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Role of NMR Techniques in Identification and Evaluation of Antioxidant Phenolic and Flavonoid Phytochemicals: A Comprehensive Review

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Abstract: Phenolic compounds and flavonoids are important natural antioxidants widely present in plants. Accurate identification, structural elucidation, and evaluation of these compounds are essential to understand their biological activity. Nuclear Magnetic Resonance (NMR) spectroscopy provides detailed structural and quantitative information in a non-destructive manner. This review highlights the application of one-dimensional (1D) and two-dimensional (2D) NMR, quantitative NMR (qNMR), and NMR-based metabolomics for the identification and evaluation of antioxidant phenolic and flavonoid compounds. Integration with in vitro antioxidant assays such as DPPH, ABTS, and FRAP is discussed. The correlation between structural features derived from NMR and antioxidant mechanisms, including free radical scavenging, metal chelation, and lipid peroxidation inhibition, is highlighted. Limitations, challenges, and future perspectives of NMR-based antioxidant research are also reviewed. This article aims to provide a comprehensive resource for researchers in pharmaceutical sciences and photochemistry. phytochemistry

Keywords: NMR spectroscopy; Phenolic compounds; Flavonoids; Antioxidant activity; Phytochemicals.

1. Introduction

Oxidative stress caused by excessive reactive oxygen species (ROS) is implicated in many chronic diseases including cardiovascular disorders, diabetes, neurodegeneration, and cancer [1,2]. Natural antioxidants derived from plants play a crucial role in neutralizing ROS and maintaining cellular redox balance [3]. Among them, phenolic compounds and flavonoids are major contributors due to their potent free radical scavenging and metal chelation properties [4,5].

The antioxidant activity of these compounds is strongly dependent on their chemical structure, including hydroxyl substitution patterns, conjugation, and glycosylation [6]. Accurate structural identification and quantification are therefore critical for understanding antioxidant mechanisms and for standardization of herbal formulations [7]. Conventional chromatographic methods provide separation and quantification but lack comprehensive structural insights. NMR spectroscopy offers a reliable, non-destructive

method for detailed structural analysis and quantification of phenolic and flavonoid antioxidants [8,9].

2. Phenolic and Flavonoid Phytochemicals as Antioxidants

Phenolic compounds consist of aromatic rings with hydroxyl groups and include phenolic acids, tannins, stilbenes, and lignans [10]. Flavonoids, a subclass of polyphenols, contain a C6–C3–C6 structure and include flavones, flavonols, flavanones, flavanols, isoflavones, and anthocyanidins [11]. The antioxidant activity of these compounds is mainly due to hydrogen atom or electron donation, radical stabilization, and metal ion chelation [12].

Structure–activity relationship studies show that ortho-dihydroxy substitutions in the B-ring of flavonoids enhance radical scavenging ability, while glycosylation often reduces antioxidant potential [13]. Therefore, precise structural elucidation is essential to correlate chemical features with biological activity.

3. Overview of NMR Techniques in Antioxidant Research

NMR spectroscopy is based on the interaction of atomic nuclei with an external magnetic field and radiofrequency radiation. It provides detailed qualitative and quantitative information about molecular structures [14]. ^1H and ^13C (Carbon – 13 nucleus) NMR are the most common nuclei used in phytochemical studies, while ^15N and ^31P are occasionally employed for specific metabolites [15].

Modern high-field NMR instruments with cryoprobes increase sensitivity and allow the analysis of low-concentration antioxidants [16]. NMR can be applied to both crude plant extracts and purified compounds, providing information on molecular connectivity, stereochemistry, and functional group environment [17].

4. Role of 1D and 2D NMR in Structural Elucidation

1D NMR spectra (^1H and ^13C) provide basic information about chemical environments of protons and carbons in phenolic and flavonoid structures [18]. Hydroxyl protons, aromatic proton patterns, and carbonyl carbons give crucial structural clues.

2D NMR experiments such as COSY, HSQC, HMBC, and NOESY are vital for establishing connectivity, confirming substitution patterns, and determining stereochemistry [19]. For example, HMBC correlations can identify linkage positions in flavonoid glycosides, which influence antioxidant activity [20].

5. Quantitative NMR (qNMR) in Antioxidant Evaluation

Quantitative NMR allows absolute quantification of antioxidant compounds without the need for calibration standards [21]. Signal integrals are directly proportional to molar concentration, making qNMR highly reproducible and accurate [22]. This method has been successfully applied for quercetin, kaempferol, catechin, gallic acid, and other phenolics [23,24]. Combining qNMR with antioxidant assays strengthens correlation between compound concentration and activity [25].

6. NMR-Based Metabolomics Approaches

NMR-based metabolomics provides comprehensive profiling of phytochemicals in complex plant matrices [26]. Multivariate statistical analysis of NMR data can identify key antioxidant markers and discriminate between samples with different antioxidant potentials [27]. This approach is widely used for quality control, standardization, and bioactivity prediction [28,29].

7. Correlation of NMR Data with Antioxidant Mechanisms

Structural features identified by NMR such as hydroxyl positions, conjugated double bonds, and glycosylation patterns are directly correlated with antioxidant mechanisms [30,31]. Metal chelation properties can be inferred by NMR-based studies showing shifts in proton or carbon signals upon metal binding [32]. This information is critical for structure–activity relationship studies.

8. Integration with In Vitro Antioxidant Assays

While NMR provides structural and quantitative information, functional antioxidant activity is measured using assays like DPPH, ABTS, FRAP, and superoxide radical scavenging [33]. Integration of NMR with these assays allows validation of antioxidant potential and identification of active components [34]. Correlation studies have shown that phenolic and

flavonoid content determined by NMR often aligns with in vitro antioxidant capacity [35,36].

Table 1: NMR Techniques and Their Applications in Antioxidant Research
Application in Antioxidant Studies

NMR Technique	Information Obtained	Application in Antioxidant Studies
^{1}H NMR	Proton environment, hydroxyl, and aromatic protons	Identification of phenolic and flavonoid structures
^{13}C NMR	Carbon skeleton, carbonyl and aromatic carbons	Confirmation of flavonoid backbone and phenolic acids
COSY	Proton–proton coupling	Determination of aromatic ring substitution
HSQC	^{1}H–^{13}C one-bond correlation	Assignment of protonated carbons
HMBC	Long-range ^{1}H–^{13}C correlation	Determination of linkage positions
NOESY	Spatial proximity	Stereochemistry and conformational analysis
qNMR	Signal integration	Absolute quantification of antioxidants
NMR metabolomics	Metabolic fingerprinting	Correlation of phenolic profile with antioxidant activity

Table 2: Major Phenolic and Flavonoid Antioxidants Identified Using NMR

Compound	Class	Key NMR Features
Gallic acid	Phenolic acid	^{1}H NMR: singlet at δ ~7.0 (aromatic H); ^{13}C NMR: δ ~168–170 (carboxylic C=O), δ ~110–150 (aromatic C)
Caffeic acid	Hydroxycinnamic acid	^{1}H NMR: δ ~6.2–7.5 (olefinic & aromatic protons); ^{13}C NMR: δ ~145–150 (phenolic C), δ ~170 (C=O)
Ferulic acid	Phenolic acid	^{1}H NMR: methoxy signal at δ ~3.8; aromatic protons at δ ~6.8–7.6
Quercetin	Flavonol	^{1}H NMR: δ ~6.2–7.8 (A & B ring protons); ^{13}C NMR: δ ~175–180 (C-4 carbonyl)
Kaempferol	Flavonol	^{1}H NMR: characteristic AA′BB′ pattern in B-ring; ^{13}C NMR: flavonoid backbone signals
Catechin	Flavan-3-ol	^{1}H NMR: δ ~4.0–4.5 (H-2, H-3); aromatic signals δ ~6.6–6.9
Epicatechin	Flavan-3-ol	Similar to catechin with stereochemical shift differences in H-2/H-3 region
Rutin	Flavonoid glycoside	^{1}H NMR: sugar anomeric proton δ ~5.1; aromatic signals δ ~6.2–7.6

Naringenin	Flavanone	^{1}H NMR: δ ~2.5–3.2 (H-3 methylene); aromatic signals in A & B rings
Apigenin	Flavone	^{1}H NMR: singlet at δ ~6.6 (H-3); ^{13}C NMR: δ ~182 (C-4 carbonyl)

Table 3: Comparison of NMR and Conventional Antioxidant Assays

Parameter	NMR-based Techniques	Conventional Spectrophotometric Assay
Principle	Magnetic resonance of nuclei in a magnetic field	Measurement of absorbance or colour change
Structural information	Detailed molecular and structural information	Not available
Identification of compounds	Direct and specific identification possible	Indirect, based on standards
Quantification	Absolute quantification (qNMR)	Relative quantification
Sensitivity	Moderate	High
Sample preparation	Minimal	Moderate to extensive
Sample destruction	Non-destructive	Usually destructive
Reproducibility	High	Moderate
Selectivity	High (compound-specific)	Low (interference possible)
Analysis time	Relatively longer	Short
Requirement of standards	Not always required	Required
Application	Structural elucidation, metabolomics, profiling	Total antioxidant capacity estimation (DPPH, ABTS, FRAP, etc.)
Cost	High instrumentation cost	Low cost

9. Limitations and Challenges

Despite its advantages, NMR spectroscopy has limitations:

- Lower sensitivity compared to MS-based methods [37]
- Requirement of high sample concentration for low-abundance compounds [38]
- Overlapping signals in complex mixtures may complicate analysis [39]
- Instrument cost and technical expertise needed [40]
- Advances in pulse sequences, high-field magnets, and chemometric analysis partially overcome these challenges [41].

10. Future Perspectives

- Future trends in NMR-based antioxidant research include:
- Ultra-high-field NMR for enhanced sensitivity [42]
- Integration with AI and chemometrics for automated structure–activity analysis [43]
- Real-time monitoring of antioxidant activity in extracts and biological systems [44]
- Combined metabolomics and bioactivity-guided fractionation for novel antioxidant discovery [45,46]
- Expansion to lesser-studied phenolics and flavonoids for functional food development [47,48]
- Coupling with other hyphenated techniques for comprehensive profiling [49,50]

11. Conclusion

NMR spectroscopy provides a powerful platform for the identification, quantification, and evaluation of antioxidant phenolic and flavonoid

phytochemicals. Integration with in vitro assays enables a holistic understanding of antioxidant mechanisms. Advances in NMR technology and data analysis promise to enhance research in natural products and pharmaceutical sciences, supporting development of standardized herbal formulations and functional foods.

Conflict of Interest

The author declares declare no conflict of interest.

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Legend:

This schematic shows integration of 1D & 2D NMR, qNMR, and metabolomics for identification, structural elucidation, and quantitative evaluation of phenolic and flavonoid antioxidants. Correlations with in vitro assays and mechanistic insights including free radical scavenging, metal chelation, and lipid peroxidation inhibition are illustrated.

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