Comparison of Total Phenolic Content, Antioxidant Activity and Extraction Yield from Apium Graveolens Waste Using Unconventional Extraction Methods

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Abstract. Apium graveolens is a medicinal and aromatic plant used in pharmaceutical industry, cosmetic industry, gastronomy and traditional medicine. Aerial parts of Apium graveolens (stems and leaves) are rich in bioactive compounds (flavonoids, phenols, furanocoumarins, volatile oils, sesquiterpene alcohols, fatty acids, etc.). It is known for its hepatoprotective, antioxidant, anticancer, antidiabetic, anti-inflammatory, antimicrobial, analgesic, anti-spasmylytic, anti-infertility, cardiotonic activities. This is why studies in this direction have not yet ceased and are increasingly pursuing the discovery of new, unconventional methods for identifying, quantifying and isolating bioactive compounds from Apium graveolens waste. The aim of this study was to determine and correlate the total phenolics, total flavonoids, antioxidant activity and extraction yield from ethanolic extracts (70%) of stems and leaves waste with different unconventional extraction methods. Apium graveolens aerial parts waste were obtained after aqueous extraction of the leaves and stems. The extraction yield was expressed as function of freeze-dried extracts, and the extraction methods were ultrasound assisted extraction, microwave assisted extraction and accelerated solvent extraction. Regarding the chosen extraction methods, numerous studies have shown, especially in the case of microwave assisted extraction, the presence of bioactive compounds in plant waste, in a reasonable percentage, therefore this study aimed to identify an optimal extraction method also in the case of Apium graveolens aerial parts waste. Total phenols content was determined by Folin-Ciocalteu spectrophotometric method, the flavonoids content waste determined by aluminium chloride spectrophotometric method and the antioxidant activity was studied using DPPH (2,2-Diphenyl-1-picrylhydrazyl) spectrophotometric method. The findings of this work indicated a positive dependence between the extraction yield of the waste material and the considerable content of bioactive compounds still present (phenols and flavonoids) for all considered extraction methods.

1. Introduction

Plants represent a rich source of various bioactive compounds with different biological properties. Thus, plants are still present in the researchers’ studies in order to improve the recovery methods of active compounds. Using different extraction methods, identification and purification methods in the...
quantitative and qualitative analysis, many targeted studies reported important results, therefore in the last time the interest for studies regarding the valorization of plants wastes has also increased.

Apium graveolens L., celery, is a medicinal and aromatic plant with many properties and many applications in traditional medicine, gastronomy, but also in pharmacology. Apium graveolens L. belongs to Apiaceae family, to the genus Apium, originally from the Mediterranean area, and is spread all over Romania territory. From a medicinal point of view, the leaves, the root and the seeds of the plant are used.

Celery is an excellent source of vitamins, phenolic compounds, volatile oil and other nutrients. The leaves contain essential oil having as major components: limonene, myrcene, 1,2 ethanediol, γ-terpinene, 2-(2-propenyl), Z-β-ocimene, trans-β-guaiene, 2,5 pyrrolidinedione, 1-phenyl, 6-butyl-1,4-cycloheptadiene, furan [1], phenolic compounds (gallic acid, vanillic acid, chlorogenic acid, caffeic acid, rutin, epicatechin, quercetin, myricetin), ascorbic acid, carotens and minerals. Celery also contains furanocoumarins, including xanthotoxin, bergapten and 5-methoxypsoralen, which are chemicals that react to sunlight [2].

Extracts or the essential oils obtained from the aerial parts of Apium graveolens have been investigated in animal or cellular experimental settings for a variety of benefits and pharmacological activities such as: antioxidant, antimicrobial, hepatoprotective, anti-spasmolytic, hypocholesterolemic activities or anxiolytic potential, etc. [3-8].

The aim of this study was to determine the total phenolics content, total flavonoids content, antioxidant activity and extraction yield from Apium graveolens aerial parts, as leaves and stems waste. The extraction of the bioactive compounds from waste plant material was an important step, therefore we adopted three extraction methods as: microwave-assisted extraction, ultrasound-assisted extraction and accelerated solvent extraction. These three extraction technologies were chosen due to the fact that it has been demonstrated by many studies as being appropriate in obtaining useful compounds from plant material.

2. Material and Methods

The plant material consisted of dried Apium graveolens leaves and stems, unprocessed and waste. The waste material was obtained by two aqueous extraction of Apium graveolens leaves and stems, at 1000C, for 3 hours the first one, and 1.5 hours the second one.

The plant material (unprocessed and waste) was powdered (Figure 1) and was extracted by three different extraction methods: microwave-assisted extraction and ultrasound-assisted extraction with 70% bioethanol (1:10), and accelerated solvent extraction. Microwave-assisted extraction (MAE) was performed using Ethos Easy Milestone equipment, at 1000C, for 55 min, pulsatory microwaves. Ultrasound-assisted extraction (UAE) was performed in an ultrasound bath for 30 min, followed by oven for 3 hours at 680C. Accelerated solvent extraction (ASE) was performed using Dionex ASE 350, all extractions were performed in 22 ml extraction cells, containing 7 g of sample. The extraction conditions are presented in Table 1. All the experiments were carried out in triplicate.

| Table 1. ASE Extraction conditions. |
| Extraction Solvent | Bio-ethanol 70% |
|---------------------|-----------------|
| Temperature         | 100°C           |
| Pressure            | 1500 psi        |
| Heat-up Time        | 5 min           |
| Static Time         | 10 min          |
| Flush Volume        | 100%            |
| Purge Time          | 90 s            |
| Static Cycles       | 3               |
Regarding extraction yield determination, the solvents from the extracts were removed in vacuo and freeze-dried for 45 hours, at -550°C using Lyo Cube 4-8 Alpha 2-4 LSC plus Christ Freeze Dryer and the extraction yield was expressed as function of freeze-dried extracts weight.

Determination of total phenolics content (TPC) from unprocessed and waste Apium graveolens leaves and stems extracts was performed using Folin-Ciocalteu method adapted after [9]. The spectrophotometric method was used to measure the absorbance of samples at 765 nm, using UV-VIS Spectrophotometer Specord 210 Plus, Analitik Jena. The total phenolics content was expressed as mg of gallic acid equivalent by using the equation obtained from gallic acid calibration curve. The obtained concentration of phenolics content was averaged from triplicate samples and the samples were diluted in a ratio 1:100.

Determination of total flavonoids content (TFC) from unprocessed and waste Apium graveolens plant material extracts was carried out by aluminium chloride method as described by [9], using 0.5 ml of sample. The absorbance was measured at 415 nm with the UV-VIS spectrophotometer. The total flavonoids content was expressed as mg of rutin equivalent by using the equation obtained from rutin calibration curve. The obtained concentration of flavonoids content was averaged from the triplicate samples and the samples were diluted with a dilution factor of 100.

Determination of antioxidant activity using α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging of the extracts was performed according to [10]. Over time, the determination of antioxidant activity by DPPH assay was often applied for various plants extracts [11], therefore it was considered a traditional method. The absorbance of the samples was read at 517 nm on the UV-VIS spectrophotometer. The determinations were carried out in triplicate and the results were averaged. The antioxidant activity was calculated using the formula:

$$ AA = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} * 100 $$

Where: $AA$ – antioxidant activity, $A_{DPPH}$ - the absorbance of the DPPH solution without sample, $A_{sample}$ - the absorbance of the sample (which contain the mixture of extract and DPPH solution).

The extraction yield was determined and the highest percentage in the case of aerial parts waste was found in the extracts obtained by UAE. The lowest extraction yield of the waste material was found in the extracts obtained by ASE method. The extraction yield values are presented in Table 2. As it can be observed from the table, extraction yields varied from 58.84 to 80.45 % from the dried unprocessed leaves and stems and from 54.41 to 65.16% from the waste leaves and stems. As the results indicated, there were significant differences between the yield obtained from waste material and from unprocessed material, by MAE method (about 17.5%), and by UAE method (about 9.8%), but there was not a substantial difference in case of ASE method (about 4.4%).
Table 2. The extraction yield of *Apium graveolens* aerial parts (leaves and stem).

| Extraction method                      | Extraction Yield (%) |
|----------------------------------------|----------------------|
| Microwave assisted extraction          |                      |
| Unprocessed plant material             | 80.45                |
| Waste plant material                   | 62.92                |
| Ultrasounds assisted extraction        |                      |
| Unprocessed plant material             | 75.04                |
| Waste plant material                   | 65.16                |
| Accelerated solvent extraction         |                      |
| Unprocessed plant material             | 58.84                |
| Waste plant material                   | 54.41                |

The total phenolics content present in the extracts from aerial parts of *Apium graveolens* unprocessed and waste was obtained by Folin-Ciocalteu method and their concentrations were expressed as equivalents of gallic acid, calculated as [12] described, adapted and multiplied with the dilution factor of 100. The resulted concentrations are presented in Table 3. The values indicate that in case of waste plant material, the highest total phenolics content was obtained using ASE method, followed by UAE method and MAE method.

Table 3. Total phenolics content and total flavonoids content of *Apium graveolens* aerial parts (leaves and stem).

| Plant type/ Extraction method                      | Total phenolics content (mgGAE/g DW) | Total flavonoids content (mgRUE/g DW) |
|---------------------------------------------------|-------------------------------------|--------------------------------------|
| Apium graveolens unprocessed / Microwave assisted extraction | 11.34                               | 11.71                                |
| Apium graveolens waste / Microwave assisted extraction | 9.47                                | 10.37                                |
| Apium graveolens unprocessed / Ultrasounds assisted extraction | 14.83                               | 23.57                                |
| Apium graveolens waste / Ultrasounds assisted extraction | 10.80                               | 11.94                                |
| Apium graveolens unprocessed / Accelerate solvent extraction | 12.46                               | 17.21                                |
| Apium graveolens waste / Accelerate solvent extraction | 11.38                               | 12.01                                |

The analytical data of the total flavonoids content of the extracts from unprocessed and waste celery aerial parts material is presented in Table 3. The concentrations were calculated as [12] described, adapted and multiplied with the dilution factor of 100, and were expressed in rutin as mg RUE/g. The obtained results varied, and the greatest total flavonoids content for the waste material was obtained using ASE method. Hence, it was observed that in this case, for both unprocessed and waste material, the ASE method offered a better total content, but even the others extraction methods offered good results, in the same range.

Due to the fact that majority of plants contain important contents of phenols and flavonoids, the scientists studied and reported the wide spectra of biochemical and pharmacological effects of these natural compounds. The concentrations of TFC and TPC obtained in this study could be compared with other similar studies results [13-18] present in literature.

More, as the results indicated, the hydroalcoholics extracts of *Apium graveolens* waste material contained considerable values of TFC and TPC. There are no results in the scientific literature until now regarding the quantitative determination of TFC and TPC from *Apium graveolens* waste material, up to our knowledge.
A substantial difference was found between TFC in waste and unprocessed plant material extracts, revealed by UAE method (23.57 mgGAE/g vs. 11.94 mgGAE/g), at close TPC values (14.83 mgGAE/g vs. 10.80 mgGAE/g). Regarding the TPC, there were no significant differences between the unprocessed plant material extracts (less than 3.5 mgGAE/g), or between the waste material extracts (less than 2 mgGAE/g). But, the variation of TPC and TFC in the extracts depend on many factors, such as extraction methods, drying process, cultivation, growth and harvesting process of the plant, soil nature, the preserving process, etc, as is well known.

As expected, the results revealed a high determination coefficient \( y = 0.0106x - 0.0657, R^2 = 0.9971 \) in the case of TPC determination, but also in the case of TFC \( y = 0.0027x - 0.0035, R^2 = 0.9993 \).

The free radical-scavenging activity of the Apium graveolens aerial parts extracts was performed through DPPH method and the results are presented in Figure 2. The obtained extracts were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The highest value of the antioxidant activity percentage from the waste material was found in the case of MAE method. The high antioxidant activities of the extracts could explain the values of the total phenolics content.

The MAE of antioxidants from Apium graveolens L. dried aerial parts was demonstrated to be effective, fast and practical, for unprocessed and waste material.

The results from the analysis of aerial parts of Apium graveolens waste showed important content of total phenols and flavonoids and good antioxidant activity, facts that confirmed the importance and the abundant presence of celery in the Romanian diet and cuisine.

3. Conclusions
In the present study, the decreasing values of antioxidant activity among the Apium graveolens aerial parts waste extracts assayed through all the described methods were found to be MAE > UAE > ASE. The opposite order was identified for the total phenolics contents and the total flavonoids content of the aerial parts waste extracts.

This study emphasizes that the waste material of Apium graveolens still contains bioactive compounds as phenols and flavonoids, making it a valuable and valorization-worthy source nowadays. More, it was found a positive dependence between the extraction yield of the waste material and the considerable content of bioactive compounds still present (phenols and flavonoids) for all considered extraction methods.
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