

Theoretical foundations of EPR spectroscopy and UV-Vis spectrophotometry – useful methods of plant antioxidant extracts from novel ecosystems

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Abstract: This article reviews the application of the methods of electron paramagnetic resonance (EPR) spectroscopy and ultraviolet–visible (UV-Vis) spectrophotometry in the examination of plant antioxidants. It proposes their use in obtaining antioxidant raw materials from plants particularly those living in harsh environments exposed to extreme external factors. It presents the principles of the operation of the EPR spectrometer and UV-Vis spectrophotometer, as well as the role of spectroscopic parameters in analysing free radicals and antioxidants for medical purposes. In this article, an X-band (9.3 GHz) EPR spectroscopy is described as well as the role of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals in the analysis of plant antioxidant properties. Antioxidants quench the EPR and UV-Vis spectra of DPPH. EPR and UV-Vis spectra decrease with increasing time of interaction of DPPH free radicals with the antioxidant plant extracts until the minimum is reached. After that the magnitude of the spectra remains constant. Extracts with stronger antioxidant properties characterise the lower minimum values of the EPR spectra amplitude and UV-Vis absorbance. The described spectroscopic methods are helpful in finding plant extracts with strong antioxidant properties for medical purposes. This work includes examples of quenching of the EPR and UV-Vis spectra of DPPH free radicals caused by their interactions with plant extracts, including examples of extracts obtained from the single raw plant materials and their combinations. We demonstrate the influence of habitat conditions on the antioxidant properties of the plant extracts. The importance of developing research into plant products obtained from disturbed environments was emphasised.

Keywords: comparative habitats studies of variety in height and slope aspect, disturbed habitat conditions, EPR spectroscopy, free radicals, medicine, plant antioxidants, UV-Vis spectrophotometry

INTRODUCTION

The processes involved in ecosystem functioning, including novel ecosystems, depend on the composition of plant species' functional traits. Plant functional traits reflect responses to specific habitat conditions by synthesising primary and secondary metabolites. Plants synthesise a variety of biochemical compounds that support the life of all heterotrophic and detritivorous organisms and enable ecosystem functioning. The quality and quantity of the biochemical compounds involved depend on the habitat conditions, which also enable an assessment of the habitat's harshness and the potential for intraspecific variation in adaptivity among individuals of the constant species. Among secondary metabolites, antioxidant intensity is an under-utilised yet highly sensitive functional trait in ecological studies, serving as a dynamic indicator of plant and organismal responses to habitat harshness. While traditional functional traits (e.g., leaf mass per area, plant height) provide insight into structural adaptation, antioxidant capacity (enzymatic and non-enzymatic) directly measures the metabolic effort required to manage reactive oxygen species (ROS) produced under extreme abiotic stress. Humans are taking advantage of the secondary metabolites that plants synthesise to survive. Plants have served as a natural source of medicinal compounds for millennia. Most of these substances are secondary metabolites, which directly influence a plant's growth or development (Bartos, 2021).

The extremely harsh conditions of a novel ecosystem provide the opportunity to follow the synthesis of antioxidants in a unique field experiment. The mineral habitats of post-mining sites are commonly spontaneously colonised by plant individuals and other organisms. The very demanding mineral habitats of these post-mining sites reveal a wide range of previously unknown plant responses to the harsh conditions (Talik *et al.*, 2018; Milewska-Hendel *et al.*, 2021; Bacler-Żbikowska *et al.*, 2025). The complex interdisciplinary aspects of a plant individual's response to its habitat conditions constrain the need for the application of various research methods that enable the identification of its adaptation processes. The useful techniques are electron paramagnetic resonance (EPR) spectroscopy and the ultraviolet-visible (UV-Vis) spectrophotometry method, which can be used to study plant antioxidants produced in post-mining habitats (Bacler-Żbikowska *et al.*, 2025).

Plant antioxidants are synthesised by individuals to protect the organism against free radicals (Miller *et al.*, 2008; Czerwiecki, 2009; Gryszczyńska, Gryszczyńska and Opala, 2011; Sieniawska, 2012; Bartos, 2021; Chwałowska *et al.*, 2024; Pilawa *et al.*, 2024). They include polyphenols, carotenoids, tocopherols, and ascorbic acid (Czerwiecki, 2009; Gryszczyńska, Gryszczyńska and Opala, 2011; Sieniawska, 2012; Bartos, 2021). These antioxidants are synthesised in response to harsh habitat conditions.

Post-mining spoil heaps represent very specific habitat conditions. They consist of piles of materials left behind after mineral extraction through mining activities. For a long time, post-mining sites have been considered to be biological deserts. However, biological and environmental studies have proved that these mineral post-mining sites are spontaneously colonised by living organisms, particularly plant individuals, which develop a set of functional features adapted to the specific habitat conditions of their new ecosystem (Woźniak and Cohn, 2007; Tropek *et al.*, 2012; Kowolik *et al.*, 2025). The colonising plant

species become assembled in non-analogous species communities, leading to the establishment of novel ecosystems *sensu* Hobbs, Higgs and Hall, (2013). The herbaceous vegetation, can have a varied amount of biomass (Ryś *et al.*, 2024) and is in a dynamic state, which is ultimately replaced in the successional process by a spoil heap forest with a diverse species composition (Woźniak *et al.*, 2022). The knowledge about mechanisms of these processes, particularly the biochemical ones, are limited in published references. The natural processes of novel ecosystem development on mineral habitats of post-mining sites are very common and effective as a result of nature-based solutions (Błońska *et al.*, 2019; Błaszczkowski *et al.*, 2021; Bakr *et al.*, 2024; Błońska *et al.*, 2024). Understanding the underlying biochemical mechanisms of these nature-based processes within developing novel ecosystems is essential for the effective management of these mineral habitats of post-mining spoil heaps (Woźniak *et al.*, 2022).

This review aims to present the current state of knowledge about possibilities of using EPR spectroscopy and the UV-Vis method in studies of the magnitude and kinetics of the interactions of plant antioxidants and free radicals. Moreover, this study aims to demonstrate the application of EPR and UV-Vis spectroscopy in research into the ability of some species of plants to survive in novel ecosystems. It proposes the application of plants from disturbed habitats as a source of antioxidants.

This work highlights the need to develop knowledge of plant metabolism in harsh environments, particularly where they are exposed to external factors such as intense sunlight, limited access to water, polluted and barren soils, and strong winds. Our preliminary research has shown the strong antioxidant properties of extracts obtained from extracts of plants growing in disturbed areas (Bacler-Żbikowska *et al.*, 2023). Antioxidant plant products are of great importance in medicine and pharmacy, which is why we have discussed this topic in this article. Multidirectional research is underway on the influence of the natural environment on the antioxidant potential of plant materials, particularly the relationship between environmental parameters and the antioxidant activity of plant metabolites, and the possibility of using plant products obtained from disturbed environments, e.g., post-mining spoil heaps, in medicine. The therapeutic use of plant products without understanding the impact of the ecosystem on their antioxidant properties would not be possible. Our work, which is illustrative in nature, aims to highlight this issue and to outline the use of beneficial research methods.

MATERIALS AND METHODS

A review of the scientific literature was conducted to gather information on the theoretical principles of EPR spectroscopy and UV-Vis spectrophotometry as useful methods for investigating plant antioxidant extracts from novel ecosystems. The search covered original articles and peer-reviewed review articles published between 1994 and 2025 in Polish and English that included the following keywords relevant to the research area: EPR spectroscopy, UV-Vis spectrophotometry, free radicals, plant antioxidants, medicine, and disturbed habitat conditions. The detailed review was conducted in March 2025. Given the authors' extensive experience applying EPR and UV-Vis methods in biology, pharmacy, and medicine, publications by the authors of the present work have also been included in the citations.

RESULTS

THEORETICAL BASIS OF ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

The phenomenon of electron paramagnetic resonance and its spectrum of free radicals

The phenomenon of electron paramagnetic resonance (EPR) is a spectroscopic technique which occurs in a magnetic field and concerns unpaired electrons (Stankowski and Hilczer, 2005; Weil and Bolton, 2007; Pilawa and Ramos, 2017). The energy levels of unpaired electrons belonging to paramagnetic molecules, e.g. to free radicals, are split in a magnetic field. This effect is due to the quantisation of the electron's angular momentum in a magnetic field. The absorption of microwave radiation is responsible for the transition of unpaired electrons to higher energy levels. Relaxation processes cause the transition of unpaired electrons from excited states to lower energy levels.

The phenomenon of electron paramagnetic resonance occurs when the following equation is fulfilled (Stankowski and Hilczer, 2005):

$$h \cdot \nu = g \cdot \mu_B \cdot B_r \quad (1)$$

where: h = Planck's constant, ν = microwave radiation frequency, g = spectroscopic splitting coefficient, μ_B = Bohr magneton, B_r = resonant magnetic induction.

According to the above equation (1), the EPR spectrum of free radicals can only be measured with the correctly correlated value of the magnetic field generated by the electromagnet with the microwave frequency. When equation (1) is met, the unpaired electrons of free radicals absorb microwave radiation, and the EPR spectrum appears. Spectrum magnitude depends on the number of free radicals in the tested system. The spectroscopic splitting coefficient (g) characterises the type of free radicals (Stankowski and Hilczer, 2005; Weil and Bolton, 2007; Pilawa and Ramos, 2017).

The use of the electron paramagnetic resonance method when study the interaction of antioxidants with free radicals

Studies on the interactions of antioxidants with free radicals are performed using an EPR spectrometer and the model DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals (Yordanov and Christova, 1994; Tirzisz and Bartosz, 2010; Pilawa and Ramos, 2017; Bartosz, 2021; Łomankiewicz *et al.*, 2021; Łomankiewicz, 2022; Zdybel, Pilawa and Chwałowska, 2023; Pilawa *et al.*, 2024). The EPR spectrometer consists of an electromagnet, a resonator, a microwave bridge, waveguides, a circulator, a detector, a power supply, and a recorder (Stankowski and Hilczer, 2005).

The chemical structure of DPPH free radicals is shown in Figure 1. Paramagnetic DPPH contains an unpaired electron. The EPR spectrum of DPPH free radicals is shown in Figure 2. Antioxidants neutralise DPPH free radicals in the reaction as shown in Figure 3. The EPR spectra of DPPH free radicals initially decrease with increasing time of contact of the antioxidant with DPPH, and after reaching the minimum value, it remains constant (Fig. 4).

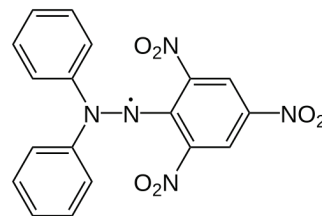


Fig. 1. Chemical structure of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, '•' = an unpaired electron; source: own elaboration

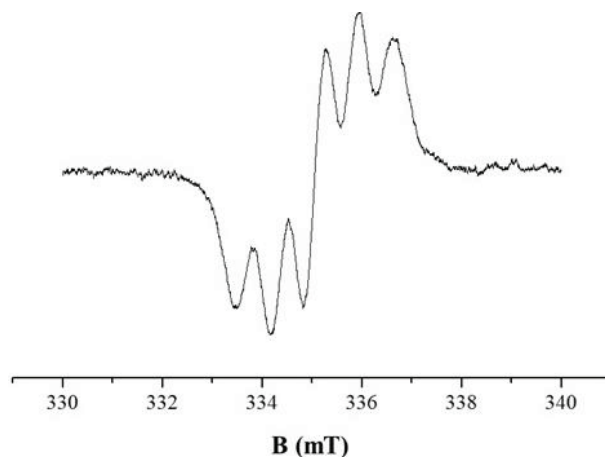


Fig. 2. Exemplary electron paramagnetic resonance (EPR) spectrum of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals; B = magnetic induction of the magnetic field generated by the spectrometer's electromagnet; source: own elaboration

THEORETICAL BASIS OF ULTRAVIOLET-VISIBLE (UV-VIS) SPECTROPHOTOMETRY

The principle of the study and phenomena accompanying the measurement of UV-Vis spectra

Studies using UV-Vis spectrophotometry concern the analysis of the absorption of electromagnetic radiation by the tested substances (Kęcki, 1998; Thomas and Burgees (eds.), 2017;

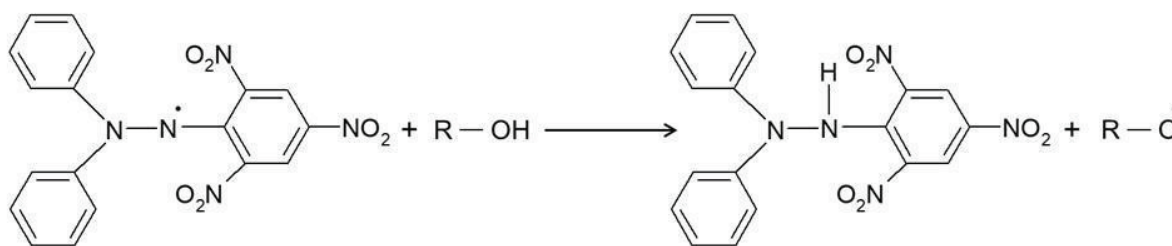


Fig. 3. Reaction of an antioxidant with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical; source: own elaboration

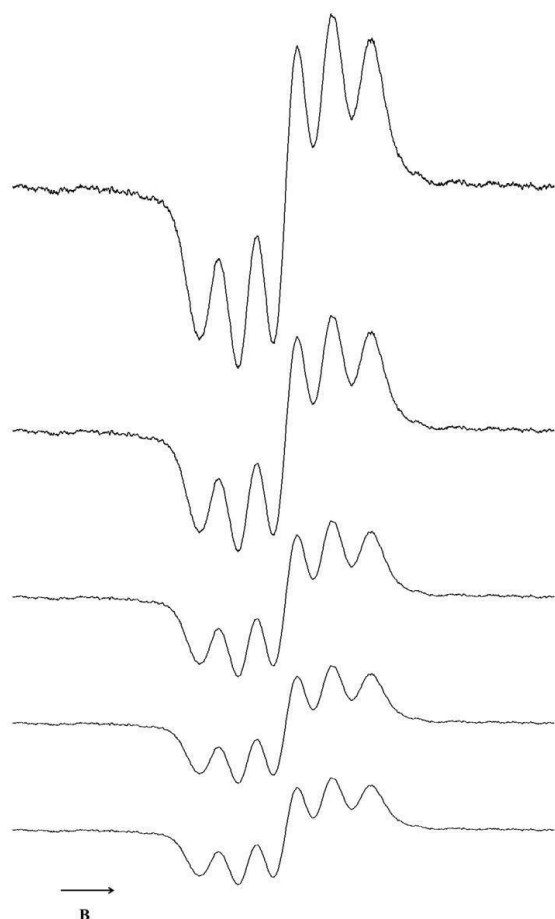


Fig. 4. Changes in electron paramagnetic resonance (EPR) spectra of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals with increasing time of interaction with an antioxidant; B as in Fig. 2; source: own study

Khemnar, 2024; Minchiotti, 2024). The UV-Vis spectrophotometer consists of a source of electromagnetic radiation in the ultraviolet and visible light range, and uses a monochromator in the form of a diffraction grating or prism, a quartz or silica cuvette containing the tested substance, and a photoelectric detector (Kęcki, 1998; Patel *et al.*, 2022). The examined irradiated sample partially absorbs electromagnetic waves, and part of the radiation passes through the sample. The photoelectric detector measures the intensity of electromagnetic radiation that is transmitted through the sample (Minchiotti, 2024).

The intensity of monochromatic light directed on the sample (I_o) is equal to the sum of the intensity of absorbed light (I_a), scattered light (I_r) and light transmitted through the sample (I_t) (Kęcki, 1998; Patel *et al.*, 2022):

$$I_o = I_a + I_r + I_t \quad (2)$$

Since $I_r \ll I_o$, the above formula becomes (Kęcki, 1998; Patel *et al.*, 2022):

$$I_o = I_a + I_t \quad (3)$$

The absorbed radiation causes the excitation of molecules and thus the transition of electrons to higher energy levels occurs. Absorption in the visible and ultraviolet ranges depends on the type of molecule and is used in qualitative and quantitative analyses of molecules (Kęcki, 1998).

The use of the UV-Vis method when studying the antioxidant properties of plant substances

Studies on the antioxidant properties of substances using the EPR method involve measuring the electromagnetic radiation transmitted through the sample in contact with the model DPPH free radicals in ethanol, or methanol solutions (Kęcki, 1998; Jabłońska and Szymczyk, 2008; Indis and Kurniawan, 2016; Thomas and Burgees (eds.), 2017; Patel *et al.*, 2022; Shi *et al.*, 2023; Khemnar, 2024; Minchiotti, 2024). The DPPH radical molecule absorbs radiation at a maximum wavelength of approximately 517 nm (Kęcki, 1998; Shi *et al.*, 2023). The maximum absorption of radiation by DPPH in contact with the tested substance corresponds to the minimum radiation intensity that is measured after leaving the tested system.

During examination of the interaction of antioxidants with DPPH using the UV-Vis method, the spectra are measured as absorbance (Abs) defined by the following formula (Kęcki, 1998; Thomas and Burgees (eds.), 2017; Khemnar, 2024; Minchiotti, 2024):

$$\text{Abs} = \log(1/T) = \log(I_o/I_t) \quad (4)$$

where: T = transmittance ($T = I_t/I_o$), I_o = intensity of the wave directed from the source on the DPPH solution, I_t = intensity of the waves transmitted through the DPPH solution.

The absorbance value decreases with an increase in the quenching of DPPH free radicals by antioxidants, for example by antioxidants in plant extracts (Indis and Kurniawan, 2016; Shi *et al.*, 2023). Examples of quench of UV-Vis absorbance with increasing interaction time of DPPH with antioxidant compounds are shown in Figure 5.

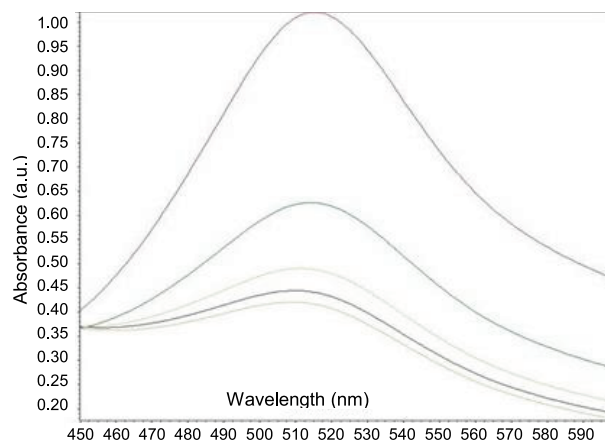


Fig. 5. Changes in UV-Vis absorbance spectra of 2,2-diphenyl-1-picrylhydr (DPPH) with increasing time of interaction with an antioxidant; a.u. = arbitral units; source: own study

SELECTED MEDICAL APPLICATIONS OF THE ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY AND ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY IN RESEARCH INTO THE ANTIOXIDANT PROPERTIES OF PLANT EXTRACTS

The spectroscopic parameters of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical EPR spectra are used in the testing of plant antioxidants effective in counteracting antioxidative stress associated with diseased states in organisms. Antioxidant plant

extracts quench the EPR spectra of free radicals. After adding the extract to the DPPH methanol (or ethanol) solution, the amplitude (A) of the EPR spectra of DPPH free radicals decreases with increasing contact time of DPPH with the plant extract, reaching a minimum value after a certain time, which then remains constant regardless of the increase in the exposure time of the extract in contact with DPPH (Fig. 6).

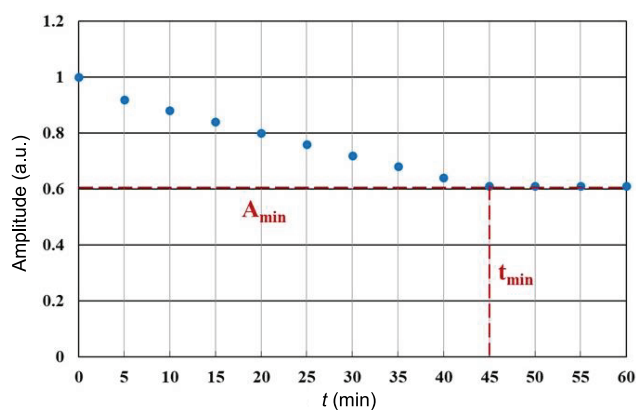


Fig. 6. Changes of amplitude of electron paramagnetic resonance (EPR) spectra of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals with increasing time of interactions of the plant extract with DPPH; a.u. = arbitrary units; $A_{\min}$ = minimal value of amplitude, $t_{\min}$ = time of reaching the minimum amplitude by the EPR spectrum of DPPH free radicals; source: own study

The minimum amplitudes of the DPPH EPR spectra depend on the magnitude of the interactions between DPPH free radicals and the antioxidant. Determining the minimum amplitudes of the DPPH EPR spectra interacting with various plant antioxidants provides the possibility of a comparison of the magnitude of their interaction with free radicals. The lower minimum amplitudes correspond to the stronger antioxidants. For the higher speeds of free radical neutralisation by plant antioxidants, amplitudes of the EPR spectra of DPPH free radicals reached faster than their minimum values. The curve in Figure 6 shows the complete kinetics of the interaction of plant antioxidants with free radicals.

For example, EPR spectroscopy was used to compare the magnitude and kinetics of interactions of infusions obtained from mixtures of globe artichoke (*Cynara scolymus*) and dandelion (*Taraxacum officinale*) flowers with free radicals (Gaj *et al.*, 2024b). The antioxidant properties of the infusions prepared from mixtures with plant component weight ratios of 1:1, 2:1, and 1:2, were examined. Gaj *et al.* (2024b) suggest that the kinetics of free radical interactions in the tested infusions are similar. According to these results, an infusion of a mixture of artichoke leaves and dandelion flowers with a weight ratio of 2:1 had the strongest free radical – quenching effect. On the other hand, an infusion of the above mentioned plant materials with a weight ratio of 1:1 in the mixture had the weakest free radical – quenching effect.

The EPR method was used to examine the interaction of infusions obtained from teasel (*Dipsacus fullonum*) leaves and root with free radicals, as well as an infusion from a complex plant material containing *D. fullonum* herb, purple coneflower (*Echinacea purpurea*) herb, and elderberry (*Sambucus nigra*) fruit (Gaj *et al.*, 2024a). The effect of UVA radiation on the antioxidant properties of these tested extracts was determined. This work showed that the antioxidant effects of the infusion made from

D. fullonum herb exposed to UVA radiation are greater than the antioxidant effects of the infusion from the herb not exposed to UVA radiation. The opposite effect was observed in the case of infusions obtained from *D. fullonum* root exposed and not exposed to UVA radiation. No change in the antioxidant properties of the infusion made from a mixture of *D. fullonum* herb, *E. purpurea* herb, and *S. nigra* fruit was observed after exposure of the plant material to UVA radiation. The infusion from *D. fullonum* root not exposed to UVA radiation had the strongest free radical – quenching effect. Among the infusions obtained from the tested plant materials exposed to UVA radiation, the infusion from *D. fullonum* herb had the strongest free radical – quenching effect.

The neutralisation of free radicals by plant extracts supporting memory and concentration, which are important in relieving the symptoms of Alzheimer's disease, was investigated (Łomankiewicz *et al.*, 2025). In this work, both EPR and UV-Vis methods were used to examine interactions of infusions of maidenhair tree (*Ginkgo biloba*), ginseng (*Panax ginseng*), yerba mate (*Ilex paraguariensis*), and green tea with free radicals. Both methods, EPR and UV-Vis, showed a dependence of antioxidant properties on the type of plant material used to prepare medicinal extracts. The strongest antioxidant properties were found for yerba mate and green tea extracts. It was found that ginseng infusion quenched free radicals the least. It was concluded that the infusions of yerba mate and green tea can be useful in the case of Alzheimer's disease (Łomankiewicz *et al.*, 2025).

Electron paramagnetic resonance (EPR) spectroscopy and UV-Vis spectrophotometry were used to examine the decrease in DPPH spectra under the influence of antioxidant water extracts from sea buckthorn (*Hippophaë rhamnoides*) and European cranberry (*Vaccinium oxycoccos*) fruit (Michalak *et al.*, 2024). The effect of UVA radiation and an elevated storage temperature on the neutralisation of free radicals by these extracts was proved.

THE EXEMPLARY APPLICATION OF ELECTRON PARAMAGNETIC RESONANCE AND ULTRAVIOLET-VISIBLE SPECTROSCOPY IN THE STUDY OF THE PROPERTIES OF PLANTS FROM NOVEL ECOSYSTEMS – A PROPOSAL FOR A NEW DIRECTION OF RESEARCH

Mineral post-mining sites play an increasingly recognised role in biodiversity conservation and in enhancing ecosystem functioning, with the ability to sequester carbon, support oligotrophic environments, and provide refugia for rare species (Woźniak *et al.*, 2022). The plant species composition of vegetation from natural and semi-natural habitat conditions differs significantly from that developing on the mineral substrates of post-mining sites. As a result, novel ecosystems *sensu* Hobbs, Higgs and Hall (2013) are becoming established on these post-mining spoil heaps. Several studies show that a strong emphasis on their artificial restoration and fast recovery of plant cover at these sites may lead to lower productivity and lower stability of this restored ecosystem compared to the situation when the same sites are allowed to naturally recover over a longer time frame (Bartuska and Frouz, 2015; Frouz *et al.*, 2015).

Bacler-Żbikowska *et al.* (2025) present the neutralisation of DPPH free radicals by infusions of *Vaccinium myrtillus* obtained from the raw materials of natural and disturbed sites. The EPR

method was used to study the antioxidant properties of infusions obtained from common bearberry (*Arctostaphylos uva-ursi*), European lingonberry (*Vaccinium vitis-idaea*), and European bilberry (*Vaccinium myrtillus*) obtained from the control and disturbed sites (Bacler-Żbikowska *et al.*, 2023). Their study results show that an infusion of *A. uva-ursi* has the strongest free radical – quenching effect, while an infusion of *V. myrtillus* has the weakest effect on free radicals. In this study, authors observed that infusions obtained from *A. uva-ursi*, *V. vitis-idaea*, and *V. myrtillus* growing in disturbed areas have stronger antioxidant properties in relation to control areas. The results obtained in the study of Bacler-Żbikowska *et al.* (2023) point to a new direction in research on plant antioxidants, in which environmental conditions are considered. The results obtained by the EPR spectroscopy were confirmed by UV-Vis spectrophotometric measurements and analysis. All the tested plant infusions mentioned earlier significantly quenched the UV-Vis spectra, which proves their strong antioxidant properties. UV-Vis examination, similar to the EPR method, confirmed differences between the antioxidant properties of the infusions from raw materials from natural sites and from disturbed sites.

We propose paying attention to the vegetation that develops on post-mining spoil areas, as it may have significant potential for medicinal plant materials. It is important to determine whether the plants from these areas could be useful for medicine and the pharmaceutical industry. Medical and environmental research should proceed simultaneously. We hope that the use of EPR and UV-Vis methods will contribute to the development of our knowledge about antioxidants in plant products and will allow the use of raw plants in medicine.

We hope that collecting a wealth of information in the future about the antioxidant properties of plant products, including plant extracts, and combining it with knowledge about the environmental conditions in which the plants, used as raw materials, were found will allow us to better understand the pathways of antioxidant processes in the plants themselves. EPR and UV-Vis methods can be helpful in this regard and complement basic research.

DISCUSSION

The article outlines two physical methods for studying the antioxidant properties of plant extracts. The presented electron paramagnetic resonance (EPR) and UV-Vis methods examine the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as a result of delivering an electron to the molecule and thus provide information on the number of free radicals neutralised by the tested plant extract (Bacler-Żbikowska *et al.*, 2025). This is an advantage of these two physical methods, which provide direct information on the changes in the free radical system resulting from interaction with a plant extract, allowing assessment of the effectiveness of a given extract in reactions with free radicals.

The limitations of EPR spectroscopy and UV-Vis spectrophotometry are that they cannot be used to directly study the processes involving free radicals and antioxidants in plant cells. A limitation of the EPR and UV-Vis methods, as well as the fluorescent TRAP (total radical trapping antioxidant parameter) method (Pellegrini *et al.*, 2003; Koss-Mikołajczyk *et al.*, 2017) and

the colourimetric ABTS method (Chaves, Santiago and Alías, 2020), is their inability to identify the types of antioxidants present in the extract. However, this is possible with High-Performance Liquid Chromatography (HPLC) (Apaydin and Arabaci, 2017; Alamami *et al.*, 2023; So *et al.*, 2023). The EPR and UV-Vis methods are used to investigate the net effects of interactions of all types of antioxidants with DPPH free radicals. The TRAP method determines the extract's ability to capture peroxy radicals (Pellegrini *et al.*, 2003; Koss-Mikołajczyk *et al.*, 2017). The ABTS method analyses the colour changes of the ABTS^{•+} cation radical due to its reaction with an antioxidant (Chaves, Santiago and Alías, 2020).

It is necessary to emphasise the influence of extraction artifacts, matrix effects and the type of solvent on the assessment of antioxidant properties of extracts performed by EPR and UV-Vis methods. Extraction artifacts may result from, among other things, the poor condition of the plant material used in the process or solvent contamination. The matrix determines, among other things, the access of the solvent to the chemical compounds that are the target of extraction. The type of solvent influences the amount and type of extracted antioxidants, which in turn influences the quenching of EPR and UV-Vis spectra. However, the applications of these methods proposed in our work concern one type of plant extract in the form of an aqueous extract, because we are interested in plant extracts that can be consumed to support the healing processes in medical patients. The proposed EPR and UV-Vis methods are used to assess chemical antioxidant capacity and do not provide information on biological antioxidant function, both methods use model DPPH free radicals. In addition, it should be noted that the study of the chemical antioxidant capacity of plant extracts is highly useful for assessing therapeutic effectiveness in medicine.

CONCLUSIONS

Electron paramagnetic resonance (EPR) spectroscopy and ultraviolet-visible (UV-Vis) spectrophotometry are applicable for research into the interactions of plant antioxidants with free radicals. The studies presented in this article, previously known from the scientific literature, have demonstrated a significant impact of the environment in which the plants, used as raw materials for extracts, influence their antioxidant properties. The influence of the local ecosystem on the interactions of the extracts from plant raw materials with free radicals is of great importance in the search for new effective antioxidants for medicine and pharmacy. Continuing the use of EPR and UV-Vis in research into the antioxidant properties of plant raw materials for medical purposes, for example, from post-mining areas, has potential benefits. The proposed research methods enable the determination of the effects of external environmental factors on plants from harsh environments on their antioxidant potential. Factors such as intense sunlight, limited access to water, polluted and barren soils, and strong winds could affect their potential and this should be further investigated. Similar studies can be applied to the comparative analysis of different species inhabiting various types of habitats. The paper focused on the application of advanced analytical methods to assess how individuals of particular plants (e.g., antioxidant extracts) respond to varied habitat conditions in disturbed habitats where novel ecosystems

develop. However, it should be noted that these methods might also be very useful in another study in which plant species respond differently to habitat conditions. A good example of changing habitat conditions might be the height or aspect of mountains. Individuals of the same species growing across a wide range of heights or aspects in mountains are exposed to varying conditions. To identify the individual response of individuals of species growing in such conditions in the mountains, can be investigated by using the EPR spectroscopy and UV-Vis spectrophotometry.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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