants.

Table 2. Optimum MW-US extraction conditions for each plant

Plants	Parameters		
	MW intensity (W)	US intensity (W)	Time (min)
Rosemary (Rosmarinus officinalis)	500	700	8
St. John's wort (Hypericum perforatum)	200	450	8
Chamomile (Matricaria recutita)	500	700	8

The evaluation of the quality of the extracts, before and after the optimization of the extraction process, was achieved by the quantification of their bioactive compounds, determined by the values of their total phenolic content (TPC), and radical scavenger activity (IC50). The results of the extracts quality evaluation are absolutely linked to their potential biological activity, confirming the literature (Papageorgiou and Vogiatzakis, 2006) on the high content of bioactive compounds contained in several herbs of the Mediterranean flora.

The extracts prepared by the combination of the optimum operating conditions of the US and MW extraction device for the selected plants, were characterized according to the aforementioned properties and presented in Table 3.

Table 3. DPPH and TPC values of the bioactive extracts

Plants	DPPH – IC50 (mg extract/g raw material)	TPC (mg GAE/g raw material)
Rosemary (Rosmarinus officinalis)	49.6703	2.9862
St. John's wort (Hypericum perforatum)	31.0580	3.7291
Chamomile (Matricaria recutita)	47.1187	3.0518

4. Conclusions

Soxhlet conventional extraction was performed in order to calculate the maximum efficiency yield, and TP values for each extract, since it is the most exhaustive technique. Nevertheless, comparing the yields, if no significant differences were observed, the larger particle size was selected; due to easier processing and less energy requirements for its production. 500 μm was found as the optimum particle size for rosemary, while for both St. John's wort and chamomile was 1000 μm. The increase of extraction time, leads to an increase of efficiency until a maximum. The US intensity increased the TPs of the extract and reduced the extraction time. However, high intensity induces the energy cost, which needs to be taken into account, thereby lower intensities with satisfactory yields observed are preferred. Hence, the optimum US intensity value for rosemary and chamomile was 700 W, and for St. John's wort 450 W. The MW intensity generally increases the extraction efficiency, and reduces experiment time. Yet, at a MW intensity of 500 W, a reduction in TPs was observed for all plants tested. The value of 200 W for MW intensity was selected as optimum for St. John's wort, and 500 W for the rest examined plants.

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