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Impact of radon-rich atmospheres on the growth and development of *Mentha spicata*

Versão final após defesa

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Declaração de Integridade

Eu, Mariana Pinto Vicente, que abaixo assino, estudante com o número de inscrição M13964 do 2º ciclo de estudos em Ciências Biomédicas da Faculdade de ciências da Saúde da Universidade da Beira Interior, declaro ter desenvolvido o presente trabalho e elaborado o presente texto em total consonância com o **Código de Integridades da Universidade da Beira Interior**.

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Universidade da Beira Interior, Covilhã, dezembro de 2025.

Dedication

I dedicate this work to my family, to my parents, my brother, and my grandmother, who never doubted me. Thank you for your unconditional support and for being my safe haven.

To my sister at heart, Alexandra Ferreira, living proof that family can also be chosen, thank you for always being there in good times and bad.

To those who made my birthday a different and more special day for me, who are undoubtedly the ones I cherish the most.

To the genuine people who walk with me. Those who have been with me since the beginning of this journey and whom I will carry with me for life. And those who appeared recently, but have already become indispensable.

Thank you for every moment of patience when I was at my limit, for the companionship that made me realise I wasn't alone, and for the fun and lightness that made the days better. For simply being true.

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Resumo

O objetivo deste trabalho é investigar o impacto de atmosferas ricas em radão (^{222}Rn) no crescimento e desenvolvimento da *Mentha spicata* L., com foco nas alterações morfológicas e bioquímicas induzidas devido a essa exposição a este gás especial enfoque. Este estudo pretende contribuir para a compreensão dos impactos ambientais deste gás radioativo natural nas plantas salientando a necessidade de investigação contínua sobre a sua toxicidade. As descobertas podem ter implicações significativas na elucidação dos mecanismos do stress oxidativo em plantas expostas à radiação. A *Mentha spicata*, reconhecida pelas suas propriedades medicinais, contém compostos fenólicos e flavonoides, como o ácido rosmarínico e a miricetrina, que desempenham um papel revelante na atenuação do stress oxidativo. Para esta investigação, as plantas foram divididas em três grupos. O Grupo A foi exposto a rochas contendo urânio, o Grupo B serviu como controlo do Grupo A, sem exposição à atmosfera radioativa, e o Grupo C foi colocado em ambiente laboratorial sem qualquer intervenção, que constitui o controlo da experiência. Foram realizadas diferentes análises, incluindo o ensaio DPPH, para determinar a capacidade antioxidante e a cromatografia líquida de alta eficiência (HPLC) para quantificar os compostos bioativos em um estudo comparativo entre plantas expostas e não expostas. A exposição ao radão reduziu a capacidade antioxidante e o teor de ácido rosmarínico na *Mentha spicata* em comparação com o controlo, indicando uma tentativa de adaptação ao stress radiológico e induzindo alterações mensuráveis no metabolismo secundário da planta. Esta resposta promove a biossíntese de compostos fenólicos com atividade antioxidante como mecanismo de defesa, abrindo perspetivas para potenciais aplicações em fitorremediação.

Palavras-chave

Radão; *Mentha spicata*; HPLC; DPPH

Abstract

The objective of this work is to investigate the impact of radon-rich (^{222}Rn) atmospheres on the growth and development of *Mentha spicata* L., focussing on the morphological and biochemical changes induced due to exposure to this special gas. This study aims to contribute to the understanding of the environmental impacts of this natural radioactive gas on plants, highlighting the need for continuous research on its toxicity. The findings may have significant implications in elucidating the mechanisms of oxidative stress in plants exposed to radiation. The *Mentha spicata*, recognised for its medicinal properties, contains phenolic compounds and flavonoids, such as rosmarinic acid and myricetin, which play a significant role in alleviating oxidative stress. For this investigation, the plants were divided into three groups. Group A was exposed to rocks containing uranium, Group B served as the control for Group A, with no exposure to the radioactive atmosphere, and Group C was placed in a laboratory environment without any intervention, which constitutes the control of the experiment. Different analyses were conducted, including the DPPH assay to determine antioxidant capacity and high-performance liquid chromatography (HPLC) to quantify bioactive compounds in a comparative study between exposed and non-exposed plants. Radon exposure reduced the antioxidant capacity and rosmarinic acid content in *Mentha spicata* compared to the control, indicating an attempt to adapt to radiological stress and inducing measurable changes in the plant's secondary metabolism. This response promotes the biosynthesis of phenolic compounds with antioxidant activity as a defence mechanism, opening up prospects for potential applications in phytoremediation.

Keywords

Radon; *Mentha spicata*; HPLC; DPPH

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UBI	Universidade da Beira Interior
MS	*Mentha spicata* L.
LabExpoRad	Laboratório de Estudo dos Efeitos da Exposição ao Radão
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
IC_{50}	Inhibitory Concentration
DPPH	2,2-difenil-1-picrilhidrazil
DPPH-H	Difenilpicrilhidrazina
HPLC	High Performance Liquid Chromatography
FRAP	Ferric Reducing Antioxidant Power
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
RNS	Reactive Nitrogen Species
DNA	Deoxyribonucleic acid
UV	Ultraviolet
Vis	Visible
DAD	Diode Array Detector
CR-39	Solid state nuclear track detector

Chapter 1

Introduction

1.1 Properties of radon

In the natural environment, where radiation is naturally present in the air, rocks, soil, and food due to their uranium, thorium, and potassium content, which emit ionising radiation according to their composition (Hu et al., 2018; Kang et al., 2019). Among the various naturally occurring radioactive elements, radon (^{222}Rn) stands out as an isotope that, together with ^{220}Rn, occurs in a gaseous form. Both are naturally occurring radioactive noble gases, classified as NORM (Naturally Occurring Radioactive Materials), which are chemically inert, colourless, tasteless, and odourless (Inácio et al., 2017; Kang et al., 2019; Vearrier et al., 2009). These are formed through the decay of uranium (^{238}U), as shown in figure 1.1, and thorium (^{232}Th) (Hu et al., 2018; Kang et al., 2019).

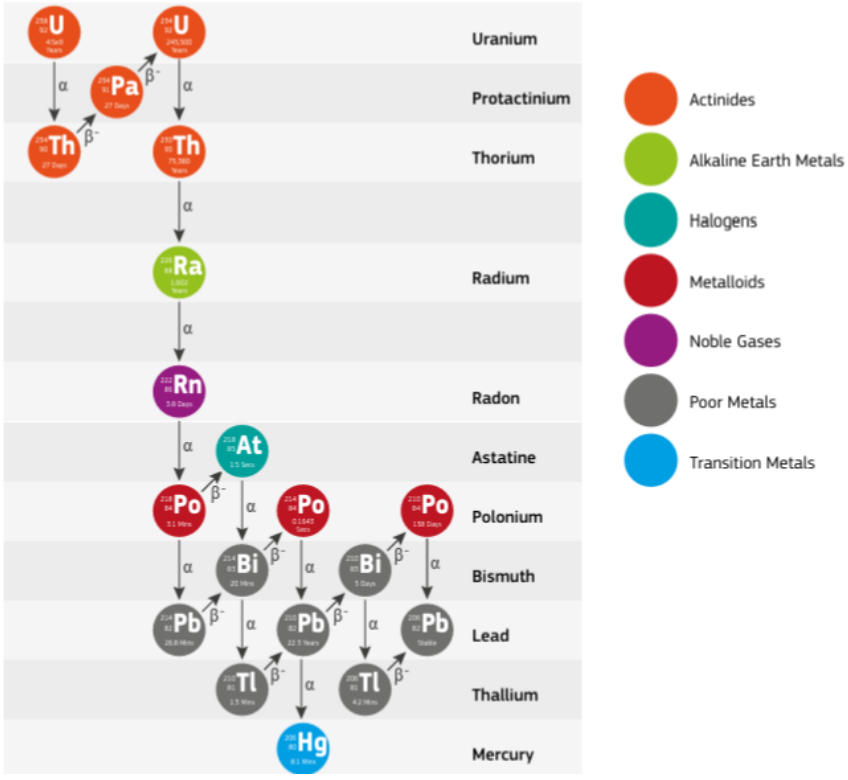

Figure 1.1: Natural ^{238}U radioactive series. Source: European Atlas of Natural Radiation

The average total dose of natural radiation received by the population is approximately 80%, of which 50% is exposure to radon (Soares et al., 2020). This gas has a high toxic

potential due to its emission of alpha particles during the decay process of its short-lived descendants, such as polonium (^{218}Po) and polonium (^{214}Po) (Soares, 2014). These particles have high energy and, when inhaled or ingested, can be deposited in the respiratory epithelium and deposit energy through alpha particles, inducing DNA damage, promoting genetic mutations and increasing the risk of developing neoplasms (Soares, 2014). Due to these effects, radon is an important risk factor, classified as a carcinogen and is currently recognised as the second leading cause of lung cancer, the first being smoking (Louro et al., 2012; Riudavets et al., 2022).

In Portugal, the susceptibility to radon exhibits a heterogeneous geographical distribution, resulting from the geological characteristics of the territory, particularly the presence of uranium-bearing granites. This variability is evident in susceptibility maps (figure 1.2) that identify areas with the highest probability of high concentrations, allowing for a more accurate spatial assessment of the risk associated with exposure to this radioactive gas.

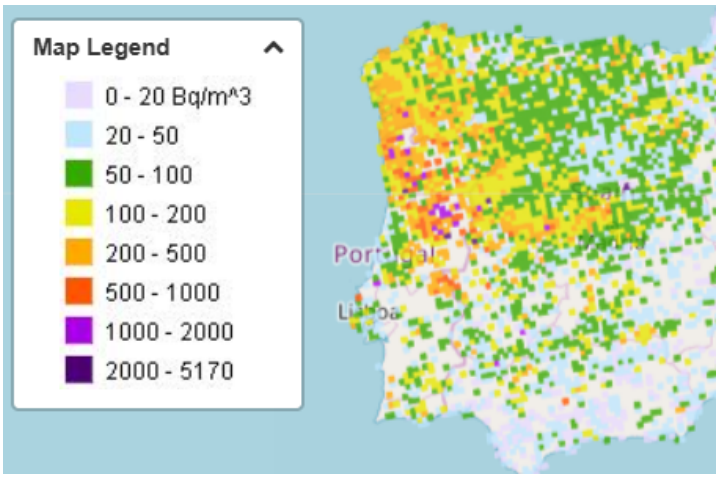

Figure 1.2: Indoor radon concentration map 2024. Source: European Commission Joint Research Centre (JRC)

Therefore, prolonged exposure to radon is particularly concerning in enclosed spaces, such as buildings and homes, where the gas can seep in through cracks in the foundation or other entry points (Elío et al., 2019; Soares et al., 2020). The concentrations of this gas in these environments tend to increase during the colder months, when ventilation is reduced and windows remain closed to combat the cold. This increase in concentration can be more pronounced in areas with good thermal insulation, which facilitates the accumulation of radon inside buildings (Bahu et al., 2021; Soares et al., 2020).

In places where susceptibility to radon is higher, it may be present in groundwater, as radon is one of the most soluble noble gases (Rani et al., 2021). For this reason, exposure to radon may be higher when contaminated groundwater is used for bathing or drinking (Rani et al., 2021; Shah et al., 2024).

Considering that the primary risks of radon exposure to living beings occur through inhalation and ingestion, it is essential to understand its presence and its impacts, both on

the environment and on the organisms that comprise it (Soares, 2014).

Given its relevance to public health, it is essential to investigate the effects of this noble gas not only in humans but also in plant organisms, particularly in species of medicinal and environmental interest, whose response to radon exposure remains largely unexplored. Several studies have shown that plants can absorb radon and contribute to air purification, making them a natural solution for reducing indoor air pollution (Chrysargyris et al., 2019; Kadhim et al., 2021; Li et al., 2018; Zainab et al., 2023).

This study aims to contribute to this understanding by investigating the response of *Mentha spicata* to radon exposure, which may open new avenues for utilising plants as phytoremediation, while also providing crucial information on environmental safety and public health.

1.2 Aromatic plants as a subject of study: *Mentha spicata* L.

Plants are essential for life on Earth, not only because they release oxygen through photosynthesis, but also due to their various medicinal effects and their ability to interact with the environment. In this context, *Mentha spicata* L. (MS), commonly known as spearmint, emerges as a potential candidate for phytoremediation applications, as it tolerates high concentrations of heavy metals (Kunwar et al., 2014).

MS is a rhizomatous perennial herbaceous plant belonging to the Lamiaceae family (S. Mahendran et al., 2013; S. K. Mahendran & Rahman, 2021), characterised by its growth up to 100 cm in height, with branched stems, intensely green serrated leaves, and terminal inflorescences, as shown in figure 1.3 (S. K. Mahendran & Rahman, 2021) and it has been widely valued in traditional medicine across various cultures for the treatment of multiple health conditions. Among its main therapeutic applications are the treatment of gastrointestinal disorders, including diarrhoea, indigestion, intestinal weakness, and abdominal pain, as well as the relief of respiratory symptoms associated with colds, flu, and sinusitis (S. K. Mahendran & Rahman, 2021). It has also been used in the treatment of headaches, flatulence, and as a carminative, antispasmodic, diuretic, and sedative agent (S. K. Mahendran & Rahman, 2021).

Figure 1.3: *Mentha Spicata* L. pot. Source: LabExpoRad

This species is widely cultivated worldwide and traditionally used in gastronomy, cosmetics, and folk medicine due to its therapeutic and aromatic properties, it is also a rich source of polyphenols with antioxidant potential, which help the body neutralise free radicals (S. K. Mahendran & Rahman, 2021; Rita et al., 2016), these molecules, being unstable and carrying unpaired electrons, which are produced naturally during cellular metabolism or by external factors such as pollution, smoking, and solar radiation. This makes them highly reactive and prone to causing damage to biological systems (Ahmad et al., 2012).

1.3 Defence mechanisms of the plants

Antioxidant compounds are characterised by their ability to delay, inhibit, or prevent oxidative processes by neutralising free radicals through electron donation, converting them into neutral and non-toxic compounds (Gulcin & Alwasel, 2023; Liu et al., 2021; Singh & Kesharwani, 2017). This mechanism is crucial for mitigating oxidative stress, a pathological state of homeostatic imbalance where excessive production of reactive oxygen and nitrogen species (ROS/RNS) exceeds cellular antioxidant capacity, resulting in the oxidation of vital macromolecules such as enzymes, proteins, nucleic acids, and lipid components (Liu et al., 2021).

As photosynthetic organisms inhabiting oxygen-rich environments, plants produce ROS in quantities substantially higher than animals, mainly as a byproduct of electron transport chains in chloroplasts and mitochondria (Gudkov et al., 2019). While ROS exert destructive effects through lipid peroxidation, protein oxidation, and nucleic acid damage, which can compromise cell viability, they simultaneously function as universal intracellular messengers in complex signalling cascades, regulating physiological processes at genetic and physiological levels (Gudkov et al., 2019). Various abiotic stressors, including drought, high salinity, extreme temperatures, ultraviolet radiation, heavy metals, and ionising radiation, can intensify ROS production, causing irreversible cellular damage

(Gudkov et al., 2019; Kozlov et al., 2024; Liu et al., 2021; Shao et al., 2008).

In response to this constant oxidative challenge, plants have evolved remarkably effective protective mechanisms, likely accounting for their significantly higher stress resistance compared to animals (Gudkov et al., 2019). Their antioxidant arsenal comprises highly diversified enzymatic and non-enzymatic systems, with ascorbate and glutathione playing the primary protective role through oxidation-reduction cycles closely coupled with antioxidant enzyme reactions, forming the crucial ascorbate-glutathione system (Gudkov et al., 2019; Kozlov et al., 2024).

Phenolic compounds represent one of the main classes of natural antioxidants and the most prevalent secondary metabolites in the plant kingdom. Distinguished by aromatic structures incorporating multiple hydroxyl groups, these molecules confer unique chemical and biological characteristics essential for plant protection against ultraviolet radiation, pathogens, parasites, and predators, and contribute to pigmentation (Dai & Mumper, 2010; Silva et al., 2023). Phenolic acids comprise benzoic acid derivatives, such as gallic acid, and cinnamic acid derivatives, including cumaric, caffeic, and ferulic acids (Liu et al., 2021). Flavonoids, the most abundant and diverse phenolic subclass, are characterised by two aromatic rings linked by a three-carbon unit and perform multifaceted functions as effective ROS scavengers, providing protection against stress-induced damage (Es-Safi et al., 2007; Liu et al., 2021).

When exposed to stress conditions, plants significantly increase the content of secondary metabolites with antioxidant activity, particularly phenolic compounds, flavonoids, terpenoids, and nitrogenous compounds (Gudkov et al., 2019; Liu et al., 2021; Sharma et al., 2019). ROS act as central regulators through molecular pathways that stimulate gene expression for key biosynthetic enzymes and increase their specific activity, establishing a sophisticated feedback system enabling dynamic adjustment of antioxidant capacity in response to environmental conditions (Gudkov et al., 2019). The distribution of these bioactive compounds varies significantly among plant organs, with roots, leaves, flowers, stems, fruits, and seeds exhibiting distinct profiles due to the specific biosynthesis of secondary metabolites (Ranjbar et al., 2020). In the MS, rosmarinic acid and caffeic acid predominate as the principal phenolic metabolites (S. K. Mahendran & Rahman, 2021), exemplifying the species-specific nature of antioxidant compound profiles in plants.

1.4 Antioxidant activity of *Mentha spicata*

The evaluation of antioxidant activity in medicinal plants represents a fundamental aspect of contemporary phytochemical research, particularly for validating traditional therapeutic applications and identifying bioactive compounds with pharmaceutical potential. MS has been extensively utilised in traditional medicine systems across diverse cultures as mentioned before. However, scientific validation of these ethnopharmacological ap-

plications necessitates rigorous quantification of their bioactive constituents and their functional properties, particularly the antioxidant capacity that underlies many reported health benefits.

The antioxidant potential of MS is intrinsically linked to its phytochemical composition, particularly the abundant presence of phenolic compounds such as rosmarinic acid, caffeic acid, and various flavonoids. These secondary metabolites function as primary defence mechanisms against oxidative stress, both within the plant and in biological systems following consumption or application. The therapeutic relevance of this antioxidant capacity extends to numerous pathophysiological conditions associated with oxidative damage, including cardiovascular diseases, neurodegenerative disorders, and inflammatory processes, positioning MS as a promising candidate for natural antioxidant formulations (Choudhury et al., 2006).

Furthermore, the antioxidant activity of MS exhibits considerable variability depending on the specific plant organ analysed (leaves, stems, flowers), developmental stage, environmental conditions, and exposure to abiotic stresses, which modulate the biosynthesis and accumulation of phenolic compounds (Scherer et al., 2013). This variability underscores the importance of systematic evaluation across different experimental conditions to optimise extraction protocols and identify the most bioactive plant materials.

To address these objectives, several analytical methods have been developed and optimised to accurately and reproducibly quantify the antioxidant capacity of pure compounds, fractions, and extracts of plant or synthetic origin (Ahmad et al., 2012; S. K. Mahendran & Rahman, 2021; Özer, 2018; Tomé & Silva, 2020). The selection of appropriate in vitro assays for initial screening is essential to efficiently identify samples with significant antioxidant potential before proceeding to more complex biological evaluations. Among the diverse methodologies available, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay has established itself as the gold standard analytical tool for evaluating free radical scavenging capacity, owing to its simplicity, rapid response, high reproducibility, and cost-effectiveness (Gulcin & Alwasel, 2023; Oliveira, 2015).

DPPH is a stable nitrogen-containing free radical whose stability results from electronic delocalisation throughout its molecular structure, conferring an intense purple colouration in solution (Gulcin & Alwasel, 2023; Oliveira, 2015). The principle of this colourimetric method is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to DPPH, promoting its reduction to DPPH-H (diphenylpicrylhydrazine), a pale yellow non-radical compound (figure 1.4).

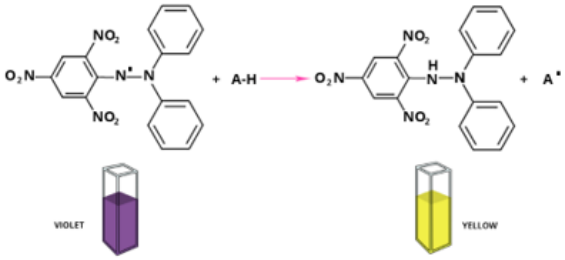

Figure 1.4: Summary of the reaction of DPPH with a general antioxidant (A-H), causing a colour change. Source: CHIMACTIV. *Antioxidant - DPPH: Principle*. AgroParisTech from: https://l1nq.com/GbmUa

This transformation results in a measurable decrease in absorbance at 517 nm, with the extent of reduction being directly proportional to the antioxidant capacity of the tested sample (Gulcin & Alwasel, 2023; Scherer et al., 2013). The antioxidant activity is quantified through the decrease in violet colour intensity, expressed either as a percentage of inhibition or through the IC_{50} value, which represents the concentration required to scavenge 50% of the DPPH radicals (Oliveira, 2015). This assay is attributed to its minimal sample requirements, rapid results, excellent reproducibility, and applicability to both isolated compounds and complex plant extracts (Oliveira, 2015). In the specific context of MS extracts, several studies have demonstrated a significant correlation between total phenolic content and DPPH radical scavenging capacity, confirming that phenolic compounds are primarily responsible for the observed antioxidant activity (Kanatt et al., 2007; Nickavar et al., 2008). For this reason, the DPPH method was chosen for this work.

1.5 Quantification of bioactive compounds

Comprehensive phytochemical characterisation of medicinal plants requires sophisticated analytical techniques capable of separating, identifying, and quantifying complex mixtures of bioactive compounds with high precision and reproducibility. Chromatography is a fundamental analytical technique based on the differential distribution of compounds between a stationary phase and a mobile phase, allowing the separation of components within complex mixtures (Snyder et al., 2011). Among the various chromatographic techniques available, high-performance liquid chromatography (HPLC) has emerged as the most widely used method for the analysis of phenolic compounds in plant matrices due to its ability to simultaneously analyse multiple compounds in a single chromatographic run, its resolution, sensitivity, versatility, and ability to perform qualitative and quantitative analyses simultaneously (Pedro et al., 2022; Xu & Wang, 2025).

The fundamental principle of HPLC involves the passage of a high-pressure liquid mobile phase through a column packed with a stationary phase, where analyte separation occurs according to differential physicochemical interactions between both phases (Pe-

dro et al., 2022; Snyder et al., 2011). Compounds with a higher affinity for the stationary phase are retained longer, whereas those with greater affinity for the mobile phase elute more rapidly, resulting in temporal separation based on unique physical and chemical properties. These interactions may involve adsorption, partitioning, ion exchange, or size exclusion, depending on the nature of the stationary phase and compound properties (Snyder et al., 2011). For phenolic compound analysis, reversed-phase HPLC (RP-HPLC) is the predominant configuration, utilising a non-polar stationary phase (typically C18-modified silica) and a polar mobile phase consisting of gradient mixtures of water with organic solvents (methanol or acetonitrile), often acidified to improve peak resolution (Do et al., 2014). This mechanism is particularly suitable for phenolic compounds, which exhibit varying polarities depending on the distribution of hydroxyl groups, glycosylation patterns, and molecular complexity. Chromatographic optimisation requires careful selection of parameters, including flow rate, column temperature, injection volume, and elution program, based on target compound characteristics such as polarity, molecular mass, and chemical stability (Pedro et al., 2022; Snyder et al., 2011).

Detection is typically achieved through diode array detectors (DAD), which enable simultaneous multiwavelength monitoring and complete UV-Vis spectral acquisition for each compound (Pedro et al., 2022). For phenolic analysis, detection wavelengths are strategically selected: 254-280 nm for benzoic acid derivatives, 310-330 nm for hydroxycinnamic acids (including rosmarinic and caffeic acids), and 350-370 nm for flavonoids, thereby allowing selective detection and compound identification through spectral comparison with authentic standards (Do et al., 2014).

Despite challenges such as peak overlap in complex polyphenol mixtures, particularly those containing multiple isomers and structurally related derivatives (Mizzi et al., 2020), HPLC offers high sensitivity, reproducibility, and robust analytical capabilities across diverse fields including pharmacology, environmental sciences, and food analysis (Snyder et al., 2011). In *Mentha spicata*, phytochemical analysis using HPLC-DAD has been extensively employed for characterising phenolic acids and flavonoids, with rosmarinic acid consistently identified as the predominant constituent (Do et al., 2014). The technique enables precise quantification through external calibration curves with authenticated standards, facilitating the establishment of quality control, comparison of extracts under different conditions, and correlation between chemical composition and biological activities. Furthermore, HPLC analysis detects compositional variations arising from differences in plant organs, developmental stages, environmental conditions, and exposure to abiotic stress, providing valuable insights into biochemical responses and the optimisation of phytochemical yield (Chai et al., 2014; Pedro et al., 2022).

For this investigation, HPLC-DAD represents the method of choice for elucidating the bioactive compounds of MS extracts, enabling both compound identification through retention time and spectral comparison with reference libraries, as well as precise quantification of major bioactive constituents to establish correlations between chemical compo-

sition and antioxidant capacity. The robustness, versatility, and high-throughput capacity of HPLC establish it as an indispensable tool for comprehensive phytochemical profiling of medicinal plants.

To evaluate the morphological and biochemical changes induced by radiation in MS, two complementary analytical techniques were employed: high-performance liquid chromatography (HPLC) to characterise phenolic compounds and the DPPH radical scavenging assay to assess antioxidant capacity. The following sections detail the experimental procedures and analytical conditions used.

Chapter 2

Materials and Methods

2.1 Preparation of the plants for exposure

Twenty-seven pots of MS were used in this experimental work. The plants were purchased from a local market in Covilhã, Portugal. They were then immediately prepared for the start of the experiment at LabExpoRad, located at UBIMedical, Covilhã, Portugal. Each experimental groups included nine plants (n=9). All experiments were conducted in triplicate for biochemical assays, and results were expressed as a mean ± Standard deviation.

The experimental chambers used in this study were made of transparent acrylic and had the following dimensions: 30,5 ± 0,05 cm in height, 28,7 ± 0,05 cm in width, and 28,5 ± 0,05 cm in depth, corresponding to a total internal volume of 24947,48 ± 128,13 cm^3. The system incorporates access holes located on two opposite sides, equipped with a controlled opening and closing mechanism, allowing measurements of radon concentration inside the chambers A to be taken without compromising the integrity of the experimental environment.

To ensure accurate and controlled experimental conditions, an environmental monitoring system was developed specifically for this study. The system consisted of sensors strategically positioned to continuously measure key atmospheric parameters, including temperature (°C), relative humidity (%), and light intensity (lux) during the exposure time. These sensors were configured to record data at regular intervals, providing real-time monitoring of the environmental conditions during radon measurements. This approach enabled the assessment of potential environmental influences on radon concentration levels and ensured the consistency and reliability of the experimental data collected across all sampling.

Following the acquisition, *Mentha spicata* plants were randomly assigned to three distinct experimental groups, each consisting of nine plants. Group A was exposed to a radon-enriched environment within a properly sealed acrylic chamber designed to prevent radioactive gas leakage from a mineral source composed essentially of quartz and jasper with pitchblende mineralisation. Pitchblende, a uranium-rich mineral containing radium, naturally exhalates radon gas through radioactive decay processes. Group B served as the confinement control, maintained in an identical sealed acrylic chamber under the same environmental conditions as Group A but without radon-emitting rock. Group C constituted the absolute control and was maintained under standard laboratory

conditions without chamber confinement or additional manipulation, as shown in figure 2.1.

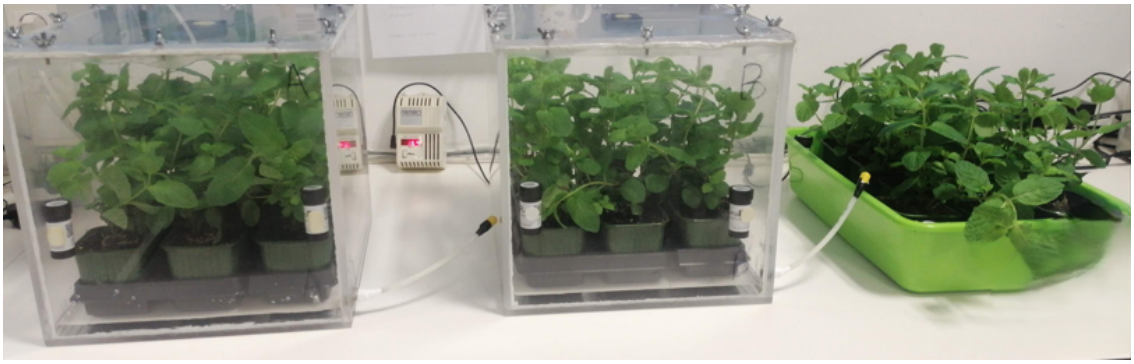

Figure 2.1: Experimental setup showing *Mentha spicata* plants in Chamber A (right) and B (center) sealed acrylic chambers during the 15-day radon exposure period, and the absolute control, Group C (left). Source: LabExpoRad

This experimental design enabled the differential evaluation of radon-induced effects (Group A vs. Group B) and confinement-related effects (Group B vs. Group C), ensuring the validity of the results through the use of appropriate comparative controls.

Throughout the 15-day experimental period, environmental conditions were continuously monitored using specific sensors positioned within the chambers in proximity to the plants, enabling real-time recording of light intensity, relative humidity, and temperature fluctuations. Plants were watered prior to chamber enclosure to minimise disturbance during the exposure period. Additionally, three passive detectors, based on CR-39 polyallyldiglycol carbonate and classified as solid nuclear trace detectors, were strategically positioned in each experimental chamber (Groups A and B) and in the laboratory environment at the onset of the experiment. These detectors provided time-integrated measurements of cumulative radon exposure throughout the entire 15-day period. The CR-39 detection mechanism relies on the interaction of alpha particles with the polymeric material, resulting in microscopic structural damage that, following appropriate chemical treatment, becomes observable and quantifiable through microscopic analysis (Bahu et al., 2021; Soares et al., 2020).

Complementarily, the RAD7 (Durrigde, USA) active detection system was employed immediately before opening chambers A and B to determine instantaneous radon concentrations at that specific moment, after 15 days of exposure. This equipment operates via an external power supply and utilises alpha spectrometry, through which alpha radiation from radon decay is captured and energetically analysed to establish environmental concentrations (Soares et al., 2020), as shown in figure 2.2. The air stream entering the RAD7 passed through a silica gel desiccant to maintain relative humidity below 10% as recommended by the manufacturer three 1-hour measurement cycles were performed, and the mean value was considered. Measurements were carried out immediately after the exposure period to minimize decay losses. The advantages of this technology include high

analytical sensitivity, rapid results, and the capability for continuous real-time monitoring (”RAD7 - Electronic Radon Detector - User Manual,” 2023).

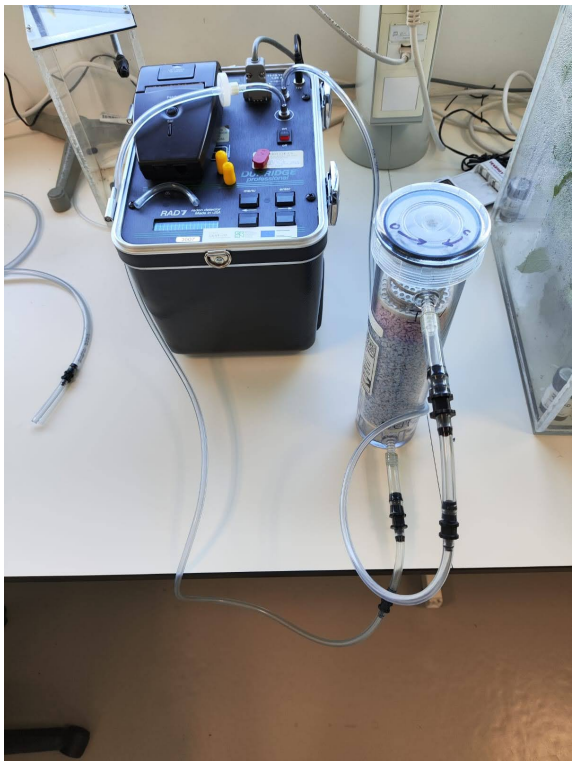

Figure 2.2: RAD7 equipment set up to measure radon inside the test chambers after 15 days of exposure. Source: LabExpoRad

This dual analytical strategy enabled both integrated and instantaneous assessment of radon concentrations, with all results expressed in the standard unit Bq/m³, ensuring comprehensive and accurate characterisation of the exposure conditions experienced by *Mentha spicata* plants throughout the experimental period.

2.2 Preparation of plants after exposure and extraction

After fifteen days of exposure, the aerial parts from MS plants of the three experimental groups (stems and leaves) were harvested and subjected to dehydration by natural drying under ambient conditions (figure 2.3).

Figure 2.3: Drying process of *Mentha spicata*.

The dry material was then subjected to mechanical fragmentation until a powdery product with a consistent particle size was obtained. The processing was conducted individually for each Group (A, B, C), ensuring the preservation of the samples.

A quantity of 2,5 g of crushed material was weighed and homogenised with 25 mL of an 80% v/v ethanol (Honeywell) in properly identified Erlenmeyer flasks. The supernatant solution was replaced every 2 days and collected in appropriately capped and labelled flasks for each sample. This process was repeated until the supernatant no longer presented any colouration, ensuring that the compounds of interest were completely solubilised in the extracting solvent. The extraction procedure was conducted over several weeks to achieve exhaustive extraction of bioactive compounds.

Figure 2.4: Erlenmeyer flasks during the extraction procedure.

After the extraction process, the solutions obtained were filtered through Whatman No. 1 filter paper using a Büchner funnel under vacuum. The filtrate was subsequently transferred to previously weighed round-bottom flasks and the solvent was removed under reduced pressure using a rotary evaporator at 40°C until dryness. The obtained dry extracts were weighed to calculate the extraction yield as (mass of extract/initial dry mass) x100. Yields are summarised in Table2.1, following (Lopes et al., 2024).

Figure 2.5: Rotary evaporator used for the elimination of the extraction solution.

Table 2.1: Sample mass data before and after extraction

	Mass (g) (± 0,01)		
Sample	**Used for extraction**	**After extraction**	**Yield (%)**
A	2,05	0,642	25,66
B	2,05	0,602	24,06
C	2,05	0,633	25,31

Based on the mass determined for each extract, the volume of absolute methanol (LAABCHEM) required for resuspension was calculated to prepare stock solutions with a standardised concentration of 25 mg/mL. The stock solutions were transferred to bottles and stored at 4°C.

2.3 Determination of antioxidant capacity

To prepare the DPPH (Sigma-Aldrich) stock solution, 0,0098 g of reagent was accurately weighed and dissolved in absolute methanol (LAABCHEM) to obtain a solution with a concentration of 0,1 mM.

The preparation of different plant extract concentrations represents a critical step in the protocol, as inadequate concentrations can compromise spectrophotometric measurement accuracy, falling outside the linear detection range of the equipment and necessitating test repetition. The experimental procedure was conducted according to a strictly controlled protocol developed for this work. For each of the five different concentrations diluted from the original stock solution, 3,9 mL of 0,1 mM DPPH solution was added, establishing a standardised volumetric ratio. Simultaneously, control samples were prepared in which the extract volume was replaced with methanol (LAABCHEM), maintain-

ing a constant total volume of the reaction mixture.

A calibration curve using Trolox (0-250µM) was prepared to express results as Trolox Equivalent Antioxidant Capacity (TEAC). The percentage of radical scavenging activity (%RSA) was calculated according to equation 3.1.

All assays were performed in triplicate to ensure reproducibility and statistical reliability of the obtained results. The reaction mixtures were incubated in darkness for 30 minutes at room temperature, conditions necessary for stabilisation of the free radical scavenging reaction.

After the incubation period, the absorbance was read in a Helios Beta spectrophotometer (Thermo Scientific, USA) at 517 nm. Control readings were taken systematically at each change in extract concentration to ensure the validity of the comparisons.

The figure 2.6 shows the DPPH reaction in one of the assays for five different concentrations of *Mentha spicata* extract, arranged in laboratory test tubes. The visual spectrum demonstrates the characteristic colour change associated with the reduction of the DPPH radical: the tubes on the left exhibit an intense purple colour (maximum presence of DPPH•, minimal antioxidant interaction), while the tubes progressively transition through violet and pink tones towards the right, culminating in a pale yellow colour (extensive reduction of DPPH•, maximum antioxidant activity). This colorimetric gradient directly correlates with the concentration-dependent antioxidant capacity of the extracts, where phenolic compounds present in the MS donate hydrogen atoms to the stable DPPH radical (2,2-diphenyl-1-picrylhydrazyl), converting the purple radical form (DPPH•) into the reduced pale yellow form (DPPH-H), measurable at 517 nm.

Figure 2.6: Test tubes during one of the DPPH tests after 30-minute reaction. The tube on the far right represents the control, while the remaining five in sequence are from one of the samples with the lowest and highest concentrations.

To complement the assessment of antioxidant capacity and identify the specific com-

pounds contributing to the observed biological activity, chromatographic analysis using high-performance liquid chromatography (HPLC) was performed.

2.4 Bioactive analysis using high-performance liquid chromatography (HPLC)

Following the evaluation of total antioxidant capacity through the DPPH assay, which provided an overall assessment of the samples' free radical scavenging ability, a detailed chromatographic analysis was conducted to identify and quantify the individual bioactive compounds responsible for the observed antioxidant activity. This approach enabled correlation between the biological activity and the specific phytochemical profile of Mentha spicata under different exposure conditions.

The analysis was performed using an HPLC 1290 Infinity system (Agilent Technologies, USA) coupled to a photodiode array detector (DAD) 1260 G4212B (Agilent Technologies, USA), equipped with a C18 column (YMC, Germany) containing the stationary phase. Detection was performed using the DAD, which enabled simultaneous monitoring of multiple wavelengths within the range of 200 to 600 nm.

The mobile phase consisted of two solvents: solvent A, the organic and polar component, composed of 10% acetonitrile in water; and solvent B, the pH-adjusting component, composed of 0,1% trifluoroacetic acid (TFA) in water. The combination of these solvents resulted in a mobile phase of variable polarity, optimising peak resolution and separation efficiency. The flow rate was maintained at 1 mL/min to ensure adequate separation of the analysed compounds, and the column temperature was held constant at 35°C throughout the analyses to ensure thermal stability and reproducibility.

For sample preparation, a 1 mL aliquot of the stock solution from each sample was dried to completeness and subsequently resolubilized in 1 mL of absolute acetonitrile. The reconstituted samples were transferred to appropriate auto-sampler vials, and the sample was injected in a volume of 50 µL, while the flow rate was 0,1 mL/min to prevent cross-contamination and maintain analytical integrity.

Twenty-one reference standards were selected based on a comprehensive literature review of previous studies on MS (Bardaweel et al., 2018; Dhifi et al., 2013; S. K. Mahendran & Rahman, 2021), representing the main bioactive compounds characteristic of this species. Compound identification was achieved by comparing retention times and detection at specific wavelengths with those of these reference standards analysed under identical chromatographic conditions. Quantification was performed by calculating the peak areas at the corresponding wavelengths and comparing them to external calibration curves constructed from reference standards at various concentrations. The analytical sequence was systematically organised, including samples from Groups A, B, C at different

positions, complemented with ultrapure water samples to monitor possible system contamination and ensure quality control throughout the analytical process.

The experimental procedures described above generated comprehensive data on plants exposed to and non-exposed to radon, as well as their antioxidant capacity and phytochemical composition. These results are organised and presented in the next section.

Chapter 3

Results and Discussion

This section presents and analyses the experimental results obtained through the application of the methodologies described above.

The discussion evaluates antioxidant activity and HPLC characterization of MS samples, focusing on the effects of radon exposure on biochemical and morphological parameters.

3.1 Direct observations of plants and measurement of radon exposure

The temporal evaluation of the plant material's conditions revealed distinct response patterns between the experimental groups. During the experiment, a differentiation between Groups A and B became evident, with the plants in Group A developing an incipient brown pigmentation, while those in Groups B and C remained morphologically stable.

The environmental conditions data is shown in table 3.1.

The temperature was measured in degrees Celsius (°C), the relative humidity in percentage (%), and the light intensity in lux. The data were recorded at 10-minute intervals throughout the entire measurement period in chambers A and B, and in the ambient environ ment C.

Table 3.1: Environmental parameters monitored during the experiments.

Group	Humidity (%RH)	Temperature (°C)	Light (Lux)
A	98,95 ± 0,35	19,01± 0,06	709,88± 20,97
B	98,21± 0,45	20,75± 1,08	661,91± 19,95
C	61,23± 1,21	18,10 ± 0,13	881,31± 21,79

The environmental monitoring revealed distinct conditions across sampling locations. Locations A and B exhibited high relative humidity near saturation (98,9% and 98,2%, respectively), while location C showed significantly lower humidity (61,2%). Temperature remained stable across all sites (18,1 - 20,7°C), light intensity varied considerably, with location C recording the highest values (881,31 lux).

After the full exposure period, radon concentrations were quantified using the RAD7 detection system, which covered both the internal environment of the chambers A and B. The experimental protocol continued with the opening of the chambers, removal of the plant

material, and packaging in appropriate and identified containers for the natural drying process.

The visual and olfactory characterisation carried out during the sample preparation confirmed substantial differences between the treatments. Group A showed signs of advanced deterioration, including widespread brown discolouration, loss of turgor, and the presence of aromatic compounds indicative of degradation processes. In contrast, Groups B and C preserved their morphological integrity, maintaining the characteristic green colour and the typical aromatic profile of MS. The quantitative results of the radon measurements, obtained through active and passive methodologies, are presented in table 3.2. The RAD7 detector (active method) has a detection limit of 4 Bq/m³. Group A shows high radon concentration detectable by both methods. Group B and C presented concentrations below the detection limit of the active method but were quantifiable using passive detectors, indicating lower radon levels in these environments

Table 3.2: Average radon concentration measurements from different experimental groups using active and passive methods.

Average Radon Concentration (Bq/m³)			
Group	A	B	C
Active method	65280 ± 930	BLD*	BLD*
Passive method	52000 ± 10000	176 ± 94	310 ± 100

*BLD: Below Limit of Detection.

The radon concentration measurements obtained reveal significant differences between the two detection systems, reflecting their distinct analytical characteristics. Group A, exposed to the pitchblende mineral source, exhibited substantially elevated radon concentrations with both methods. The observed discrepancy between RAD7 and CR-39 measurements can be attributed to their operational differences: RAD7 provides instantaneous radon concentrations at the specific moment before chambers opening, whereas passive detectors integrate cumulative exposure over the entire 15-day experimental period inside the chambers and in the environment. It can also be concluded that Chamber A in relation to Chamber B provides a good comparison because it shows a significant difference in the amount of radon.

Although both techniques are complementary, the discrepancy between instantaneous (RAD7) and time-integrated (CR-39) measurements must be considered when interpreting cumulative exposure data.

These quantified exposure levels establish a solid foundation for subsequent analysis of radon effects on the antioxidant capacity and phytochemical profile of MS, enabling correlation of observed biochemical alterations with well-characterised radiological conditions.

The DPPH assay were conducted in triplicate and the results expressed as mean ± standard error. The statistical analysis was performed using linear regression and Microsoft Excel. The calculation of radon concentration using the active method was performed with

the assistance of CAPTURE software, and the results are expressed as mean ± standard deviation determined directly in the software.

3.2 Antioxidant activity results

The antioxidant activity of MS extracts was evaluated through the DPPH free radical scavenging assay, with the results expressed as the percentage of radical scavenging activity (%RSA) and the inhibitory concentration at 50 (IC_{50}) (table 3.3).

The percentage of radical scavenging activity (%RSA) was calculated (3.1):

$$\%RSA = \frac{\text{Ac-As}}{\text{Ac}} \times 100 \quad (3.1)$$

Where %RSA represents the percentage of DPPH radical scavenged; Ac corresponds to the average absorbance of the control samples for each concentration; As represents the average absorbance of each concentration of the test samples.

High %RSA values indicate a greater antioxidant capacity of the evaluated extract.

Antioxidant activity was additionally quantified using the IC_{50} parameter, defined as the concentration of the antioxidant required to reduce the initial concentration of the DPPH radical by 50%. This parameter is an inverse indicator of antioxidant capacity: lower IC_{50} values correspond to higher antioxidant activity, since lower concentrations of extract are sufficient to neutralise the same amount of DPPH radicals (Gulcin & Alwasel, 2023; Molyneux, 2003; Nickavar et al., 2008).

Table 3.3: DPPH Radical Scavenging Activity (%RSA) and IC_{50} values of *Mentha spicata* extracts from experimental groups

Samples	**A**	**B**	**C**
%RSA	58,34 ± 3,93	65,47 ± 8,44	54,73 ± 3,07
IC_{50} (µg/mL)	442,49 ± 3,93	302,66 ± 8,36	445,88 ± 3,31

The IC_{50} values were determined by graphical interpolation from the relationship the concentration of the extracts to the respective percentage of inhibition.

The results are expressed both in terms of IC_{50} (µg/mL) and as a percentage of RSA, allowing for a comprehensive assessment of the antioxidant capacity of the extracts. The interpretation of these parameters requires careful analysis, considering the inherent variability in biological systems and the influence of experimental conditions on the activity of bioactive compounds present in MS extracts.

The evaluation of antioxidant capacity revealed significant differences between the three

experimental groups. Group B (control of Group A) exhibited the highest antioxidant activity with 65,47 ± 8,44 %RSA, followed by Group A (exposure to radon) with 58,34 ± 3,93 %RSA, and by Group C (absolute control) with 54,73 ± 3,07 % RSA, as shown in Figure 3.1.

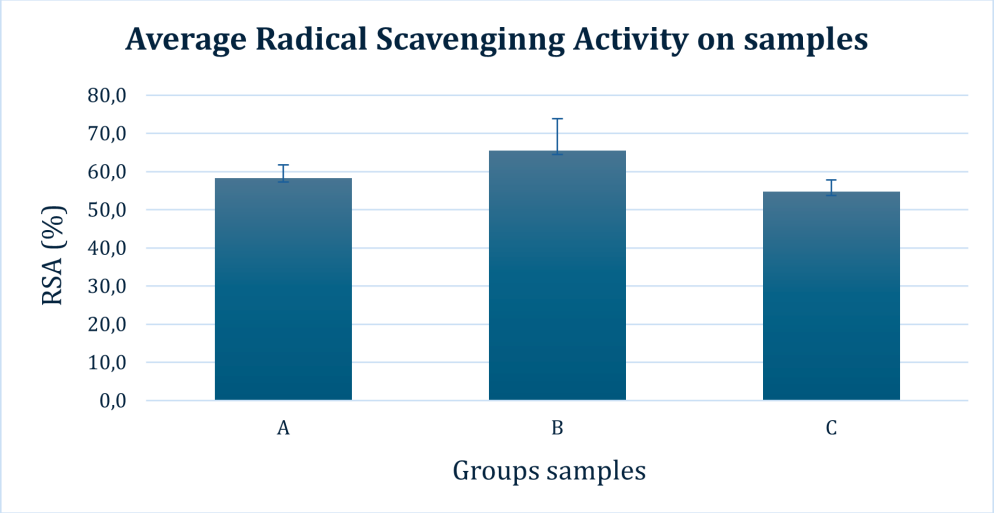

Figure 3.1: Average radical scavenging activity (%RSA) of Mentha spicata concentration extracts from experimental groups evaluated by the DPPH assay.

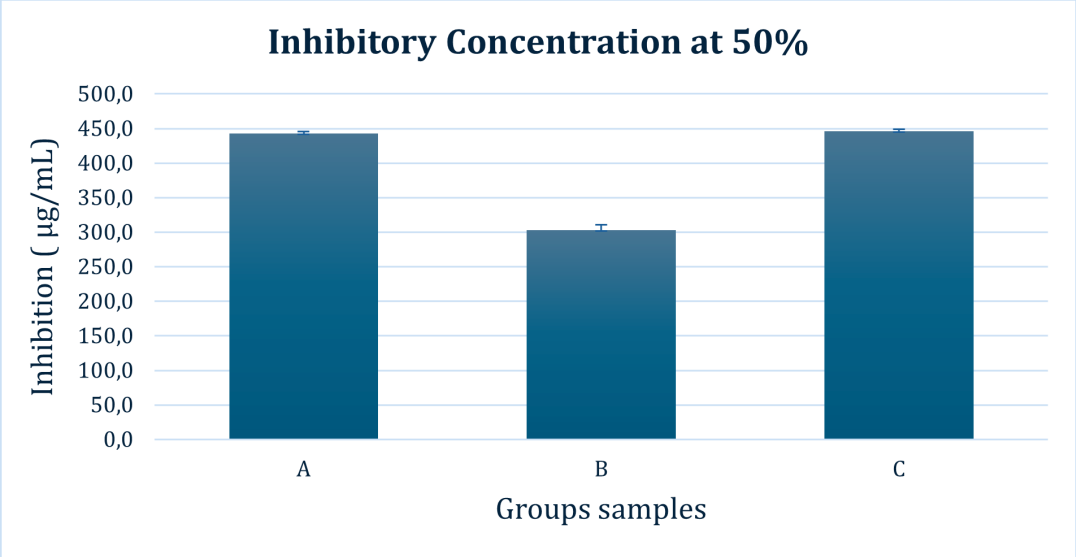

Figure 3.2: Half-maximal inhibitory concentration (IC_{50}) values of *Mentha spicata* extracts from experimental groups.

The higher RSA in Group B suggests that moderate confinement may have triggered a mild stress response, increasing the production of antioxidant metabolites. This agrees with literature reports describing stress-induced enhancement of phenolic biosynthesis in *Mentha* species. Group A, despite exposure to radon, showed intermediate %RSA values, potentially reflecting a balance between confinement-induced stress (like Group B)

and the effects of ionising radiation. Radon exposure and its decay products generate reactive oxygen species (ROS) that can overwhelm cellular antioxidant systems, resulting in an antioxidant capacity lower than that of Group B, but still higher than the absolute control. Group C, maintained under standard laboratory conditions without confinement or additional stressors, exhibited the lowest antioxidant activity, establishing the baseline for non-stressed plants.

The IC_{50} values, which represent the concentration of the extract needed to inhibit 50% of DPPH radicals, corroborate the %RSA results, as shown in Figure 3.2. Group B showed the lowest IC_{50}, confirming superior antioxidant efficiency, followed by Groups A and C. The difference between Groups A and C demonstrates that exposure to radon, combined with confinement, promotes an antioxidant response that surpasses the absolute control, although less pronounced than that of isolated confinement.

The graphics obtained for each group confirm the robustness and reliability of the analytical method. The regression equations (Group A: y = 159,96x + 24,585; Group B: y = 110x + 39,839; Group C: y = 177,11x + 11,636) reflect the differential sensitivities of the extracts to the DPPH assay, with Group B showing the lowest slope, indicative of greater antioxidant efficiency that requires lower extract concentrations to achieve the same radical scavenging capacity and, therefore, it can be assessed that Group B has an antioxidant capacity approximately 12% higher than Group A, indicating that exposure to radon influences the metabolic response. This conclusion is reinforced by the results obtained in the determination of the IC_{50}. Group B shows an IC_{50} of approximately 300 μg/mL, where Group A shows a value of approximately 430 μg/mL.

3.3 HPLC analysis

Chromatographic analysis allowed for the identification and quantification of phenolic compounds present in MS extracts from the three experimental groups (Figure3.3). The results reveal significant differences in the phytochemical profile between the groups, providing a molecular basis for the variations observed in antioxidant capacity.

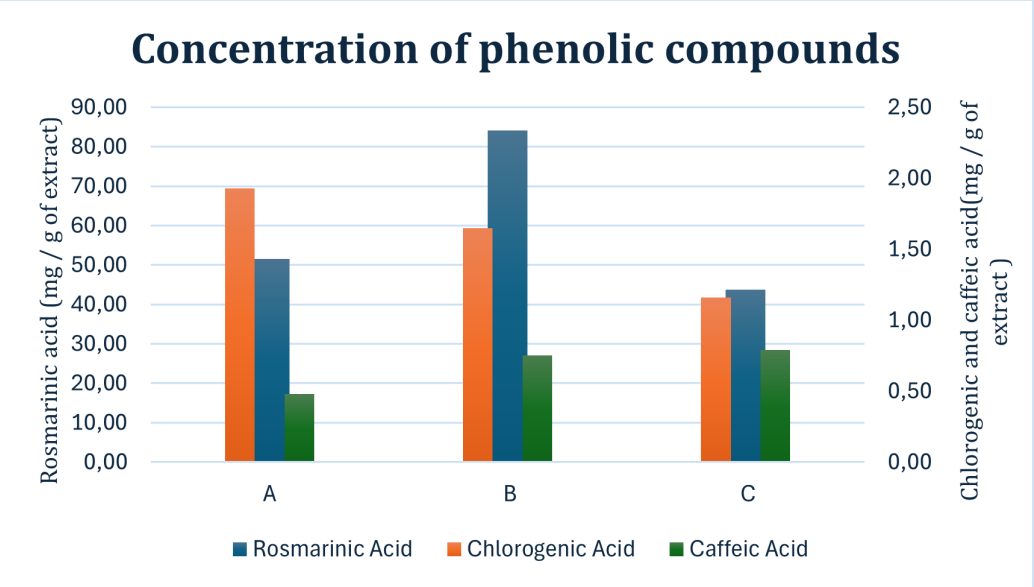

Figure 3.3: Concentration of the three most abundant phenolic acids identified by HPLC in plant extracts. Rosmarinic acid showed the highest concentration across all samples, followed by chlorogenic acid and caffeic acid. A, B, and C represent the three different sample groups. Values are expressed in mg/g of extract. Group B exhibited the highest phenolic acid content, particularly for rosmarinic acid (84,12 mg/g of extract).

The chromatographic profile obtained revealed the presence of 21 compounds; however, eleven compounds, such as rosmarinic acid, emerged as the predominant phenolic compounds in all experimental groups, with concentrations substantially higher than those of the other identified compounds.

Table 3.4: Quantitative HPLC-DAD analysis of phenolic acids and flavonoids in extracts of *Mentha spicata* L. extracts from experimental sample groups.

Compound	Sample A (mg/g extract)	Sample B (mg/g extract)	Sample C (mg/g extract)
Rosmarinic Acid	51,48	84,12	43,72
Chlorogenic Acid	1,93	1,65	1,16
Caffeic Acid	0,48	0,75	0,79
Myricetin	6,09	8,03	3,81
Epicatechin	2,36	2,05	1,45
Kaempferol	1,42	1,90	2,06

Group B exhibited the highest concentration of rosmarinic acid (84,12 mg/g of extract), followed by Group A (51,48 mg/g of extract) and Group C (43,72 mg/g of extract). This pattern is consistent with the results of the DPPH assay, as rosmarinic acid is recognized as a potent natural antioxidant, widely described in the literature as the main contributor to the antioxidant activity in species of the genus *Mentha*. The confinement markedly stimulated the biosynthesis of this compound, while exposure to radon resulted in intermediate values between both controls. This pattern suggests that ionizing radiation can simultaneously exert stimulating and degrading effects on phenolic compounds, attenuating the beneficial effect of confinement stress observed in Group B.

Chlorogenic acid, a significant natural antioxidant, exhibited relatively consistent concentrations among the groups (A: 1,93 mg/g; B: 1,65 mg/g; C: 1,16 mg/g), with Group A displaying the highest value. This result suggests that exposure to radon may have specifically stimulated biosynthetic pathways related to this compound, possibly as a response to radiation-induced oxidative stress.

Caffeic acid showed low and relatively homogeneous concentrations among the groups (A: 0,48 mg/g; B: 0,75 mg/g; C: 0,79 mg/g), with an inverse trend compared to the other compounds, being slightly higher in the absolute control.

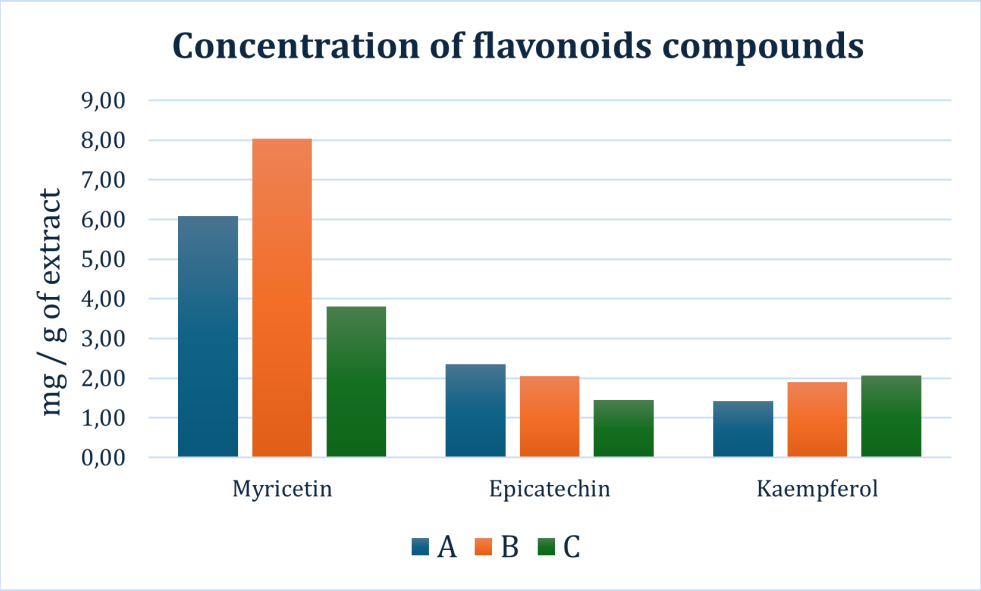

Figure 3.4: Concentration of the three most abundant flavonoids identified by HPLC in plant extracts. Myricetin was the predominant flavonoid in all samples, with significantly higher levels compared to epicatechin and kaempferol. A, B, and C represent the three different sample groups. Values are expressed in mg/g of extract. Group B showed the highest myricetin concentration (8,03 mg/g of extract), while epicatechin and kaempferol were present in lower and more uniform concentrations across all groups.

Myricetin showed higher concentrations in Group B (8,03 mg/g) compared to Groups A (6,09 mg/g) and C (3,81 mg/g), as shown in Figure 3.4. Group C showed the highest concentration of this flavonoid, contrary to the general trend observed for other compounds. This inversion may suggest that confinement or exposure to radon has a negative impact on the biosynthesis, or confirms the imbalance of homeostasis of this specific compound and the others.

Kaempferol, a significant flavonoid with antioxidant and anti-inflammatory properties, exhibited relatively uniform concentrations among the groups (A: 1,42 mg/g; B: 1,90 mg/g; C: 2,06 mg/g), with a slight tendency toward reduction in the groups subjected to stress.

Epicatechin, although present in moderate concentrations, was more abundant in Group A (2,36 mg/g), followed by Group B (2,05 mg/g) and Group C (1,45 mg/g), reinforcing the

general pattern of flavonoid biosynthesis stimulation under stress conditions.

The R^2 values for myricetin (R^2 = 0.9999), kaempferol (R^2 = 0.9998), epicatechin (R^2 = 0.9997), rosmarinic acid (R^2 = 0.9995), chlorogenic acid (R^2 = 0.9986), and caffeic acid (R^2 = 0.9971) indicated the validation of the method and ensured accurate quantification and reliability.

These results collectively indicate an imbalance in plant homeostasis, specifically that ROS production exceeds elimination capacity, resulting in oxidative stress and visible degradation in the plant, as observed in Group A. To understand the reason for the discrepancy between the values obtained, further analysis is needed on the defence mechanisms used under stress caused by ionising radiation, in order to understand which substances are produced or consumed in greater quantities.

3.4 DPPH vs HPLC

A strong positive correlation was observed between the integrated analysis of the chromatographic data and DPPH assay results, in particular, the total phenolic compound content, rosmarinic acid, and antioxidant capacity. Group B, which simultaneously exhibited the highest concentrations of rosmarinic acid, catechin, and quercetin, also displayed the highest RSA and the lowest IC_{50} value.

These results suggest that moderate confinement increases the antioxidant capacity of MS by stimulating the biosynthesis of phenolic compounds, with a particular emphasis on rosmarinic acid as the main mediator of this adaptive response.

3.5 Limitations and Methodological Considerations

It is important to recognize that a comprehensive assessment of antioxidant capacity requires the application of multiple complementary methodologies, as the mechanisms of antioxidant action are complex and multifaceted. In this context, although the DPPH assay has provided valuable data on free radical scavenging activity, this method should be considered an initial screening tool. The combination with other methods, such as FRAP (Ferric Reducing Antioxidant Power) or ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), which evaluate different aspects of antioxidant activity, would have allowed for a more complete characterisation (Munteanu & Apetrei, 2021).

The FRAP assay is particularly suitable for evaluating water-soluble antioxidants through the reduction of ferric complexes, while the ABTS method, based on the sequestration of the cationic radical ABTS•+ (absorption at 734 nm), offers greater versatility in terms of solubility, being applicable in both aqueous and lipophilic media (Kaddour et al., 2022;

Munteanu & Apetrei, 2021). Future studies that incorporate the combined application of these methods will enable a more comprehensive and reliable assessment of the antioxidant capacity of MS under these stress conditions, providing a more robust basis for evaluating the defence mechanisms and responses to ionising radiation.

Future work should increase the number of biological replicates per group to strengthen statistical power.

Conclusions

This study evaluated the effects of radon exposure on the morphology and biochemical profile of *Mentha spicata* L., integrating radiological and phytochemical analyses. The results demonstrate that exposure to radon-rich atmospheres induces measurable physiological and metabolic alterations in this species.

The plants exposed to radon (Group A) showed a reduction in antioxidant capacity and in rosmarinic acid content compared with the confined control (Group B), indicating that ionizing radiation affects the equilibrium of secondary metabolism and triggers oxidative stress. Confinement alone stimulated the biosynthesis of phenolic compounds, particularly rosmarinic acid, catechin and quercetin, revealing an adaptive response to moderate environmental stress. These patterns were confirmed by consistent trends in both DPPH and HPLC analyses.

Although the results are conclusive within the tested conditions, differences in relative humidity and illumination between chambers represent confounding factors that may have influenced plant responses. Future experiments should control these environmental parameters, increase biological replication and incorporate complementary assays (FRAP, ABTS) and LC-MS analysis to provide a more complete characterization of antioxidant defenses under radiation stress.

In summary, this work provides quantitative baseline data on the interaction between natural radioactivity and plant antioxidant systems. It contributes to the emerging field of plant radiobiology and supports future research exploring the potential of aromatic species such as *Mentha spicata* L. as bio-indicators of environmental radiation and, eventually, as candidates for phytoremediation studies. These findings also highlight the importance of understanding plant responses to natural sources of ionizing radiation, contributing to environmental risk assessment and to the development of sustainable strategies for radiological protection.

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