DOI: 10.46793/SBT28.315DJ

CELERY (*APIUM GRAVEOLENS* L.) AS A SOURCE OF PHYTOCHEMICALS WITH ANTIOXIDANT AND ANTIBACTERIAL EFFECTS

Vesna Đurović[1], Leka Mandić[1], Marija Igrošanac[1], Mirjana Radovanović[1], Marijana Pešaković[2], Jelena Mladenović[1], Dragutin Đukić[1]

Abstract: The aim of this work was to investigate the content of phytochemicals in celery root and its antioxidant and antibacterial potential. The content of total phenols in celery root was 2.99 mg GAE g^{-1}, total flavonoids 1.38 mg RE g^{-1}, total tannins 1.87 mg GAE g^{-1} and vitamin C 12.7 mg 100 g^{-1}. Antioxidant activity measured by the DPPH method and expressed in % inhibition were 42.26 % or 2.38 μmol TE g^{-1}. Antibacterial activity was tested on pure cultures of *Enterococcus faecium, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritidis, E. Coli* and *Serratia marcescens*. *Listeria monocytogenes* showed the highest sensitivity while the *Salmonella enteritidis* and *E. coli* were the most resistant.

Keywords: celery, antioxidant, antibacterial activity

Introduction

Many aromatic plants have been used as vegetable and flavoring ingredients in cooking and in traditional medicine. Celery (*Apium graveolens* L.) is an aromatic plant from the Apiaceae (Umbelliferae) family. The whole plants including leaf, stem, seed and root are used in cooking as soups and salads due to its aroma and essential oil (Aboody, 2021). According to the assessment report of the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency on *Apium graveolens* has been known as a medicine since ancient times in Greece.

Celery is a good source of vitamins A and C, carotenes, tocopherols and secondary metabolites such as flavonoids (including quercetin, apigenin, kaempferol, isorhamnetin and luteolin), alkaloids, terpenoids, tannins, saponin, phenolic acids (caffeic acid, p-coumaric acid, ferulic acid) and volatile compounds (limonene, myrcene, α-pinene, β-selinene) (Kooti et al., 2014; Al-Asmari et al., 2017). Thanks to the presence of these components, celery has a high antioxidant potential

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (djurovicvesna@yahoo.com)

[2]Fruit Research Institute Čačak, Kralja Petra I 9, 32102 Čačak, Serbia

(Kooti and Daraei, 2017) and the ability to remove OH and DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) (Kooti and Daraei, 2017; Emad et al., 2022).

Celery is used to treat cardiovascular diseases, strengthen the heart and lower blood pressure. It has anticoagulant properties. Celery root was used to reduce potassium and increase calcium in cardiac tissues (Khalil et al., 2018). Celery has many pharmacological activities: antimicrobial, antifungal, antiparasitic, anti-inflammatory, antioxidant, antidiabetic, anti-spasmolytic, hepatoprotective, cardioprotective, neuroprotective, cytoprotective and analgesic activity, etc. (Khairullah et al., 2021). The essential oils of celery have anti-bacterial effects on *Streptococcus faecalis, Staphylococcus albus, Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae* and *Streptococcus pyogenes* (Khalil et al., 2018).

The content and chemical composition of celery depend on genetic and environmental factors, as well as applied agrotechnical measures such as fertilization, irrigation, growing method and harvesting method (Powanda et al., 2015).

In this study, celery root was investigated as an aromatic herb in traditional Serbian cuisine, which is known to have strong pharmacological activity and a high content of antioxidants. They were tested for their content of biologically active compounds (the content of ascorbic acid, tannin, phenolic and flavonoid compounds) and their antioxidant and antibacterial activity.

Material and methods

Sample preparation and analysis

The samples (*Apium graveolens* L.) were harvested at full maturity in 2021 from individual producers, who cultivated them in the vicinity of the town Trstenik, Rasina district, Serbia. The sample (roots) was ground and dried. Samples were extracted with 50% ethanol at 25 ℃in an ultrasonic bath for 30 min., and after that 24 h maceration at room temperature. The extracts were filtered and then centrifuged at 5000rpm for 10 min. The clear supernatants were used for the determination of the total phenolic, flavonoids, tannin content and antioxidant activity. For antibacterial activity, the extracts were evaporated in a nitrogen atmosphere under a vacuum, in a water bath at 60 ˚C.

Determination of dry matter content in the samples was performed by gravimetric method at 105̊C , drying to constant mass. The ash content was determined by burning at 550 ˚C for 14 h.

The total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999; Liu et al., 2002). The tannin content in the sample was determined using (PVPP) insoluble polyvinyl-polypirrolidone (Makkar, 2003). The flavonoid content (FC) was determined using Zhishen et al. (1999) method. The antioxidant capacity of the extracts was studied through the evaluation of the free radical-scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical (Brand-Williams et al., 1995). The estimation of ascorbic acid was determined by the titration method (Tillmans' method).

The antibacterial properties of plant extract were tested against Gram-positive bacteria (*Enterococcus faecium* ATCC 6057, *Listeria monocytogenes* ATCC 13932 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *E. coli* WDCM 0013 and *Serratia marcescens* ATCC 43862). The strains used in the tests were sown 24 h before the experiments. After this period, the bacterial inoculum was suspended in saline, corresponding to 0.5 of the McFarland scale, approximately 1.5×10^8 CFU/mL. The minimum inhibitory concentration tests were performed using the microdilution method with resazurin as an indicator (Javadpour et al., 1996). Resazurin is a molecule that serves as a redox indicator. In response to the metabolic activity of living cells, resazurin (blue) is reduced to resorufin, which has a pink color and is fluorescent. The MIC is defined as the lowest concentration at which no growth is observed (Oliveira et al., 2022).

Results and discussion

The content of moisture, dry matter and ash in celery roots was shown in Table 1. It can be especially noted that the celery root has a high content of ash or mineral substances (8.94%). This is in accordance with literature data showing that celery root has a significant content of minerals such as K, P, CA, Se, Ni and others (Qureshi et al., 2014). Researchers state that every part of the plant contains minerals necessary for the human body, with the most of them being in the leaves (Dong and Zhao, 2004).

Table 1. Content of moisture, dry matter and ash in the samples

Sample	Moisture content, %	Dry matter content, %	Ash content, %
Celery root	7.22	92.78	8.94

In this study total phenols, flavonoids, tannins and vitamin C content as well as antioxidant activity were detected in celery roots. The results are shown in Table 2.

Table 2. Content of total phenols, flavonoids, tannins, vitamin C and antioxidant activity

Samples	Total phenolics, mg GAE g^{-1}	Total flavonoids, mg RE g^{-1}	Total tannins, mg GAE g^{-1}	DPPH, % inhibition	DPPH, µmol TE g^{-1}	Vitamin C, (mg/100g)
Celery root	2.99±0.07	1.38±0.17	1.87±0.01	42.26±3.61	2.38±0.24	12.70±0.15

The vitamin C content of celery root was 12.7 mg 100g^{-1}. Similar results for vitamin C are reported by Qureshi et al. (2015). However, the content of vitamin C is significantly higher in the leaves compared to the roots (Meng-Yao et al., 2017). The content of total phenols in celery root was 2.99 mg GAE g^{-1}, total flavonoids 1.38 mg RE g^{-1} and total tannins 1.87 mg GAE g^{-1} (Table 2). Tang et al. (2015) and Jung (2011) reported amounts of phenolic compounds in celery (4.64 g GAE 100 g^{-1}). Antioxidant activity measured by the DPPH method and expressed in % inhibition were 42.26% or 2.38 µmol TE g^{-1} (Table 2). Phenolic compounds and flavonoids are mainly responsible for the antioxidant activity of plants (Masuoka et al., 2012). There are many studies that have demonstrated the antioxidant activity of Apium plants (seeds, roots and leaves) in vitro and in vivo (Salehi et al., 2019).

This study also investigates the antimicrobial activity of *Apium graveolens* against six bacterial strains. The MIC and MBC values of celery extract were used to evaluate the bacteriostatic and bactericidal properties. MIC and MBC values were determined based on bacterial growth on MHA (Muller Hinton Agar). MIC and MBC of celery root extract are shown in Table 3.

Table 3: The effect of the extract on the tested bacterial cultures

Bacteria	MIC mg/mL	MBC mg/mL	Dovicin µg/mL
1. *Enterococcus faecium* ATCC 6057	100	>100	<0,24
2. *Listeria monocytogenes* ATCC 13932	50	>100	<0,24
3. *Staphylococcus aureus* ATCC 25923	100	>100	0,24
4. *Salmonella enteritidis* ATCC 13076	>200	>200	1,95
5. *E.coli* WDCM 0013	>200	>200	0,92
6. *Serratia marcescens* ATCC 43862	100	>100	<0,24

The results showed different degrees of inhibition of antibacterial activity against some of the tested microorganisms. From the obtained results it can be seen that *Listeria monocytogenes* ATCC 13932 showed the highest sensitivity while the most resistant were *Salmonella enteritidis* ATCC 13076 and *E. coli* WDCM 0013.

The structure of the cell wall of Gr^- bacteria is more complex compared to the structure of Gr^+ bacteria, because it contains a thin layer of peptidoglycan, surrounded by an outer membrane made of lipopolysaccharides, while in Gr^+ bacteria there is no outer membrane, but a thick layer of peptidoglycan (Silhavy et al., 2010). The strong hydrophobic outer membrane of Gr^- bacteria acts as a strong permeability barrier (Nikaido, 2003) and limits the diffusion of active substances from the agent (Pierozan et al., 2009).

A. graveolens contain tannins, phenols, flavonoids, vitamin C (Table 2), in accordance with that, literature data show that phytochemicals in plants have antimicrobial activity, especially flavonoids (Mickymaray, 2019). The main mechanism of the antibacterial action of the phenols and flavonoids is DNA inhibition, membrane degradation and deterioration of energy metabolism (Al Aboody et al., 2020). This is consistent with literature data which show that extract of celery leaves and roots demonstrated antibacterial activity against different bacteria (Sipailiene et al., 2005, Emad et al., 2022) and that Apium plants have a role in the prevention of microbial growth (Maxia at al., 2012; Edziri at al., 2012; Baananou et al., 2013).

Conclusion

Recently, there has been a growing interest in using natural antioxidants from plant sources instead of synthetic compounds. Various plants from the Apiaceae family including *Apium graveolens* present a good source of antioxidants such as phenolic acids, flavonoids, tannins, stilbenes, coumarins, lignans, carotenoids, tocopherols and ascorbates. Of these phenolic compounds, especially phenolic acid and flavonoids make the main contribution to scavenging free radicals as well as inhibition of different gram-positive and gram-negative bacteria.

Acknowledgment

This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract numbers: 451-03-47/2023-01/ 200088 and 451-03-47/2023-01/200215)

References

Aboody M.S.A. (2021): Cytotoxic, antioxidant, and antimicrobial activities of Celery (*Apium graveolens* L.). Bioinformation. 17(1): 147–156.doi: 10.6026/97320630017147.

Al Aboody M.S., Mickymaray S. (2020): Anti-Fungal Efficacy and Mechanisms of Flavonoids. *Antibiotics*, *9*, 45. https://doi.org/10.3390/antibiotics9020045.

Al-Asmari A.K., Athar M.T., Kadasah S.G. (2017): An Updated Phytopharmacological Review on Medicinal Plant of Arab Region: *Apium graveolens* Linn. Pharmacogn Rev. 11(21):13–18. doi: 10.4103/phrev.phrev_35_16. PMID: 28503047; PMCID: PMC5414449.

Baananou S., Bouftira I., Mahmoud A., Boukel K., Marongiu B., Boughattas N.A. (2013): Antiulcerogenic and antibacterial activities of Apium graveolens essential oil and extract. Nat. Prod. Res. 27, 1075–1083.

Brand-Williams W., Cuvelier ME., Berset C. (1995): Use of the radical method to evaluate antioxidant activity, Lebensmittel- Wissenschaft und Technologie, 28, 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.

Edziri H., Ammar S., Souad L., Mahjoub M.A., Mastouri M., Aouni M., Mighri Z., Verschaeve L. (2012): In vitro evaluation of antimicrobial and antioxidant activities of some Tunisian vegetables. S. Afr. J. Bot. 2012, 78, 252–256.

Emad A.M., Rasheed D.M., El-Kased R.F.; El-Kersh, D.M. (2022): Antioxidant, Antimicrobial Activities and Characterization of Polyphenol-Enriched Extract of Egyptian Celery (*Apium graveolens* L., Apiaceae) Aerial Parts via UPLC/ESI/TOF-MS. Molecules, *27*, 698.

Jung W.S. (2011): "In vitro antioxidant activity, total phenolics and flavonoids from celery (*Apium graveolens*) leaves," Journal of Medicinal Plants Research, vol. 5, pp. 7022–7030.

Javadpour M.M, Juban M.M, Lo W.C, Bishop S.M, Alberty J.B, Cowell S.M, Becker C.L, McLaughlin M.L. (1996): De novo antimicrobial peptides with low mammalian cell toxicity. J Med Chem. 2; 39(16):3107-13. doi: 10.1021/jm9509410. PMID: 8759631.

Khairullah A.R., Solikhah T.I., Ansori A.N.M., Hidayatullah A.R., Hartadi E.B., Ramandinianto S.C., Fadholly A. (2021): Review on the Pharmacological and Health Aspects of Apium Graveolens or Celery: An Update. Sys Rev Pharm 2021;12(1):606–612.

Kooti W., Ali-Akbari S., Asadi-Samani M., Ghadery H., Ashtary-Larky D.A. (2014): Review on medicinal plant of *Apium graveolens*. Adv Herb Med. 1:48–59.

Kooti W., Daraei N. (2017): A Review of the Antioxidant Activity of Celery (Apium graveolens L). J Evid Based Complementary Altern Med. 22(4):1029–1034. doi: 10.1177/2156587217717415.

Khalil N.; Ashour M.; Fikry S.; Singab A.N., A, Osama S (2018): Chemical composition and antimicrobial activity of the essential oils of selected Apiaceous fruits. Future Journal of Pharmaceutical Sciences, (), S2314724517300171–doi:10.1016/j.fjps.2017.10.004.

Liu M., Li X.Q., Weber C., Lee C.Y., Brown J., Liu R.H. (2002): Antioxidant and antiproliferative activities of raspberries. J. Agric. Food Chem. 50: 2926–2930.

Nikaido H. (2003): Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 67(4):593-656. doi: 10.1128/MMBR.67.4.593-656.2003. PMID: 14665678; PMCID: PMC309051.

Makkar H.P.S. (2003): Quantification of tannins in tree and shrub foliage: a laboratory manual. Publisher Springer Dordrecht, ISBN: 978-94-017-0273-7. https://doi.org/10.1007/978-94-017-0273-7.

Maxia A., Falconieri D., Piras A., Porcedda S., Marongi B., Frau M.A., Goncalves M.J., Cabral C., Cavaleiro C., Salgueiro L. (2012): Chemical Composition and Antifungal Activity of Essential Oils and Supercritical CO2 Extracts of *Apium nodiflorum* (L.) Lag. Mycopathologia, 174, 61–67.

Masuoka N., Matsuda M., Kubo I. (2012): Characterisation of the antioxidant activity of flavonoids. Food Chem., 131, 541–545.

Meng-Yao L., Xi-Lin H., Feng W., Guo-Fei T., Zhi-Sheng X., Ai-Sheng X. (2017): Advances in the research of celery, an important Apiaceae vegetable crop. Critical Reviews in Biotechnology, 1–12. doi:10.1080/07388551.2017.1312275

Mickymaray S. (2019): Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds against Clinically Important Pathogens. *Antibiotics*. 8(4):257. https://doi.org/10.3390/antibiotics8040257.

Oliveira L.C.C, Rodrigues F.A.A, dos Santos Barbosa C.R, dos Santos J.F.S, Macêdo N.S, de Sousa Silveira Z, Coutinho H.D.M, da Cunha F.A.B (2022): Antibacterial Activity of the Pyrogallol against *Staphylococcus aureus* Evaluated by Optical Image. *Biologics*. 2(2):139–150. https://doi.org/10.3390/biologics2020011.

Pierozan M.K., Pauletti G.F., Rota L., Santos A.C. A., Lerin L.A., di Luccio, M., Oliveira J.V. (2009): Chemical characterization and antimicrobial activity of essential oils of salvia L. species. Ciência e Tecnologia de Alimentos, 29(4), 764–770, doi:10.1590/s0101-20612009000400010.

Powanda M..C., Whitehouse M.W., Rainsford K.D. (2015): Celery seed and related extracts with anti-arthritic, anti-ulcer, and anti-microbial activities. In Novel Natural Products: Therapeutic Effects in Pain, Arthritis and Gastro-intestinal Diseases, Springer: pp 133–153.

Tang E.L.H., Rajarajeswaran J., Fung S., Kanthimathi M. (2015): "Petroselinum crispum has antioxidant properties, protects against DNA damage and inhibits proliferation and migration of cancer cells," Journal of the Science of Food and Agriculture, vol. 95, no. 13, pp. 2763–2771, 2015.

Qureshi K., Tabassum F., Neelam., Amin M., Akram M.Z., Zafar M. (2014): Investigation of Mineral Constituents of Apium graveolens L available in Khyber Pakhtunkhwa Pakistan. Journal of Pharmacognosy and Phytochemistry. 3(4): 234–239.

Re R., Pellegrinni N., Proteggente A., Pannala A., Yang A., Rice-Evans C. (1996): Antioxidant activity applying an improved ABTS radical decolonization assay. Free Radical Bio. Med. 26: 1231–1237.

Salehi B., Venditti A., Frezza C., Yücetepe A., Altuntaş Ü, Uluata S., Butnariu M., Sarac I., Shaheen S.A. Petropoulos S.R., Matthews K., Sibel Kılıç C., Atanassova M., Oluwaseun Adetunji C., Oluwaseun Ademiluyi A., Özçelik B., Valere Tsouh Fokou P., Martins N.C., Cho W., Sharifi-Rad J. *Apium* (2019): Plants: Beyond Simple Food and Phytopharmacological Applications. Applied Sciences. 9(17):3547. https://doi.org/10.3390/app9173547.

Silhavy T.J., Kahne D., Walker S. (2010): The bacterial cell envelope. Cold Spring Harb Perspect Biol., 2 (5):a000414. doi:10.1101/cshperspect.a000414.

Singleton VL., Orthofer R., Lamuela-Raventos RM. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152–178.

Sipailiene A., Venskutonis P.R., Sarkinas A., Cypiene V. (2005): Composition and antimicrobial activity of celery (Apium graveolens) leaf and root extracts obtained with liquid carbon dioxide. Acta Horticulturae. 677(677):71-77. doi:10.17660/ActaHortic.2005.677.9.

Zhishen J., Mengecheng T., Jianming W. (1999): The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chem., 64, pp. 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2.