Total Phenolic Content and Radical Scavenging Potential of Celery Root and Celeriac Stalk and Leaf Extracts

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In the study, celery root and celeriac stalk and leaf extracts were prepared using heat-assisted extraction, and the total polyphenol concentration (TPC) and anti-DPPH radical potential of the obtained extracts were determined. The TPC values of the extracts were from 2.67 to 13.43 mg gallic acid equivalent/g, following the trend: ethanol celeriac leaf sample>water celeriac leaf sample>ethanol celeriac stalk sample>water celery root sample. Ethanol celery root and water celeriac stalk samples showed significantly lower TPC. The anti-DPPH activity follows the trend: ethanol celeriac leaf sample>water celeriac leaf sample>water celery root sample>water and ethanol celeriac stalk samples>ethanol celery root sample. The IC_{50} values (the concentration of the sample necessary to neutralize 50% of free radicals) varied in a range of 63.9 to 326.4 mg/mL. Both phenolic yield and antioxidant activity achieved the highest levels in the ethanol celeriac leaf sample.

Keywords: celeriac leaf, celeriac stalk, celery root, extraction, polyphenols, radical scavenging activity

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1. INTRODUCTION

Celery (*Apium graveolens*), as an edible herb, was used in traditional therapy for cardiovascular diseases, high blood glucose levels, and hypertension, and as an antifungal, anti-inflammatory, anticoagulant, antioxidant, and antitumor agent (Al-Asmari et al., 2017; Tyagi et al., 2013)[1,2]. Various parts of celery are also employed in hepatic, spleen, and brain diseases, pain, and sleep problems (Al-Asmari et al., 2017). The constituents of celery include glycosides, steroids, polyphenols (furanocoumarins and flavones), as well as trace elements (sodium, potassium, calcium, and iron) (Hussain et al., 2013; Tyagi et al., 2013) Celeriac (*Apium graveolens* var. *rapaceum*) shows anticancer properties due to the presence of flavonoids, volatile oil, vitamins, and minerals in the root, stalk, and leaf(Turner et al., 2021). In addition, celeriac is a rich source of phthalides with health benefits on the central nervous system and cardiac performance, including anti-thrombotic modulation and protection against cerebral ischemia and high blood pressure (Lin et al., 2005; Turner et al., 2021).

Heat-assisted extraction represents a novel procedure for extracting different biologically active components from various herbal materials. Namely, the employment of thermal energy enhances the efficiency of the extraction by cell disruption, increasing membrane permeability, and breakdown interactions between polyphenols and other compounds, such as lipids or proteins (Jovanovic et al., 2017; Mustafa and Turner, 2011). Additionally, high temperature causes a decrease in the viscosity of the extraction solvent, allowing better penetration of the extraction medium into the plant material (Jovanovic et al., 2017; Miron et al., 2011). In comparison to traditional extraction protocols, heat-assisted extraction provides faster kinetics reducing time and energy costs (Jovanovic et al., 2017, 2017). In modern times, novel extraction procedures (ultrasound-, microwave, and enzyme-assisted extractions, sub- and supercritical extractions, extraction employing natural deep eutectic solvents, etc.) can be used for the extraction of phytoconstituents from celery root and celeriac stalk and leaf (Rahaman et al., 2023). However, the mentioned techniques require expensive devices and reagents or time-consuming pre-treatment. Since the celery root and celeriac stalk and leaf are dominantly edible plants with medicinal properties and are used in culinary, the focus of the present research was on the extraction method that can be performed in ordinary conditions without complex apparatus. Namely, heat-assisted extraction, although a traditional extraction technique, can provide a satisfactory extraction yield by using simple and one-step operational protocol. The presented manuscript also follows

world trends in the field of green chemistry, due to the employed extraction mediums (water and ethanol). Namely, according to the literature, ethanol, water or hydroethanolic mixtures are GRAS (Generally Recognized As Safe) solvents (Gil-Martín et al., 2022). In addition, celeriac stalks used for the extract preparation represent agro-food waste. Also, according to Rupérez and Toledano study (2003), both stalks alone and stalks with leaves represent two celery residues from the food industry.

Therefore, in the present study, heat-assisted extraction from *A. graveolens* root and *A. graveolens* var. *rapaceum* stalk and leaf was performed and the polyphenolic yield and DPPH radical neutralization potential of the prepared extracts were investigated.

2. MATERIALS AND METHODS

2.1. Extraction protocol

Celery root and celeriac stalks and leaves, purchased in the local market (Belgrade, Serbia), were washed and wiped off to remove excess water and subsequently cut and shredded using a mixer grinder. Fresh plant material was used for the extraction. Heat-assisted extraction from fresh and shredded celery root and celeriac stalks and leaves was performed at 60°C in the incubator shaker (IKA, Germany) and water or 30% ethanol (Fisher Science, United Kingdom), at a ratio of 1:20 g/mL (1 g of plant material and 20 mL of the extraction solvent), during 20 min. The samples were filtered and stored at 4°C before further analytical experiments.

2.2. Total polyphenol content

The total polyphenol concentration (TPC) was measured in a modified Folin-Ciocalteu method(Sari et al., 2023). The absorbance of the mixture (extract, water, Folin-Ciocalteu reagent, and sodium carbonate) was read at 765 nm against a blank (UV-VIS Spectrophotometer UV-1900i, Shimadzu, Japan) after incubation of 2 h. The data was expressed as mg of gallic acid equivalents per g of fresh herbal material (mg GAE/g). Sodium carbonate was from Fisher Science (United Kingdom), while Folin-Ciocalteu reagent and gallic acid were from Merck (Germany).

2.3. DPPH radical neutralization capacity

The antioxidant activity of the prepared extracts was examined in the DPPH test [13], and 2,2-diphenyl-1-picrylhydrazyl – DPPH was from Sigma-Aldrich (Germany). In the DPPH test, 200 μL of the extract and 1800 μL of ethanol DPPH$^{\bullet}$ radical solution was mixed. The absorbance was measured at 517 nm after the incubation of 20 min, and the data are presented as IC_{50}, the concentration of the sample necessary to neutralize 50% of DPPH radicals.

2.4. Statistical data processing

Statistical data processing was done in STATISTICA 7.0 software (one-way ANOVA and Duncan's *post hoc* test). The statistically significant differences were at $p<0.05$, n=3.

3. RESULTS AND DISCUSSION

The TPC and DPPH radical neutralization potential of celery root and celeriac stalk and leaf extracts were examined. The data are shown in Table 1. In the preliminary study, ultrasound-assisted extraction and different extraction buffers were employed but the polyphenol content and antioxidant capacity of the extracts were significantly lower in comparison to the extracts obtained using heat-assisted extraction and water-ethanol mixture (data not shown), probably because of the degradation potential of ultrasound waves, as well as production of free radicals by ultrasound.

As can be seen from Table 1, the polyphenol yield was from 2.67 to 13.43 mg GAE/g of fresh plant material, and the trend was as follows: ethanol celeriac leaf extract>water celeriac leaf extract>ethanol celeriac stalk extract>water celery root extract. Ethanol celery root and water celeriac stalk samples showed significantly lower phenolic concentrations. In the case of celery root, water extract showed significantly higher TPC in comparison to ethanol parallel. In contrast, ethanol celeriac extracts had significantly higher values of phenolics compared to aqueous extracts using both plant organs (stalks and leaves). The same trend can be observed for the antioxidant capacity (shown in Table 1). The anti-DPPH potential of the extracts follows the trend: ethanol celeriac leaf extract (73.9 ± 2.6 mg/mL)>water celeriac leaf extract (97.4 ± 2.5 mg/mL)>water celery root extract (161.9 ± 2.9 mg/mL)>water and ethanol celeriac stalk extracts (~210 mg/mL)>ethanol celery root extract (326.4 ± 9.7 mg/mL). Therefore, it can be concluded that antioxidant potential follows the trend of the phenolic content in most cases (higher TPC=higher antioxidant activity, *i.e.*, lower IC_{50} value) and both phenolic yield and antioxidant potential achieved the highest values in ethanol celeriac leaf extract. Septiana et al. (2023) have shown that the application of ethanol as an extraction solvent in celery extracts provided higher TPC and DPPH free radical scavenging potential in comparison to water. Since different extraction mediums gave the best results for different plant materials, every herbal matrix requires investigation of the appropriate extraction solvents for achieving the highest extraction efficiency in terms of TPC and antioxidant capacity (Batinić et al., 2022). Additionally, the differences in the TPC can be due to the use of various parts of the plant matrix in the process. Namely, the concentration of flavonoid compounds, as a large group of phenols with antiox-

Table 1. The total polyphenol concentration (TPC) and DPPH radical neutralization potential of celery and celeriac extracts.

Plant material	Extraction medium	TPC (mg GAE*/g)	IC_{50} DPPH (mg/mL)
Celery root	water	3.94±0.08^{d}	161.9±2.9^{c}
	30% ethanol	2.67±0.07^{e}	326.4±9.7^{e}
Celeriac stalk	water	2.75±0.13^{e}	209.3±2.6^{d}
	30% ethanol	4.66±0.17^{c}	211.5±3.3^{d}
Celeriac leaf	water	12.21±0.41^{b}	97.4±2.5^{b}
	30% ethanol	13.43±0.18^{a}	73.9±2.6^{a}

*GAE, gallic acid equivalent; IC_{50}, the concentration necessary to neutralize 50% of free DPPH radicals; analysis of variance (one-way ANOVA) and Duncan's *post hoc* test (the differences marked as different letters in each column were considered statistically significant at $p<0.05$, n=3).

idant potential, varies between different species, as well as the organ of the used herb (Anokwuru et al., 2011; Batinić et al., 2022). Golubkina et al. study (2020) reported that the TPC was higher in celery leaf and stalk samples in comparison to root parallels. Also, the study that dealt with industrial celery by-products showed that the stalk possessed a higher polyphenol yield and radical scavenging potential than the root (Beltrán Sanahuja et al., 2021). The neutralization of DPPH radicals is possible by the reducing potential of flavonoid compounds (Hirano et al., 2001) presented in the obtained extracts. According to the literature data and HPLC analysis, quercetin glucosides are the main compounds in celery root extracts, while the assay also quantified lower content of other flavonoids, including apigenin and luteolin glucosides, and naringenin (Nikolić et al., 2011). Various studies have shown that catechin, epicatechin, rutin, and quercetin are present in celery leaf extracts (Ashoush et al., 2017; Ingallina et al., 2020). Malonylated and acetylated derivatives of flavonoid compounds were also revealed by HPLC analysis in celeriac products (Kaiser et al., 2013). The fact that some extracts obtained in the present study showed better anti-radical activity despite lower TPC (the absence of a strict correlation between TPC and antioxidant activity) can be explained by the presence of some non-phenolic compounds which can enhance the reactions of neutralization of free radicals (Özyürek et al., 2011).

4. CONCLUSION

The aim of the present study was to compare different extraction mediums (water and water-ethanol mixture) and various plant parts in terms of polyphenol yield and antioxidant capacity. Therefore, celery root and celeriac stalk and leaf extracts were prepared using heat-assisted extraction, and the phenolic content and anti-DPPH radical capacity of the extracts were measured. Different extraction solvents gave the best results for different herbal sources; thus, it can be concluded that every material requires the investigation of the appropriate solvent to achieve the highest phenolic yield and antioxidant potential. The highest phenolic yield and antioxidant activity were obtained for ethanol celeriac leaf extract, therefore further analyses will be aimed to perform the chemical characterization of the above-mentioned sample and its components responsible for the radical scavenging, and other biological properties. Thus, the most prominent extract will be chosen for further experiments which should include a wider spectrum of *in vitro*, *in silico*, or *in vivo* tests related to the biological potential of the extract.

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CONFLICT OF INTEREST

The authors declare that they have no financial or commercial conflict of interest.

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