

Techniques for the Extraction of Bioactive Compounds from Lebanese *Urtica dioica*

Hanan Bandar, Akram Hijazi*, Hassan Rammal*, Ali Hachem, Zeinab Saad, Bassam Badran.

Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE), Lebanese University, Lebanon

Address for Correspondence

Doctoral School of Science and Technology, Research Platform for Environmental Science (PRASE), Lebanese University, Lebanon.

E-mail: hijazi_akram@hotmail.com

ABSTRACT

The use of bioactive compounds in different commercial sectors such as pharmaceutical, food and chemical industries assures the need of the most appropriate and standard method to extract these active components from plant materials. In the present study, conventional methods and numerous new methods (maceration, reflux, soxhlet, hydrodistillation, Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE)) using different solvents have been developed for the extraction of bioactive compounds from *Urtica dioica* grown in Lebanon. Our results revealed that the extraction method, solvent and time had a significant effect on the amount of the extracted compounds. In terms of extraction method applied, microwave-assisted extraction was the more effective technique compared to the other methods. The extraction time was reduced, less solvent was used and the amount of extracted compounds was increased.

Keywords: *Urtica dioica*, bioactive compounds, Extraction, Microwave-Assisted Extraction.

INTRODUCTION

Bioactive compounds of plants are produced as secondary metabolites¹. Every living body, from one cell bacterium to million cell plants, processes diverse chemical compounds for their survival and subsistence. Secondary metabolites, which are a group of compounds other than primary metabolites believed to help plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings². The production of secondary metabolites in

different species is mainly selected through the course of evaluation and the particular need of that species. Humans use secondary metabolites as medicines, flavorings and recreational drugs³. The importance of the antioxidant properties of some of these bioactive compounds and their possible uses in processed foods as a natural antioxidant have reached a new high in recent years.

Urtica dioica is a herbaceous perennial flowering plant native to Europe, Asia, northern Africa, and North America,

and is the best-known member of the nettle genus *Urtica*. The plant has a long history of use as a medicine and as a food source⁴. Conventional extraction is usually performed using reflux, cold maceration, soxhlet and simple distillation techniques. These methods which have been used for many decades are very time consuming and require relatively large quantities of solvents⁵. Extraction using non-conventional methods (microwave assisted extraction and ultrasound assisted extraction) can result in a yield increase in shorter time using less solvent⁶.

This study aimed to provide a comparison between different techniques used for the extraction of bioactive compounds from the Lebanese *Urtica dioica* using different solvents.

MATERIALS AND METHODS

Plant Preparation

Fresh plants were gathered from South Lebanon on spring season between March and June in 2012. Then, plants were well cleaned and washed with water and then dried in the shade and at room temperature. After this period, leaves and stems of the plant have been grinded and transformed to powder by a grinder. The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

Maceration method

1g of powdered leaves and stems of *U. dioica* were blended with 50 ml of different solvents (hexane, dichloromethane, acetone, ethanol and water) for different periods (14, 24 and 48 h) with agitation at room temperature. After, the extracts were taken and filtered by using a 0.45 millipore filter paper. Then, the extracts were concentrated using a rotary evaporator at 40°C under reduced pressure. Finally, the extracts were weighted and stored at -20°C till their usage in the different tests.

Reflux method

The extraction method used for dried samples had as follows: 50 ml of each solvent (hexane, dichloromethane, acetone, ethanol and water) were added to 1 g of dried sample in a round bottom flask. The mixture was stirred carefully for different period (14, 24 and 48 h). The extraction mixture was then refluxed for 6-8 h. Each extraction was repeated three times for both methods with all solvents. All this work was carried out in the dark (flasks were covered with aluminum foil). After that, the extracts were filtered by Buchner funnel under reduced pressure and they have been taken, measured and used for several phytochemical screening.

Soxhlet method

1 g of leaves and stems was extracted in 50 ml solvent (hexane, dichloromethane, acetone, ethanol and water) by soxhlet extraction technique for 2, 14 and 24 hours. The extracts were filtered and the filtrate was evaporated under reduced pressure yielding crude⁷.

Ultrasound Assisted Extraction (UAE) method

1 g of powdered stems and leaves of *U. dioica* was loaded into a 100 ml flask and 50 ml solvent (hexane, dichloromethane, acetone, ethanol and water) were added. UAE was performed at 400 W, at 35, 50 and 60°C for 10, 25 and 60 min.

Microwave Assisted Extraction (MAE) method

A domestic microwave oven (KOG-3767, DAEWOO), used in this study, had a total capacity of 850 W⁸. Plant samples (0.5 g) were mixed with the same solvents (25 ml) as in the other methods in flat bottom, threaded round bottom top PFA vials. Each vial was inserted alone to the microwave oven into a PFA beaker. The resulting mixtures were irradiated with microwaves (750W

power) according to the method of Pan *et al.*⁹ with some modifications to achieve 2, 4 and 6 min of irradiation: 45 sec power on followed by 30 sec power off and then by 15 sec power on. After each irradiation of 60 sec, the sample is allowed to cool at room temperature. Before measuring the extracts mass, the samples were filtered quickly through a 0.45µm membrane filter and concentrated using a rotary evaporator at 40°C under reduced pressure¹⁰.

RESULTS AND DISCUSSION

Conventional extraction techniques

Maceration extraction

The mass of the extracted compounds from 1g of *U. dioica* leaves and stems with solvents including hexane, dichloromethane, acetone, ethanol and water were compared in 14, 24 and 48 hours period in order to optimize the extraction conditions. The mass of the extracted compounds (in mg) using maceration method is summarized in Table 1.

Effect of solvent type

The non-polar solvent hexane extracted the highest yield (20 mg) at 24 h. Dichloromethane gave the highest extraction yield at 48h (44.6 mg). Based on the information given in Table 1, acetone demonstrated the highest yield at 48 h (27.1 mg). As shown in Table 1, ethanol was the most efficient organic solvent; it gives the highest extraction yield at 48 h (49.1 mg). For the water which is the most polar solvent, the highest yield (169.6 mg) is obtained at 48h. Because highly-polar solvents (e.g. water) and non polar ones (e.g hexane) are not appropriate for extracting a high polar content. Moreover, the use of water as the only solvent yields to an extract with a high content of impurities (e.g. organic acids, sugars, soluble proteins) along with polar compounds which could interfere in the identification and quantification. On the other

hand, the absolute alcoholic solvents decrease the extraction yield. So, application of water combined with other organic solvents makes it a moderately polar medium ensuring the optimal conditions for extraction. Besides, using water in combination with alcohols leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent finally improves the extraction yield¹¹. Acetone could not be a suitable solvent in extracting polar compounds like phenols due to its nonpolar entity, and based on what mentioned above, it is understood that methanol and ethanol extracts contain higher polar compounds than water. Many studies have confirmed that also in other plant species polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones¹².

Effect of extraction time

As seen in Table 1, there was a certain correlation between increasing of time and yield extraction. By which as time increases (from 14 h till 48 h) the extraction product with different solvents (acetone, dichloromethane and ethanol) increases, but with hexane it increases from 14 h till 24 h then it remains constant. With respect for the extraction with water as time increases, extraction product decreases from 14h till 48 h.

We can conclude that the optimal extraction time depended on solvent type. This observation was well explained by Fick's second law of diffusion, the final equilibrium will be achieved between the solute concentrations in the plant matrix and in the bulk solution (solvent) after a certain time meaning that an excessive extraction time is not useful to extract more compounds and prolonged extraction process might lead to oxidation due to light or oxygen exposure¹³.

Soxhlet extraction

In soxhlet as maceration, the solvent type and the extraction time have an effect on the extraction. The mass of the extracted compounds (in mg) using soxhlet method is summarized in Table 2.

Effect of solvent type

As shown in Table 2, the non-polar solvent hexane extracted the highest yield (31.4mg) at 48 h. Dichloromethane gave the highest extraction yield at 24h (37mg). In Table 2, acetone demonstrated the highest yield at 14 h (82.4mg). Ethanol was the most efficient organic solvent; it gives the highest extraction yield at 24 h (116.8mg). For water which is the most polar solvent, the highest yield (461.8mg) at 24h.

Effect of extraction time

As seen in Table 2, there was certain correlation between the increasing of time and the yield extraction. By which as time increases (from 2 h till 24 h) the extraction products with different solvents (dichloromethane, water, hexane and ethanol) increases, but with acetone the extraction yield increases as time increases from 2 h till 14 h then from 14 h till 24 h it remains nearly constant. These results were well explained by Fick's second law of diffusion¹³.

Reflux extraction

In reflux as the above mentioned methods, the solvent type and the extraction time have an effect on the extraction. The mass of the extracts using reflux method is summarized in Table 3.

Effect of solvent type

In the present study, *U. dioica* stems and leaves were extracted in hexane, dichloromethane, acetone, ethanol and water using hot extraction (reflux). 1 g of *U. dioica* stems and leaves in 50 ml solvent yield 34.9 mg at 24h, 58 mg at 24h, 45.7mg at 48h,

68.7mg at 24h and 388.4mg at 24h, respectively in the used solvents as seen in Table 3.

Effect of extraction time

Table 3 shows certain correlation between increasing of time and yield extraction. As time increases (from 14h till 24h) the extraction product with different solvents (dichloromethane, water, hexane and ethanol) increases, but with acetone the extraction yield increases as time increases from 14h till 48h. The final equilibrium will be achieved between the solute concentrations in the plant matrix and in the bulk solution after a certain time meaning that an excessive extraction time is not useful to extract more compounds since most organic chemicals are quite volatile, and if heated they will evaporate and be lost.

Non-conventional extraction techniques

Ultrasound assisted extraction

In UAE as in the conventional techniques, the solvent type and the extraction time have an effect on the extraction. The mass of the extracted compounds using ultrasound method is summarized in Table 4.

Effect of solvent type

As shown in Table 4, the non-polar solvent (hexane) extracted the highest yield (34.9mg) at 60 min. Dichloromethane gave the most extraction yield at 60 min (41.4 mg). Based on the results given in Table 4, acetone demonstrated the highest yield at 60 min (30.4 mg). Ethanol was the most efficient organic solvent; it gives the highest extraction yield at 60 min (58mg). The polar solvent water extracts the highest yield at 60 min (402mg).

Effect of extraction time

Table 4 shows certain correlation between increasing of time and yield

extraction. When time increases (from 10 min till 60 min) the extracted products with different solvents (hexane, dichloromethane, acetone, ethanol and water) increases.

Microwave extraction

As in the two latest methods, in microwave the solvent type and the extraction time have an effect on the extraction. The mass of the extracts using microwave method is summarized in Table 5. The most content was optimally obtained from ethanol after 2 min (100mg). With acetone, dichloromethane, water and hexane as extracting solvents, the highest extraction yield was detected at 6 min (54.2mg), 6 min (54.6 mg), 6 min (520mg) and 6 min (37.4mg) respectively. Hence, as for extraction, 2 min extraction with ethanol was deemed to be the optimal extraction time. In case of using acetone, water, dichloromethane and hexane, this would be recommended to be chosen at 6 min.

Effect of solvent type

Non-polar solvents remain transparent to microwave due to their lower dielectric constant and dissipation factor in comparison to the polar solvents, thus producing no heat under microwave and are of no efficiency in extraction with MAE¹⁴. That is why in case of using acetone, the amount of extract was low. Among the polar solvents, water undergoes greater microwave absorption and efficiently converts it into heat due to its high dielectric constant. Ethanol has lower values than water. In comparison to water, ethanol is preferred due to its greater capability in solving the bioactive compounds and higher heating efficiency¹⁵.

Effect of extraction time

As seen in Table 5, there was certain relation between increasing of time and yield extraction. As time increases from 2 min till 6 min the extracted products with different solvents (hexane, dichloromethane, acetone

and water) increases, but with ethanol the extraction yield remains constant (100 mg) as time increases from 2 min till 6 min. The longer exposures caused higher values of extraction yield, whereas further increase in irradiation time not only resulted in no improvement in the extraction performance, but sometimes led to a fall in the concentration yield. These prolonged exposures always involve the risk of degradation by heating¹⁶.

CONCLUSION

In terms of the organic solvent applied, ethanol was the most effective one, producing the highest extraction yield and hexane gave the lowest yield in extracting bioactive compounds by these methods. Furthermore, there was an increase in the yield of extracted compounds with increasing extraction time. Within conventional methods, soxhlet method had the highest extraction yield and the maceration method had the lowest one. Non-Conventional extraction techniques gave high extracted product within few minutes while using conventional methods several hours are needed to obtain high extraction yield. As a result the UAE and MAE are the most effective techniques. Hence, the microwave assisted method has many advantages compared with other methods due to its reduced extraction time, higher extraction efficiency, less labor and high extraction selectivity which makes it a favorable method in extraction of bioactive compounds from *Urtica dioica* leaves and stems.

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Table 1. Total extracts produced by cold maceration extraction technique

Solvent Time (h)	Hexane	Dichloromethane	Acetone	Ethanol	Water
14	19 ± 0.36	42 ± 1.401	22.2 ± 0.53	32.1 ± 1.05	223 ± 1.361
24	20 ± 0.577	41.9 ± 0.665	22.3 ± 1.011	39 ± 1.058	194.7 ± 0.793
48	20 ± 1.040	44.6 ± 0.81	27.1 ± 1	49.1 ± 0.435	169.6 ± 0.702

Table 2. Total extracts produced by soxhlet extraction technique

Solvent Time (h)	Hexane	Dichloromethane	Acetone	Ethanol	Water
2	17.3 ± 0.208	34.6 ± 1.628	68.8 ± 0.53	84 ± 2.15	253 ± 2.358
14	26.6 ± 0.305	35 ± 2.753	82.4 ± 1.285	84 ± 1.26	252.6 ± 1.193
24	31.4 ± 0.721	37 ± 1.154	80 ± 2.516	116.8 ± 2.25	461.8 ± 1.6

Table 3. Total extracts produced by reflux extraction technique

Solvent Time (h)	Hexane	Dichloromethane	Acetone	Ethanol	Water
14	25.6 ± 0.3	24.2 ± 0.642	25.8 ± 1	63.2 ± 1.792	273.4 ± 3
24	34.9 ± 1	58 ± 1.014	22.3 ± 1.014	68.7 ± 2.227	388.4 ± 2.247
48	34.5 ± 0.5	56.7 ± 1.014	27.1 ± 0.36	68 ± 0.53	238.7 ± 0.602

Table 4. Total extracts produced by ultrasound assisted extraction technique

Solvent Time (min)	Hexane	Dichloromethane	Acetone	Ethanol	Water
10	20 ± 0.115	35.5 ± 0.763	25 ± 0.53	28.4 ± 0.305	257.5 ± 4.481
25	26 ± 0.321	20.4 ± 0.503	26.5 ± 1.322	41.3 ± 0.7	313 ± 3.214
60	34.9 ± 0.351	41.4 ± 1.026	30.4 ± 0.984	58 ± 0.2	402 ± 2

Table 5. Total extracts produced by microwave assisted extraction technique

Solvent Time (min)	Hexane	Dichloromethane	Acetone	Ethanol	Water
2	20.8 ± 0.461	51 ± 2.645	30 ± 1.527	100 ± 1.058	281.4 ± 3.304
4	20 ± 0.577	50 ± 1.311	31.2 ± 0.642	100 ± 0.6	512 ± 5.892
6	37.4 ± 0.503	54.6 ± 0.611	54.2 ± 0.602	100 ± 19.424	520 ± 2.683