THE EFFECT OF RADIATION PRESERVATION ON MACROMOLECULE CONTENT, ANTIMICROBIAL ACTIVITY AND RADICAL - SCAVENGING POTENTIAL OF *PORTULACA OLERACEA* L. LEAVES EXTRACT

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ABSTRACT

The Portulaca oleracea L. (P. oleracea) phytochemical composition (contains omega-3 fatty acids, quercetin, rutin, gallo tannins, proteins) is responsible for its biological effects, e.g. antioxidative, antibacterial, anti-inflammatory and antiradiation activity. The plant has a C4 metabolism and it is known for its tolerance to different stressors, such as salinity, no water, high - temperature conditions, and 9 kGy radiation.

The purpose of the present investigation was focused on the inhibitory effects of P. oleracea leaves extracts against 5 - 10 kGy radiation - induced abiotic stress, and assess to total phenol, flavonoid, and tannins content; antibacterial potential (Staphylococcus aureus ATCC25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) and antioxidant capacity. The free dried, powdered P. oleracea samples were ^{60}Co irradiated, 50 % ethanol solvent (v/v) extracted and compared to 0 kGy extract. 10 kGy irradiated extract provide to be potentially effective against bacterial strains and possessed stable antioxidant activity, towards DPPH ($p < 0.002$), ABTS$^{\bullet+}$ ($p < 0.05$), FRAP ($p < 0.05$) and NO ($p < 0.002$). In addition, highly sensitive Electron Paramagnetic Resonance (EPR) was used to evaluate antiradical capacity. Single, symmetrical signal ($g = 2.0023$) was recorded in 10 kGy irradiated P. oleracea extract, comparable to the EPR signal in non-irradiated sample and depended directly on the antiradical potential. Moreover, in vitro P. oleracea inhibited the superoxide anion ($\bullet O_2^-$), hydroxyl (•OH), alkyl radicals, and exhibited antioxidant properties against 10 kGy irradiation.

Keywords: P. oleracea, gamma radiation, bacterial strains, antioxidants, protectors.

INTRODUCTION

Portulaca oleracea L. *(P. oleracea)* belongs to the *Portulacaceae* family, commonly known as purslane is endemic plant to Europe. *P. oleracea* is an annual herbaceous plant, with therapeutic and dietary benefits [1]. The plant contains bioactive phytochemicals such as β-carotenoids, phenolic, O and C - flavonoids (rutin, myricetin, quercetin, apigenin), minerals, ascorbic acid, tocopherols, glutathione, vitamins E and B, and ω - 3 and ω - 6 fatty organic acids with proven antioxidant properties [1, 2]. *P. oleracea* is an excellent food source due to its relatively low concentration of elements such as nitrogen, phosphorus, sulfur, potassium, calcium, magnesium, iron, and copper [2, 3]. The stems, leaves, and seeds consumption of *P. oleracea* possessed hepatoprotective, gastroprotective, neuroprotective, antiseptic, analgesic, anti-inflammatory, anticancer, antidiabetic, and antioxidant properties [1, 3]. Purslane leaves are characterized with high nutritional potential (23 - 24 % protein, fiber, starch, essential amino acids) [4], dietary minerals and antioxidant molecules

(vitamin A/C, riboflavin, niacin, pyridoxine, thiamine, α-tocopherol, and pantothenic acid), worked as free - radical scavengers and protectors against abiotic stress induction [4, 5]. In addition, several studies have reported antifungal and antibacterial activity in Gram - positive bacteria of *P. oleracea* in different plant parts [6, 7].

Gamma radiation (γ) is a source of ionizing energy, with a harmful effect on biological systems caused by the direct ionization or indirect generation of highly reactive oxygen/nitrogen species (ROS/RNS) [4, 8]. High doses γ - radiation directly interact with water molecules in plant cells, leading to ROS/RNS overproduction and morphologically, biochemically, or physiologically cellular modifications [8]. The 5 - 10 kGy radiation increase the ROS/RNS concentrations by radiolysis process and caused structural and metabolic cellular damages, and thylakoid membranes expansion, photosynthesis change, antioxidant system modulation, and phenolics accumulation [9]. The leave irradiation also disrupts protein synthesis, gas and water exchange and hormone balance [9, 10]. In contrast, several reports conveyed that the 5 - 10 kGy irradiations increased the glucose, fructose, flavonoid, phenolic content, vitamins, and antioxidant enzymes activity in plants and an alternative method for food preservation [10, 11].

The present study aimed to analyze the effect of 5 kGy and 10 kGy γ - irradiation preservation on Bulgarian *P. oleracea* leaves extract, to assess the macromolecule content, antibacterial stability in bacterial strains *(Staphylococcus aureus* ATCC25923*, Escherichia coli* ATCC 25922*, and Pseudomonas aeruginosa* ATCC 27853*),* their antioxidant and antiradical potential, and compared to 0 kGy extract.

EXPERIMENTAL

Plant material and extraction procedure

The *P. oleracea* leaves were harvested from the Stara Zagora region, Bulgaria, during July - August 2022. The raw material was dried in shade, at 26ºC and 4 % water content. The air - dried plant material was mechanically ground with a laboratory mill to obtain a homogeneous powder. The ground raw material is placed in a thermostated water bath shaker (T = 50ºC) under constant stirring, in a solid/liquid ratio $\xi = 0.1$ mL g^{-1} and extractant 50 % ethanol (EtOH, p.a. ≥ 99.8 %), and 90 minutes' time extraction. The stirring rate was $n = 4$ s^{-1} to ensure maximum diffusion of the extracted component into the solution.

Radiation preservation

Two parallel *P. oleracea* leaves samples (10 g each) were irradiated at 5 - 10 kGy using a ^{60}Co mobile irradiation chamber (0.873 kGy h^{-1} dose rate) with vertical axe rotation and dimensions: 14.5 cm diameter and 22 cm height (Radiobiology, Stara Zagora, Bulgaria). Non-irradiated *P. oleracea* extracts were used as controls.

Total phenolic, flavonoid, and tannin content analysis

The phenolic content (TPC) in 0 - 5 -10 kGy irradiated *P. oleracea* extracts is estimated using the Folin-Ciocalteu method [12] at 750 nm absorbance (Epoch BioTek - 22, USA), defined as mg gallic acid equivalent per gram extract (mg GAE g^{-1}). The flavonoid content (TFC) in non- and irradiated extracts was carried out using the aluminium chloride ($AlCl_3$) method [13] at 415 nm absorbance, expressed in mg quercetin equivalent per gram extract (mg QE g^{-1}). The tannin content (TTC) in non- and irradiated *P. oleracea* was carried out following the vanillin-hydrochloride procedure [14] at 700 nm absorbance, expressed in mg tannic acid equivalent per gram extract (mg TAE g^{-1}).

Antimicrobial activity

The antimicrobial activities of 0 - 5 -10 kGy irradiated *P. oleracea* extracts were detected using the agar well diffusion method. The 20 mL of molten Muller Hinton agar medium (Himedia, Mumbai, India) was used to culture selected Gram (+) positive (*Staphylococcus aureus* ATCC25923, SUP, India) and Gram (−) negative bacteria (*Escherichia coli* ATCC 25921, SUP, India and *Pseudomonas aeruginosa* ATCC 27853, SUP, India). The 100 µL (200 mg L^{-1}) of 0 kGy and 5 - 10 kGy treated extracts and standard antibiotic (gentamicin) as positive control were added to the incubated plate using filter paper (1 mm x 0.11 RD). The plates were incubated for 24 h at 37°C (aerobic conditions), the experiment was repeated 3 times, and the mean inhibition zone (IZ, mm) was recorded [15].

Antiradical activity

To study the antiradical activity, two methods were

used: 1) The DPPH (2,2-dipheny l,1-picrylhydrazyl), ABTS•+ (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), FRAP and nitric oxide ion (NO) scavenging activities were determined spectrophotometrically by Cuendet et al. [16], Adhikari et al. [17], Oyaizu [18], Shirwaikar et al. [19], respectively; and 2) The spectral stability and capability of *P. oleracea* components to terminate the superoxide anion ($\bullet O_2^-$) and hydroxyl (•OH) radicals generated by chemical reaction directly in the *in vitro* system were determined by the spin-trapping EPR (Bruker ER-116-DS), according to the Karamalakova et al. [20], Zhao [21] and Wang et al. [22].

Statistical analysis

Statistical analysis was performed with Statistica 8.0, Stasoft, Inc., one - way ANOVA, and Student t - test to determine the significant difference among data groups. The results were expressed as means ± standard error (SE) and the $p < 0.05$ value was considered statistically. EPR spectral processing was performed using Bruker Win - EPR and Sim - Fonia software.

RESULTS AND DISCUSSION

Secondary metabolites such as low molecular mass organic compounds (phenolic compounds, flavonoids, terpenoids, and vitamins) are easily biosynthesized, helping plants to interact with biotic and abiotic stressors, and activated protective mechanisms [23].

γ - irradiation, through accelerated cell proliferation, increases the ROS/RNS formation and induces oxidative stress (OS) responses. This enables plants to increase the phytochemicals concentration and the production of protective enzymes, vitamins, and antioxidants, i.e. to proportionally increase the secondary metabolites concentrations secondary metabolites [24]. In this context, 10 kGy irradiation is the maximum dose used for disinfection and sterilization of plant food products, to foodborne pathogens inactivation and oxidative stability incensement [25].

γ - irradiation preservation increase TPC, TFC, and TTC activities

The most suitable parameters for the bioactive components extraction from the plants are extraction agent - 50 % ethanol, extraction time 90 minutes and temperature 50°C [26, 27]. The TPC, TFC, and TTC activities of 0 kGy and 5 - 10 kGy irradiated extracts are summarized in Table 1. The TPC of the three *P. oleracea* extracts ranged from 3.23 ± 0.07 to 4.58 ± 0.01 mg GAE g^{-1} ($p < 0.002$), with non-irradiated *P. oleracea* having the lowest TPC content, while 10 kGy irradiated sample recorded the maximum TPC. Likewise, under TFC conditions, non-irradiated extract showed the lowest (1.804 ± 0.003 mg QE g^{-1}), while the 10 kGy irradiated *P. oleracea* had the highest (2,17 ± 0.04 mg QE g^{-1}, $p < 0.002$) flavonoid content. Interestingly, in terms of secondary metabolite activation, non-irradiated *P. oleracea* had low polyphenol and flavonoid content and therefore reduced antioxidant properties compared to 5 - 10 kGy treated extracts. Phenolic and flavonoid metabolites increased at 10 kGy, probably due to high ROS/RNS induction and the need for enhanced activation of biosynthetic enzymes and vitamins, especially phenylalanine ammonia lyase. *P. oleracea* compounds neutralized γ - irradiation induced damages by cellular regeneration and increased oxidative stability [8, 28]. On the other hand, the maximum TPC and TFC, recorded at 10 kGy may have been affected by the solvent (ethanol) polarity or by the mature growth stage of *P. oleracea* [15]. Several studies have suggested that TPC and TFC in *P. oleracea* leaves are at their maximum concentration at the developmental stage, which contributes to the complete ROS/RNS inhibition, maximum enzyme activation, and to remove γ - radiation toxicity. Also, the increased phenolic flavonoids content probably indicates the γ - radiation inability to break all O - H chemical bonds in the molecules and to induce cellular level changes [8, 15]. The TTC increased at 10 kGy irradiated extracts by 38.97 ± 0.09 % $p < 0.001$ (Table 1). The increased phenols and flavonoids stability leads to stability in the tannins structure [8]. The tannins complete react with proteins and metal ions from the leaf mass and protection of host cells from OS changes caused by 10 kGy radiation.

γ - irradiation preservation increase antibacterial and antioxidant activity

Several studies indicated that *P. oleracea* contains a trehalose - binding lectin exhibiting different antibacterial activity levels in Gram (+) positive and Gram (-) negative bacteria [1]. The corresponding inhibition zone values (IZ, mm) are presented in Table 2. The gentamicin - IZ is fully consistent with the CLSI criteria. 200 mg L^{-1}

Table 1. Total phenolic, flavonoid, and tannin content of the non-irradiated and 5 - 10 kGy irradiated *P. oleracea* extracts.

Radiation dose (kGy)	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	TAC (mg TAE g^{-1})
0	3.237	1.804	37.91
5	4.09	1.97	38.56
10	4.58	2.17	38.97

The TPC defined as mg gallic acid equivalent per gram extract (mg GAE g^{-1}), TFC expressed in mg quercetin equivalent per gram extract (mg QE g^{-1}), TTC expressed in mg tannic acid equivalent per gram extract (mg TAE g^{-1}).

Table 2. Antimicrobial screening test results (IZ ± SD) of non-irradiated and 5 - 10 kGy irradiated *P. oleracea* extracts against bacterial strains - *S. aureus* ATCC25923, *E. coli* ATCC 25921, *P. aeruginosa* ATCC 2785.

Radiation dose (kGy)	*S.aureus*	*P. aeruginosa*	*E.coli*
0	12.06 ± 0.2**	26.4 ± 1.0***	8.7 ± 0.6**
5	13.09 ± 0.3***	24.5 ± 0.3***	8.9 ± 0.2***
10	17.2 ± 1.0***	26.34 ± 1.0***	8.9 ± 0.3***
Positive control (gentamicin)	14.2 ± 0.7	14.0 ± 0.5	17.6 ±1

*IZ - inhibition zone, mm; *low antibacterial activity (IZ = 8 - 10 mm); **medium antibacterial activity (IZ = 11-14 mm); *** high antibacterial activity (IZ ≥ 15 mm)*

of 5 - 10 kGy irradiated *P. oleracea* extract registered slight incensement in antimicrobial activity in the tested *S. aureus ATCC25923*, and relatively low statistically insignificant, antibacterial activity in *E. coli ATCC 25922* and *P. aeruginosa ATCC 27853* bacterial strains. The non-irradiated extract registered relatively low activity against *S. aureus ATCC25923* and *E. coli ATCC 25922* and maximal activity against *P. aeruginosa ATCC 27853* bacterial strains. Many studies reported that the different TPC, TFC, and TTC concentrations in the *P. oleracea* exhibited different antibacterial activities against Gram (+) and Gram (-) bacteria, showing antibacterial activity in Gram (+) strains [1, 7, 15], (Table 2).

Radiation preservation (up to 10 kGy) as a cold process, retained the color, flavor, taste, and aroma of the plant - food products, and eliminated microbial contamination without toxicological risk [29]. Regarding the preservation method, DPPH ($p < 0.002$), $ABTS^{\bullet+}$ ($p < 0.05$), and FRAP ($p < 0.05$) (Table 3) scavenging activities were significantly increased in 10 kGy, compared to non-irradiated extract. The 10 kGy irradiated *P. oleracea* had the strongest antioxidant activity, which was related to the phenolic, flavonoid, and tannin compounds retention. The probable stimulation of the adaptive protection by increased iron (III) (Fe^{3+} to Fe^{2+}) electron - donating activity and additional secondary metabolites re-activation by the *P. oleracea* extract after 10 kGy impacts is another possible mechanism. Several studies reported that the antiradical activity of irradiated *P. oleracea* extract increased dose-dependently [29]. Moreover, 9 kGy and 10 kGy irradiation preservation increased phenolic, flavonoids, and tannin compounds' extractability and protected against lipid peroxidation and microbial activation [29].

Abiotic (γ - radiation) stresses perturb cellular redox homeostasis by ROS/RNS accumulation in cellular organelles, rendering OS metabolic disorders, unless the plants by scavenging detoxified efficiently from ROS/ RNS. Plants, in order to counteract abiotic stressors, synthesize low-molecular compounds that work as cellular redox - balancers/ adapters on NO chain - reactions initiation and OS preventers [30, 31]. The highest NO - scavenging activity in *P. oleracea* extracts was at 10 kGy irradiation ($p < 0.002$) in comparison with 0 - 5 kGy extracts. *P. oleracea*, contains macro - and low - molecular compounds that detoxified NO radicals and stopped NO

Table 3. Antioxidant and antiradical potential of non-irradiated and 5 - 10 kGy irradiated *P. oleracea* extracts.

Radiation dose (kGy)	DPPH (%)	ABTS•+ (%)	FRAP (%)	NO (%)	EPR g-value	EPR $•O_2^-$, (%)	EPR •OH, (%)
0	37.04	64.5	0.08	15.4	2.0023	73.4 ± 0.91	59.6 ±1.73
5	39.19	68.1	0.086	17.2	2.003	76.2 ± 1.07	61.22 ± 1.8
10	39.47	72.4	0.097	18.56	2.0023	96.11 ± 1.5	75.09 ± 1.27
	R^2=0.54	R^2=0.795	R^2=0.782	R^2=0.814	-	R^2=0.851	R^2=0.866

Antioxidant activity and antiradical potential of P. oleracea extracts: DPPH scavenging activity, % from control; ABTS•+ scavenging activity, %; Nitric oxide assay, %; EPR spectra g- value; EPR $•O_2^-$ scavenging activity, (%); EPR •OH scavenging activity, (%).

- chain reactions, especially after high abiotic stress [31].

In addition, to confirm the *P. oleracea* compounds stability and antiradical-adaptive effects, especially after γ - induced toxicity, we investigated the EPR spectral signals. As expected, in the 0 kGy and 10 kGy extracts, a singlet - symmetrical signal with g = 2.0023, and almost identical intensity resulted (Table 3). 5 kGy irradiated *P. oleracea* shown singlet line intensity but with a slight change in the g value (g = 2.0031).

For the first time, to fully determine the Bulgarian *P. oleracea* protective ability against γ - radiation generated highly reactive radicals such as $•O_2^-$ and •OH, we used spin- trap BMPO (5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide). This approach reflects the direct antioxidant activity of the plant extract. After the splitting constants calculation ($_aN$ = 13.4 G and aH_β = 12.1 G) the spin - adduct was identified as the BMPO/ •OOH [21, 22]. The highest antiradical activity of *P. oleracea* extracts towards BMPO/ $•O_2^-$ (sextet; 96.11 ± 1.57 %) and BMPO/ •OH (quartet; 75.09 ± 1.27 %) were found at 10 kGy irradiation. The *P. oleracea* presents inhibited the $•O_2^-$ and •OH production, and exhibited antioxidant properties against 10 kGy irradiation. Several studies discuss that addition of plant extracts leading to a decrease in spin - adduct concentration as a result of competitive reactions between antioxidants, generated radicals and the spin - trap BMPO [32]. In general, 10 kGy preserved *P. oleracea* extract showed the highest antioxidant and antiradical activities, mainly attributed to the phenolic and flavonoid compound, as well as ascorbic acid, α-tocopherol, pigments, and solvent ability to inhibit the polyphenol oxidase action, which causes the *P. oleracea* macromolecules non - oxidation [31, 32].

CONCLUSIONS

Ethanoic *P. oleracea* extracts, non-treated and 10 kGy irradiated possessed equal oxidative stability in macromolecules and could be used as excellent natural antioxidants with great potential in radiation preservation. Moreover, the results can also provide the effectiveness of *P. oleracea* extracts for antimicrobial activity. For the first time high sensitive EPR spectroscopy was used to study *in vitro* antiradical abilities of *P. oleracea* leaves extracts against the highly reactive $•O_2^-$ and •OH radicals. In the studied *in vitro* systems the *P. oleracea* extract manifested strong antioxidant properties, especially after 10 kGy irradiation. We consider further thorough studies should be conducted to identify the extract ingredients involved in the antioxidant properties and to clarify the mechanism causing Fenton system production.

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