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AN OVERVIEW OF ADVANCED 2,2-Diphenyl-1-picrylhydrazyl (DPPH) ANALYSIS TECHNIQUES

Gervacia Jenny Ratnawaty [1*], Ratih Indrawati [1], Wahdaniah [1], Khusna Arif Rakhman [2]

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak, Indonesia

[2]Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Khairun, Ternate, Indonesia

*Corresponding author: jennyratnawaty19@gmail.com

Abstract

Antioxidants play a crucial role in preventing oxidative damage, necessitating reliable analytical techniques for their evaluation. Oxidative stress, resulting from an imbalance between free radicals and antioxidants in the body, has been associated with various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Therefore, accurately assessing antioxidant activity is essential for developing health-related products and validating their efficacy. This study reviewed and compared various analytical methods used to determine antioxidant activity, emphasizing their advantages, limitations, and applicability across different sample types. Traditional spectrophotometric assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, remain widely used because of their simplicity and cost-effectiveness, although they often suffer from matrix interferences. Advanced techniques, including gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography coupled with DPPH (HPLC–DPPH), electrochemical methods, microfluidic systems, and miniaturized paper-based assays, provide improved sensitivity, accuracy, and efficiency. These methods enable a more comprehensive assessment of antioxidant activity by offering deeper insights into reaction mechanisms and compound interactions. The integration of multiple analytical approaches can further enhance antioxidant characterization, supporting applications in the food, pharmaceutical, and biomedical industries. This review highlights the importance of selecting appropriate analytical techniques based on research objectives and sample characteristics, while also outlining future directions for advancing antioxidant detection methodologies.

Keywords: *antioxidant analysis, DPPH assay, analytical techniques*

Introduction

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis, also known as the DPPH assay, is a widely used spectrophotometric method based on the stable free radical DPPH. It serves as a simple and accessible preliminary screening tool for evaluating the antioxidant properties of natural extracts (Gulcin & Alwasel, 2023; Munteanu

& Apetrei, 2021; Rajesh, 2021). However, it is important to note that the DPPH assay is limited to in vitro applications, as the reactive behavior of polyphenolic compounds observed in vitro may not accurately represent in vivo responses due to differences in biological environments (Bottoni et al., 2022). Antioxidants play a crucial role in protecting biological systems by neutralizing free radicals, reactive molecules associated with oxidative stress. Free radicals form when oxygen interacts with certain compounds and can damage essential cellular components, such as DNA, proteins, and cell membranes. Antioxidants mitigate this damage by donating electrons to free radicals, thereby stabilizing them (Moon & Shibamoto, 2009; Siddartha & Gupta, 2020). Consequently, the DPPH method helps evaluate the potential health benefits of foods and supplements, particularly their ability to combat oxidative stress, which has been linked to diseases such as cancer, Alzheimer's, and cardiovascular disorders (Munishamappa et al., 2017).

The principle of DPPH analysis relies on the reactivity of the DPPH radical, a stable free radical characterized by its deep violet color, which is reduced to the yellow, non-radical form diphenyl-picrylhydrazine (DPPH-H) upon reaction with antioxidants, as shown in Figure 1. This color change serves as an indicator of antioxidant activity, with the extent of discoloration directly correlating with the antioxidant concentration in the sample (Safaryan et al., 2016).

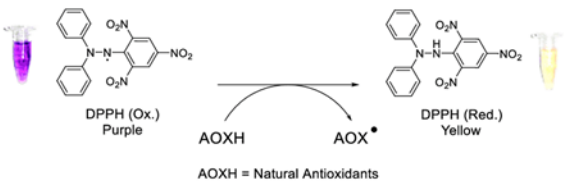

Figure 1. DPPH reaction with natural antioxidants. Reproduced from Arce-Amezquita et al. (2019), published by the Mexican Society of Soil Science, licensed under CC BY-NC-ND.

The mechanism underlying DPPH analysis involves electron transfer (ET) and hydrogen atom transfer (HAT) processes. Antioxidants donate a hydrogen atom to the DPPH radical, leading to its stabilization and a corresponding decrease in absorbance (Ayuni et al., 2024).

One of the main advantages of the DPPH assay is its rapidity and simplicity. It requires minimal sample preparation and can be performed on various extract types without extensive purification (Gómez et al., 2019). However, conventional DPPH assays also face several limitations, including reproducibility issues and challenges in accurately measuring complex samples due to interference from colored pigments (Rahman et al., 2018). The DPPH radical itself is prone to gradual decomposition and is highly sensitive to temperature, pH variations, and light exposure. To maintain stability, DPPH solutions must be freshly prepared in an organic solvent before analysis. Deng et al. (2018) reported that DPPH solutions should be used within two hours of preparation to prevent degradation. Similarly, Xie and Schaich (2014)found that DPPH absorbance decreases within 90 minutes under temperature fluctuations and light exposure, whereas solutions stored in the dark remain stable.

Given these limitations, advanced analytical techniques have been developed to provide more reliable, efficient, and comprehensive assessments of antioxidant potential. Nevertheless, a lack of systematic review on these advanced DPPH-based approaches remains, leaving a gap in understanding their comparative performance and applicability. Therefore, this study aimed to present a comprehensive review assessing the strengths, limitations, and suitability of these methods for evaluating antioxidant potential across diverse biological and chemical contexts.

Research Method

This study employed a literature review approach to compare findings from multiple research articles. Relevant publications were retrieved from PubMed, Web of Science, and ScienceDirect, all

indexed by Scopus, focusing on studies published within the past decade (2013–2023). The search was conducted online over a one-month period using the keywords "DPPH," "techniques," "analysis methods," "antioxidant," and "review." Only articles published in English were included.

The selection process followed specific inclusion and exclusion criteria. Articles were included if they focused on DPPH-based antioxidant analysis methods, particularly those comparing traditional and advanced analytical techniques. Eligible studies also presented experimental data or methodological developments and were limited to peer-reviewed original research articles and systematic reviews. Exclusion criteria included articles that did not employ DPPH as a primary analytical method, were written in languages other than English, or were classified as conference abstracts, editorial notes, commentaries, or studies lacking sufficient methodological detail or validation data.

An initial total of 217 articles was identified using the specified keywords and filters. After screening titles and abstracts, 89 articles were selected for full-text review. Of these, 48 articles met all inclusion criteria and were incorporated into the final analysis. The selected studies were examined in detail to extract and compare data on the analytical techniques employed, such as gas chromatography–mass spectrometry (GC–MS), high-performance liquid chromatography coupled with DPPH (HPLC-DPPH), and electrochemical methods, as well as the advantages and limitations of each technique. Additional comparisons were made regarding the applicability of each method to different sample types and matrices, as well as the reported outcomes in antioxidant detection and characterization.

Results and Discussion

Gas Chromatography

Gas chromatography (GC) is used to separate volatile and semi-volatile compounds present in complex mixtures. The method involves heating the sample and carrying it through a chromatographic column using an inert gas as the mobile phase. The compounds interact differently with the stationary phase in the column, leading to their separation based on volatility and chemical properties (Viet et al., 2021). After separation, the eluted compounds are collected either directly within the GC system or in collection vials. These fractions are then incubated with a DPPH solution. The interaction between antioxidants present in each fraction and the DPPH radical produces a measurable color change, indicating the scavenging of DPPH radicals (El-Nasr & Mokhtar, 2023; Ahmad et al., 2018). GC is often coupled with mass spectrometry (GC-MS) to provide structural identification of the separated compounds. The combination of retention time data from GC and mass spectral data from MS enables precise identification of antioxidants within the sample (Gandhi et al., 2023; Grover et al., 2021).

For instance, the study by Zhang et al. (2024) evaluated the antioxidant capacity of Tulipa edulis extracts using DPPH analysis in conjunction with cellular and transcriptomic approaches, as illustrated in **Figure 2**. The crude extract was purified using Sephadex LH-20 gel, and the antioxidant activity of the purified fractions was assessed using the DPPH radical scavenging assay. To further investigate its protective effects, human umbilical vein endothelial cells (HUVECs) were exposed to oxidative stress induced by 35 mmol/L glucose and treated with the active component (F2) from T. edulis. The results demonstrated that F2 exhibited strong antioxidant activity, as confirmed by DPPH analysis using GC-MS.

GC is particularly suitable for analyzing volatile and semi-volatile compounds, which are often relevant in the study of essential oils and flavor compounds that may exhibit antioxidant properties. This allows a broader range of compounds to be evaluated as potential antioxidants (Valle et al., 2016). However, GC is limited to volatile compounds, making it unsuitable for the analysis of non-volatile or thermally

unstable substances that may be critical in antioxidant research. Such limitations can result in potentially significant antioxidants being overlooked (Kim et al., 2023). Moreover, GC is less effective for separating polar antioxidants without prior chemical modification, such as derivatization (Angulo-López et al., 2023).

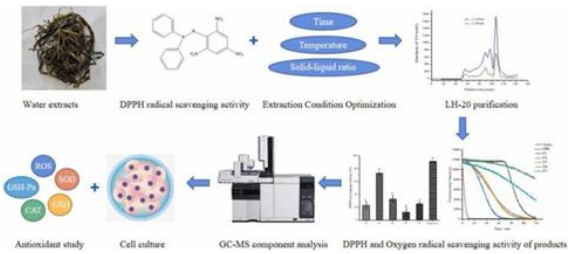

Figure 2. GC-MS DPPH analysis of water extracts from Tulipa edulis. Reproduced from Zhang et al. (2024), published by Tsinghua University Press, licensed under CC BY-NC-ND.

High-Performance Liquid Chromatography (HPLC)

The mechanism of High-Performance Liquid Chromatography (HPLC)–DPPH analysis involves using the stable DPPH radical as a reagent to assess the antioxidant capacity of compounds separated by HPLC. The basic principle is that as antioxidants react with DPPH, they reduce it, resulting in a measurable color change corresponding to a shift in absorbance. This change can be monitored in real time as compounds elute from the HPLC column (Baldan et al., 2017; Liu et al., 2023).

There are two primary HPLC–DPPH assay methods: offline and online. In the offline HPLC–DPPH method, the sample (e.g., a plant extract) undergoes extraction, typically using organic solvents, before analysis. The extract is then filtered and prepared for injection into the HPLC system. The mixture is introduced into the HPLC column, where individual components are separated based on their interactions with the stationary phase and their chemical properties, such as polarity. The elution is performed under controlled conditions, allowing for precise identification of compounds based on their retention times (Quispe et al., 2017). After separation, the compounds collected in individual fractions are mixed offline with a DPPH solution. As antioxidants within these fractions react with DPPH, the violet color of DPPH diminishes. This change can be quantitatively measured by assessing variations in absorbance using a mass spectrophotometer or mass spectrometry (MS), resulting in what is commonly referred to as the HPLC–MS method. The degree of decolorization reflects the antioxidant capacity of the separated compounds. Chromatograms obtained before and after the DPPH reaction are compared, where a significant reduction or disappearance of peak areas corresponding to specific compounds indicates their antioxidant activity (Hu et al., 2019; Zuo et al., 2021).

Although HPLC–MS provides high analytical resolution and sensitivity, its separation power is generally lower than that of GC–MS (Quanson et al., 2016). GC–MS often achieves higher sensitivity and resolution due to its ability to separate low-volatility organic compounds and its lower detection limits (Angulo-López et al., 2023). Moreover, the HPLC–DPPH method is time-consuming, as it involves sequential separation and reaction steps similar to those in GC–MS. Delays between separation and assay can lead to the decomposition of unstable antioxidants, potentially affecting the accuracy of results. In addition, the multistep handling process may introduce variability across assays.

In the online method, the DPPH solution is delivered directly into the HPLC system, where it immediately interacts with the antioxidant compounds post-separation, as illustrated in **Figure 3**. This approach enables real-time monitoring of antioxidant activity as compounds are eluted. The online HPLC–DPPH technique provides a simpler and more reproducible alternative to traditional methods for determining antioxidant capacity. It allows for the rapid identification of antioxidant components in natural products at the chromatographic level, facilitating subsequent purification and characterization (Quispe et al., 2017; Liu et al., 2023; Pedan et al., 2016).

Meanwhile, the offline HPLC–DPPH technique represents a hybrid approach that combines HPLC separation with the DPPH radical scavenging assay to evaluate the antioxidant capacity of compounds extracted from various sources.

Compared with the offline method, the online HPLC–DPPH technique allows faster analysis, as the entire process occurs in a single continuous run. This integration enables rapid assessment of multiple samples, significantly enhancing throughput and reducing processing time. Online systems can also lower the detection limits for antioxidants, allowing researchers to detect lower concentrations of antioxidant activity that might not be measurable using offline methods (Lee et al., 2015; Pedan et al., 2016). Furthermore, traditional DPPH assays are prone to interference from colored or complex matrices. The chromatographic separation provided by HPLC minimizes these interferences, resulting in cleaner data and more accurate quantification of antioxidant activity (Quispe et al., 2017).

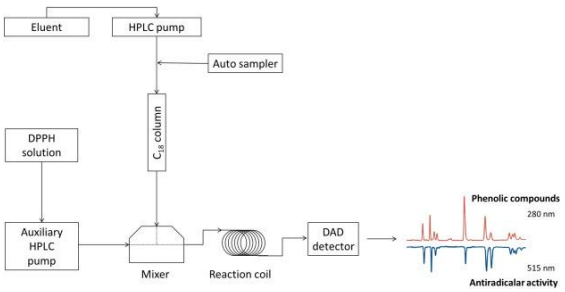

Figure 3. Online HPLC–DPPH screening method. Reproduced from Braham et al. (2020), published by Elsevier, licensed under CC BY 4.0.

However, online systems often require sophisticated instrumentation, as well as precise calibration and maintenance, to ensure that the DPPH reaction is well controlled and consistent throughout the elution process (Ruiz-Rivera et al., 2015). This method may also face challenges when analyzing highly polar compounds or those requiring specific solvent conditions, which can limit the range of antioxidants that can be effectively evaluated compared with offline methods (Wang et al., 2017).

Microfluidic

Microfluidic techniques in DPPH analysis represent a significant advancement in the evaluation of antioxidant activity. Microfluidics involves the manipulation of very small fluid volumes (typically in the microliter range) within microchannels, allowing precise control over reaction conditions, mixing efficiency, and sample analysis (Abd Rahman et al., 2018).

Abd Rahman et al. (2018) conducted a study using a microfluidic Lab-on-a-Disc (LoD) system that integrated centrifugal forces to drive the flow of the DPPH solution and test samples through distinct reaction chambers, as illustrated in **Figure 4**. The LoD, a type of centrifugal microfluidic device and part of the Micro Total Analysis System (μTAS), enables the miniaturization and automation of chemical and biological analyses. LoD technology offers several advantages, including simplified assay procedures, rapid analysis, and cost efficiency. Unlike Lab-on-a-Chip (LoC) systems that rely on syringe pumps, the LoD operates in a closed, "all-in-one disc" format, reducing reagent and sample consumption while minimizing processing time and human error (Hosseini et al., 2016; Sayad et al., 2016).

The study's findings demonstrate that sequential sample loading and mixing within the closed LoD system reduce human error and minimize volume loss typically associated with manual pipetting. The "load-and-run" approach of the LoD method eliminates repetitive pipetting, mixing, and loading steps required in conventional DPPH assays (Abd Rahman et al., 2018).

A centrifugal motor and a high-speed camera are connected to the computer to regulate the disc's rotational speed (RPM) and to enable visualization of the process. A reflector transmits signals to a digital RPM sensor for accurate monitoring (Abd Rahman et al., 2018).

Ceylan et al. (2023) also reported that microfluidic techniques help mitigate common interferences encountered in traditional DPPH assays, such as those caused by colored compounds or turbidity

in complex samples. The controlled environment of a microfluidic system minimizes such complications, resulting in clearer and more reliable measurements of radical scavenging activity.

Figure 4. The complete experimental setup includes a computer-controlled system and a custom Lab-on-a-Disc (LoD) spinning test system. Reproduced from Abd Rahman et al. (2018), published by Multidisciplinary Digital Publishing Institute, licensed under CC BY.

Phonchai et al. (2016) further demonstrated that recent innovations in microfluidic chip design allow the integration of complementary analytical techniques, such as measuring total phenolic content (TPC) alongside DPPH assays. Moreover, because microfluidics enables precise control of environmental parameters (e.g., flow rates, mixing speeds), it provides better insight into the kinetics of antioxidant reactions. This facilitates more nuanced investigations of reaction mechanisms, including whether scavenging primarily occurs via electron transfer or hydrogen atom transfer (Li et al., 2022). Overall, microfluidic systems operate with extremely small fluid volumes (microliters to nanoliters), minimizing sample and reagent consumption. This makes the technology not only cost-effective but also environmentally sustainable (Xu et al., 2020).

However, the design and fabrication of microfluidic devices can be technically challenging and require specialized materials, equipment, and expertise. Maintaining consistent performance of key components, such as pumps and valves, can also be difficult (Liu et al, 2021). Moreover, microfluidic methods are generally more suitable for analyzing compounds that can tolerate the processing conditions within the microchannels. Many antioxidants, particularly unstable ones, may degrade during analysis or require specific solvent systems, which can complicate assay design (Abd Rahman et al., 2018). Due to the small dimensions of microfluidic channels, achieving optimal reagent mixing can be more challenging than in macro-scale systems. This limitation may result in incomplete reactions or variable outcomes, especially when the reaction time is insufficient (Ward & Fan, 2015). In addition, microfluidic systems are susceptible to clogging caused by particulate matter in samples or by bubble formation within the channels, which can lead to inconsistent results and necessitate frequent maintenance or cleaning protocols (Yoon et al., 2016).

Electrochemical

Electrochemical DPPH analysis employs electrochemical techniques, primarily voltammetry, to evaluate the antioxidant capacity of compounds by monitoring their interactions with the DPPH radical. Among these, cyclic voltammetry is one of the most widely used methods. This technique measures the current response to an applied potential at an electrode, providing detailed information on the redox behavior of DPPH and its interaction with antioxidant compounds. Studies using cyclic voltammetry have demonstrated that DPPH undergoes a reversible, diffusion-controlled electron transfer process. Modifications such as incorporating multi-walled carbon nanotubes on a glassy carbon electrode (CNT–GCE) have been shown to enhance electron transfer efficiency. Flow-injection analysis (FIA) combined with amperometric detection has also been applied to assess antioxidant capacity in plant extracts and wines by continuously monitoring DPPH consumption. Other electrochemical techniques, such as batch-injection analysis (BIA) with amperometric detection, offer portable and precise alternatives to FIA. BIA simplifies the analytical process by eliminating the need for pumps and valves, making it particularly suitable for food,

environmental, and pharmaceutical applications (Oliveira et al., 2016).

The reduction and oxidation peaks observed in voltammograms correspond to the electron transfer processes occurring during radical scavenging. The electrochemical behavior of DPPH can be analyzed under varying pH conditions and solvent systems to optimize sensitivity and selectivity. One of the principal advantages of electrochemical methods is their ability to deliver rapid analysis. Automated systems incorporating microtiter plates can conduct multiple assays simultaneously, further enhancing efficiency (Ngueumaleu et al., 2023).

Electrochemical techniques typically require smaller amounts of reagents than conventional spectrophotometric DPPH assays, while offering faster analysis times. This efficiency reduces operational costs and enables the analysis of limited or valuable samples, an advantage in pharmacognosy and drug discovery. Furthermore, unlike traditional spectrophotometric assays that can be influenced by turbidity or coloration, electrochemical methods exhibit higher resistance to such interferences, enabling accurate assessments even in complex food matrices or biological samples (Ziyatdinova & Kalmykova, 2023). Another major strength of electrochemical methods lies in their ability to directly measure electron transfer during the radical scavenging process. This allows for kinetic insights into antioxidant reaction mechanisms, providing valuable information on reaction rates and pathways that are not easily obtainable through conventional spectrophotometric techniques (Arvapalli et al., 2021; Deutchoua et al., 2019).

However, electrochemical measurements can be influenced by other electroactive substances within the same sample matrix, potentially causing inaccurate readings or overestimations of antioxidant activity (Deutchoua et al., 2019; Koc et al., 2022). Consequently, results must be carefully validated against more established methods such as spectrophotometry. Establishing reliable calibration curves for electrochemical sensors can also be challenging, particularly when dealing with complex or variable sample matrices. Data interpretation may become difficult due to differences in redox potentials and reaction kinetics among various antioxidants (Koc et al., 2022; Oliveira et al., 2016). Additionally, electrodes used in electrochemical assays can experience fouling or passivation over time, leading to decreased sensitivity and necessitating regular maintenance or replacement (Fu et al., 2021; Hu et al., 2023).

Miniaturized and Paper-Based Assays

Miniaturized DPPH assays typically involve the reduction of the total assay volume, allowing for high-throughput analyses while requiring smaller quantities of both samples and reagents compared to conventional methods. The miniaturization process often utilizes microplates or microfluidic devices that can automatically handle multiple samples concurrently, as illustrated in **Figure 5** (Becker et al., 2019).

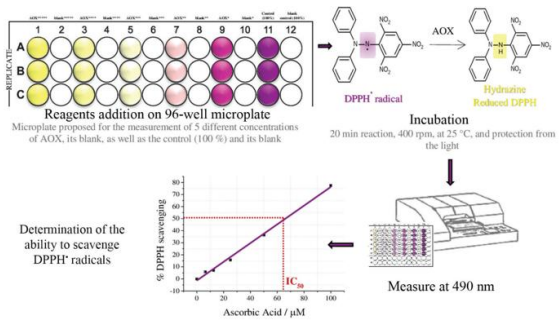

Figure 5. Miniaturized DPPH assay scheme. Reproduced from Becker et al. (2019), published by Sociedade Brasileira de Química, licensed under CC BY.

The reduction in sample size not only conserves valuable materials, such as plant extracts or biological fluids, but also accelerates analytical throughput. Enhanced automation and integration with robotic systems enable researchers to conduct a large series of tests simultaneously, which is crucial for comprehensive antioxidant profiling (Adiyanto et al., 2023). The design of miniaturized assays, particularly in paper-based formats, simplifies analytical procedures and reduces the need for

extensive training. This accessibility allows technicians and researchers with minimal expertise to perform reliable analyses, expanding the usability of these methods in diverse environments, including educational institutions (Soum et al., 2019).

Sirivibulkovit et al. (2018) developed a paper-based DPPH assay by incorporating DPPH reagents onto a paper substrate. The device was fabricated using a lamination method that created a 5-mm-diameter circular test zone embedded with DPPH reagent. The analysis was performed in a single step by applying an antioxidant or test sample directly onto the test zone. Upon reduction by the antioxidant, DPPH radicals were converted into stable DPPH molecules, resulting in a color change from deep violet to pale yellow. The intensity of the violet color was inversely proportional to the antioxidant activity of the sample and quantified using imaging software. The method demonstrated high precision and a low detection limit when analyzing six standard antioxidants: gallic acid, trolox, ascorbic acid, caffeic acid, vanillic acid, and quercetin. Validation against the conventional spectrophotometric DPPH assay using seven tea samples showed no significant difference in gallic acid equivalent values at the 95% confidence level, confirming the reliability of the developed method for assessing antioxidant activity in real samples. Additionally, the paper-based device exhibited stability for up to 10 days when stored at 2–4°C.

Traditional methods often face challenges such as interference from colored compounds or turbidity in complex matrices; however, paper-based assays can be designed to minimize these issues, thereby improving the specificity and reliability of antioxidant detection. Furthermore, the establishment of a clear visual endpoint allows for immediate interpretation of results, making these assays particularly useful for rapid screening applications (Darachai et al., 2019). Paper-based devices also offer exceptional portability, allowing them to be used in field settings or remote locations without the need for complex laboratory equipment, thereby expanding their applicability to environmental monitoring and clinical assessments (Nguyen et al., 2018).

Despite these advantages, miniaturized and paper-based formats may not be suitable for samples requiring extensive processing or filtration, which limits their use for more complex matrices. Their performance may also be inferior to other analytical techniques when analyzing highly concentrated or viscous solutions (Ionita et al., 2015). The stability of DPPH and other reagents can be compromised within a paper matrix if not stored properly or when exposed to environmental fluctuations, thereby affecting measurement reliability (Sirivibulkovit et al., 2018). Moreover, inconsistent color development and uneven liquid absorption in the paper substrate can lead to variability in quantitative analysis (Tay et al., 2022). Consequently, accurate absorbance measurements may be influenced by the physical and optical properties of the paper material itself.

Computational Methods

Computational approaches, particularly those based on artificial intelligence (AI) and machine learning, have emerged as powerful tools for analyzing large datasets generated from DPPH assays. These techniques enable the identification of complex patterns, classification of compounds according to their antioxidant capacities, and correlation of molecular structures with functional activity. For instance, multivariate regression and classification models can be used to evaluate the relationship between the chemical structure of antioxidants and their efficiency in scavenging DPPH radicals (Abdulwahab et al., 2015).

By leveraging datasets containing known antioxidants and their corresponding DPPH scavenging activities, machine learning models can be trained to predict the antioxidant potential of novel compounds. Such predictive analytics accelerate the discovery of new antioxidant agents in food science and pharmaceutical

research, thereby reducing the time and resources required for experimental validation (Angeli et al., 2023).

Real-world applications have demonstrated a strong correlation between AI predictions and experimental DPPH results. For example, Fontoura et al. (2023) applied Random Forest and Partial Least Squares Regression (PLSR) models to predict the antioxidant activity, including DPPH radical scavenging, of Bertholletia excelsa bark extracts based on extraction parameters and RGB image data. The models achieved high accuracy, with R^2 values ranging from 0.85 to 0.99, highlighting the capability of AI algorithms to reliably forecast DPPH assay outcomes.

Computational methods can also be employed to optimize extraction protocols for antioxidant compounds from various natural sources. Machine learning algorithms can analyze the effects of variables such as temperature, extraction time, and solvent composition on antioxidant yield and DPPH activity, leading to optimized and reproducible extraction processes (Singh et al., 2023).

AI-integrated systems can further facilitate real-time monitoring of DPPH assays, enabling immediate adjustments in experimental conditions based on sensor feedback. This approach improves data accuracy and enhances the reliability of quantitative antioxidant assessments (Yang & Berdine, 2023).

A major advantage of computational methods lies in their ability to process large datasets efficiently, enabling high-throughput screening and analysis of antioxidant compounds. AI and machine learning algorithms can also reduce human error and analytical variability, resulting in more consistent and reproducible comparisons across samples and experimental conditions. Moreover, these models can uncover complex, non-linear relationships, such as structure–activity correlations, that are difficult to detect using conventional statistical approaches (Singh et al., 2023; Yang & Berdine, 2023). In addition, the integration of AI-driven predictive models with laboratory workflows reduces reagent use and experimental costs, providing a more sustainable and resource-efficient approach to antioxidant research (Angeli et al., 2023).

Nevertheless, the reliability of AI and machine learning methods depends heavily on the quality, diversity, and representativeness of the training data. Insufficient or biased datasets can lead to overfitting, poor generalization, and inaccurate predictions. Implementing these computational approaches also requires significant computational resources and interdisciplinary expertise in chemistry and data science, which may not be readily available in all laboratory settings (Angeli et al., 2023; Yang & Berdine, 2023). Furthermore, results obtained through AI-based predictions must still be validated experimentally, which can offset some of the time and cost advantages of computational screening (Singh et al., 2023).

Conclusion

Recent advancements in antioxidant analysis techniques have significantly enhanced sensitivity, accuracy, and efficiency. Traditional methods such as the spectrophotometric DPPH assay remain widely used due to their simplicity; however, they are susceptible to interferences from complex sample matrices. Advanced approaches, including GC–MS, HPLC–DPPH, electrochemical methods, microfluidic systems, miniaturized and paper-based assays, as well as computational modeling, have emerged as promising alternatives for DPPH-based analysis. Each technique presents distinct advantages and limitations, highlighting the importance of selecting an appropriate method based on the sample's characteristics and specific analytical objectives. The integration of multiple techniques can yield a more comprehensive evaluation of antioxidant activity, providing deeper insights into reaction mechanisms and expanding potential applications across food science, pharmaceuticals, and biomedical research.

Recommendations

Researchers are encouraged to select antioxidant analysis techniques according to the physicochemical nature of the target sample. For example, GC–MS is preferable for analyzing volatile compounds, while HPLC–DPPH is better suited for complex plant extracts. On the other hand, electrochemical methods are advantageous for real-time measurements in biological samples.

Future studies should prioritize optimizing microfluidic platforms and miniaturized assays by addressing current challenges such as reagent mixing, channel clogging, and signal stability. Computational modeling should also be advanced to facilitate predictive analysis and elucidate reaction mechanisms. Furthermore, novel techniques must undergo rigorous validation against established assays, such as the spectrophotometric DPPH method, to ensure accuracy, reproducibility, and inter-method comparability. Efforts should also focus on developing low-cost, portable antioxidant detection systems to enable widespread application in food quality control, pharmaceutical formulation, and environmental monitoring.

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