

Simplified Practical Identification of Polyphenol Types in Natural Dietaries Based on 1D and 2D Nuclear Magnetic Resonance

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Received November 10, 2023; Revised December 11, 2023; Accepted December 18, 2023

Abstract Polyphenol groups have gained great interest as health-promoting and ailment-preventing agents in food ingredients. Their large range of distribution in the plant kingdom offers prospective development for many kinds of food products. From the beginning of the development processes, polyphenol group identity must be confirmed in order to guarantee the quality of their bioactivity, concentration consistency from batch to batch, and molecular stability. 1D and 2D nuclear magnetic resonance (NMR) provide robust and efficient finger printing of each polyphenol type at the skeleton level. Therefore, understanding the molecular skeleton of polyphenol types and their corresponding NMR typical signal has clearly become a necessity. However, for those in the initial involvement stage of polyphenol studies who do not possess adequate fundamental concepts in organic molecule structure, this will be a burdensome task. The goal of this review is to facilitate understanding how to use ¹H and ¹³C NMR spectra to figure out the type of polyphenol based on the type of skeleton derived from possible biosynthesis pathways. Since simple polyketide polyphenol groups are very rarely found in a settled group, shikimic acid-derived compounds such as hydroxy benzoic acid and ferulic acid become the entry point to insight into more complex groups: coumarin, lignan, diarylheptanoid, Anthraquinone, phloroglucinol, xanthonoid flavonoid, stilbenoid, glycosides, and combined polyphenols with terpenoids. 2D NMR methods, in particular COSY and HMBC spectra data, are highlighted for determining the attachment site of each skeleton identity. Flavonoid, lignan, and stilbenoid oligomer possibilities possessing spatial orientation are also featured.

Keywords: polyphenols, natural dietaries, healthy food product, ¹H, ¹³C NMR, HMBC

Cite This Article: Azis Saifudin Habibie, Habibie, Muhammad Aswad, Yasuhiro Tezuka, and Ken Tanaka, "Simplified Practical Identification of Polyphenol Types in Natural Dietaries Based on 1D and 2D Nuclear Magnetic Resonance." Journal of Food and Nutrition Research, vol. 11, no. 12 (2023): 742-751. doi: 10.12691/jfmr-11-12-6.

1. Introduction

Polyphenols are a natural product compound group constructed by benzyl rings ornamented with hydroxyl moieties. Sometimes hydroxyl group undergo substitution to become alkoxy. They are consumed on a daily basis by humans because they are naturally present widely in fruits, cereals, vegetables, beverages, etc. with concentrations ranging from 1-25% [1,2]. Compared to the other natural compound groups, polyphenols have gained the most attention because their long-term consumption is linked to large contributions to human health. Moreover, when consumed daily from edible plants, natural polyphenols give an indication of a relatively nontoxic effect. According to a plethora of

ongoing clinical reports and fundamental in vitro and in vivo studies, they evidently exert numerous biological activities that are vital to supporting normal metabolisms [3]. Furthermore, data analysis from epidemiological studies and associated meta-analyses have fundamental outcomes that long term diets with rich natural polyphenols associated with some health promotive contribution and linked with the prevention against chronic diseases development such as cognitive defect [3] cardiovascular diseases in particular atherosclerosis [4,5], numerous cancer progression [6-8], osteoporosis [9,10], digestive system disorders [11,12], preventing overweight [13] and many crucial effects closely to the promotive status of human growth and general health. Therefore, it is critical for domestic policy that the governmental authority provide appropriate guidance on polyphenol quality in agricultural product treatments and processed

food products. Furthermore, the developing formulas of modern health food products, functional foods, dietary supplements, and even cosmetics have been leveraging numerous plant components rich in polyphenols as raw materials. Since polyphenols are widely distributed in numerous plant families, genera, and species, from terrestrial to marine biota, there is great potential for their further studies and utilization in a broad range of industrial products. Although considered just phenolic entities, polyphenols possess diverse structural combinations in nature. Therefore, it is urgent to certify their identity of the raw materials, the processed formula, and the final products. It is mandatory from the initial stage of study to establish their authentic identity. In spite of numerous advanced methods for natural food quality control and processing that have been introduced, nuclear magnetic resonance (NMR) spectrometry appears to be irreplaceable for providing robust molecular identity to natural products. NMR spectra provide precise and distinctive data from the atomic fingerprinting level, in particular hydrogen and carbon atoms, to their constructed skeleton. A fundamental understanding and practical concept of structural molecules corresponding to typical NMR spectra will be very valuable for those in the initial stages of involvement in natural polyphenol dietary research and who have an inadequate organic chemistry background. This review's goal is to feature the basic building block structure of polyphenol groups and their combinations based on the putative biosynthesis pathway, to envisage their typicality based on 1D NMR by means of ^1H and ^{13}C NMR spectra, and further to elaboratively analyze the characteristics and distinguish the properties of each polyphenol type according to 2D NMR spectra, in particular the key HMBC (Heteronuclear Multiple Bond Correlation) correlations. Last but not least, it should be noted here that an adequate literature study on chemotaxonomy is fundamentally important to reconcile the polyphenol structural determination, especially referring to NMR spectra data of reported metabolites from previous organism families, genera, and species.

2. Building Block of Polyphenols

Although more than 9,000 polyphenols have been identified, they can be simply classified into limited subclasses based on their typical configuration. As a consideration, there are two main biosynthetic pathways that produce polyphenols: the polyketide pathway and the shikimic pathway [14]. The hydroxyl functional group further ornaments the phenyl ring, which serves as the main building block as a result of these two pathways. Fundamentally, the polyketide pathway yields compounds with an even carbon number, which C2 and its multiple ($\text{C2} \times n$) represent. When two or three hydroxyls substitute for its benzyl and their positions are in *meta*

with one another, it takes on a more specific character [15,16]. Sometimes methoxyls replace those hydroxyl hydrogens to produce less polar compounds. However, the simple structure of a polyketide compound with a single benzyl ring is rare. Numerous reports claim that the polyketide pathway is responsible for producing anthraquinones. The vast majority of polyphenolic polyketides are found as combined structures with a shikimic derivative skeleton. Meanwhile, the shikimic pathway results in aromatic amino acids such as tryptophan, phenylalanine, and tyrosine [17]. These aromatic amino acids further form the phenylpropanoid skeleton, which is symbolized by C9 (C6 representing the phenyl ring and C3 for the propanoid side chain) or, most likely, its modifications, C7 (C6 representing the phenyl and C1 representing the single side carbon). When a hydrogen of a phenyl ring is substituted by a hydroxyl or alkoxy, its position will be in the *para* position toward the propanoid side chain. Meanwhile, if two or more hydroxyls are present, their positions are *ortho* one another [18]. The hydrogens in hydroxyls are sometimes substituted by methoxyls similar to those of polyketide aromatics. The exception to the cyclication formation of benzyl mode is given to xanthenes, in which hydroxyls are usually in *meta* position, although based on plausible biosynthetic pathways, they result from the shikimic pathway [19]. Simple polyphenol subclasses formed from the shikimic pathway are simple phenyl propanoids such as benzoic acid derivatives (gallic acid, vanillin, anisaldehyde, etc.) and caffeic acid derivatives (eugenol, saffrol, ferulic acid, etc.). Among C9 skeletons in particular, very often they are able to combine among them to form the $\text{C9} \times 2$ formula, such as lignans, diarylheptanoids (curcumin), and dicoumarin. The combination between polyketides and phenylpropanoids results in flavonoids ($\text{C2} + \text{C9}$), stilbenes ($\text{C2} + \text{C9}$), and more complex structures of flavonoid oligomers called non-hydrolysable tannins with a formula of $(\text{C2} + \text{C9}) \times n$. All those polyphenol types are able to conjugate with sugars to result in numerous glycosides. Nevertheless, carbon numbers are not always fixed according to the original formula; depletion or addition of one carbon may occur in C2 derivatives such as emodin anthraquinone type (Figure 1) or the side chain of C9. It should be noted that the other pathways, such as the mevalonic and deoxyxylulose pathways, may produce polyphenols, although in an insignificant number. Nevertheless, terpenoids produced from these pathways are very often combinators of polyphenols. Since polyphenols bearing halogen, nitrogen, and sulfur atoms are very rare in edible plants, these types are excluded from this review. Last but not least, new discoveries in biosynthetic pathways could make these theoretical ideas conflict with each other. However, for practical NMR interpretation, efforts to estimate structure based on recognizing patterns in building blocks are very useful.

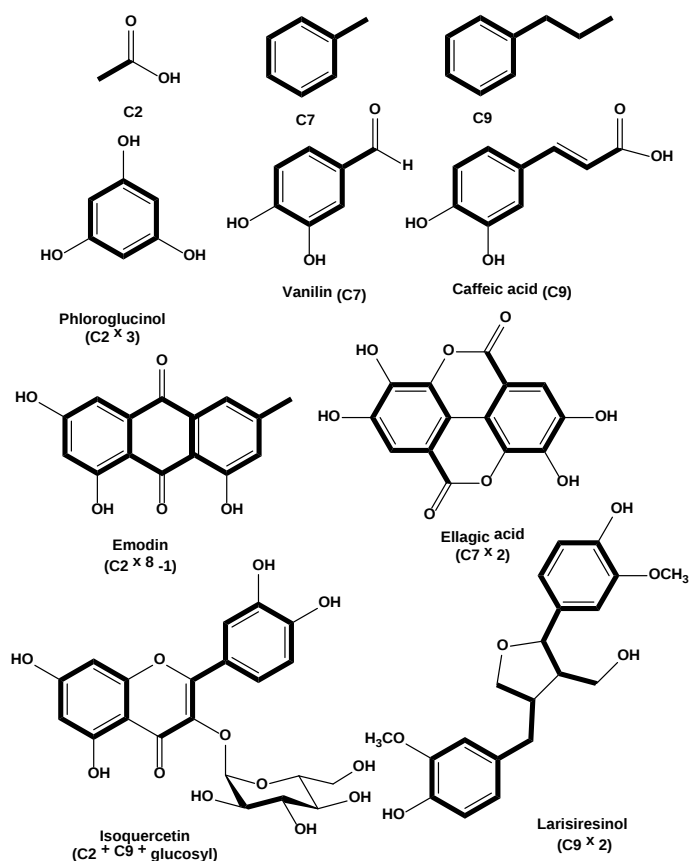


Figure 1. Polyphenol building blocks, as indicated by bold lines depicted according to putative biosynthesis pathways, can be categorized into polyketide (C2), phenylpropanoid (C9 or C7) derivatives, and their combinations

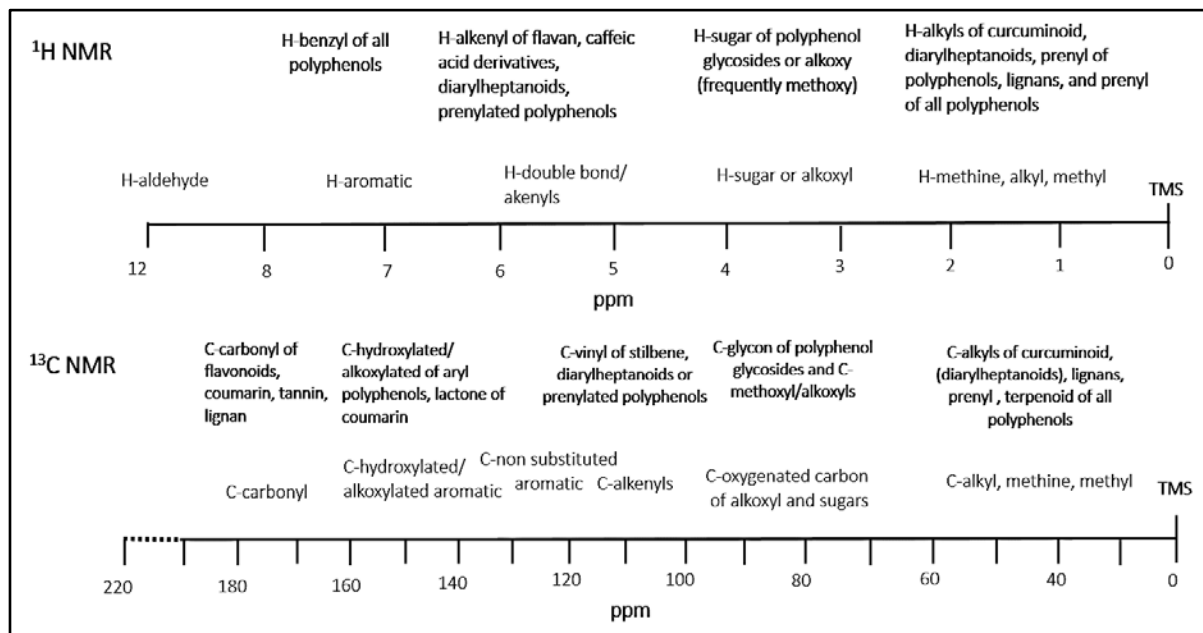


Figure 2. Typical chemical shift signal positions of polyphenol skeleton types in ¹H NMR spectra based on plausible biosynthesis pathways in the ¹³C NMR spectrum. Each typical metabolite has a similar trend. Usually, tetramethylsilane (TMS) is used as an internal standard

3. The Role of 1D and 2D NMR for Determining the Building Blocks of Polyphenols

Based on the putative biosynthetic approaches, there are two main building blocks of polyphenols: polyketides and

phenylpropanoid polyphenols. However, both building blocks have a benzyl ring as their main structure. The role of 1D NMR spectrometry by means of mean ¹H and ¹³C NMR spectra data is to estimate the fundamental backbone of the metabolite structure. The first important piece of information is the hydrogen and carbon chemical shift signal positions of typical polyphenol groups. In aromatic structures, magnetic anisotropy is the main factor

that determines the hydrogen/carbon chemical shift instead of electronegativity power. In aliphatic structures, electronegativity power is the main factor that determines how well the neutrons are shielded. As a consequence, their hydrogen signals are observable around δ_H 6.5 to 8 ppm, while their carbon signals are around δ_C 110 to 160 ppm, respectively (Figure 2). The centers of hydroxylated or alkoxyated aryl carbon are recognizable between δ_C 140 and 165 ppm. So, using the formula $C2 \times n$ in the ^{13}C NMR spectra, we can figure out that polyphenolic polyketides have six signal numbers at δ_C 110 to 160 ppm. In the case of symmetric aryls, at least two hydrogen and/or carbon signals are overlapped. The second most important thing is the strength of their aromatic hydrogen signal. An asymmetric aromatic ring has short hydrogen signals with an integration value of 1, which means that each single hydrogen is bonded to a carbon atom. On the other hand, symmetric aromatic rings at least have one integration value of 2. There are sometimes methoxyl substituents, which are easy to spot because they show up as high signal peaks with an integration value of 3 at δ_H 3–4 ppm. Meanwhile, the hydrogen signal of hydroxyls is undetected because hydrogen is fast moving in many NMR solvents. The third fundamental data point is the splitting pattern of aromatic hydrogen. A single hydrogen in it will have a singlet peak shape called singlet (s). Scrupulous attention should be paid when the hydrogen number is more than one since their splitting patterns follow the ABX system due to *ortho*, *meta*, and *para* coupling [20]. Their typical coupling constant values can be estimated around $J = 6$ –10, 1–3, and 0–1 Hz, respectively. Since the *meta* hydroxyl/alkoxyl position is one of the main characteristics of polyketide aryl hydrogen, the corresponding typical coupling can be estimated accordingly. It can also be figured out for phenylpropanoid aromatic hydrogens, which tend to have two *ortho* positions and one of the hydroxyls in a *para* position toward the carbon side chain (C3 or C1). For polyphenolic phenylpropanoid derivatives in particular, the hydrogen signal can be observed at δ_H 5–6, 3–4, and 0.5–2.5 ppm for vinylic, oxygenated carbon, and alkyl, respectively. Figure 2 shows the positions of the hydrogen and carbon chemical shift signals, as well as the putative metabolite groups that go with them. Lastly, but most importantly, giving firm NMR spectra of previously reported metabolites from the same species, genus, and family is helpful for understanding structural and chemotaxonomic horizon mapping.

In regard to 2D NMR spectra applications, its basic function is to examine the bond connectivity between two adjacent hydrogens by using COSY (Correlated Spectroscopy), correlate the protons in a bond to a heteronucleus of ^{13}C based on HMQC/HSQC (Heteronuclear Multiple/Single Quantum Coherence), and correlate hydrogen contiguity with 1 to 3 adjacent carbons based on key HMBC (Heteronuclear Multiple Bond Coherence) correlation. A cross-peak in the spectra in 2D NMR indicates the correlations. The most important part of 2D NMR is the key HMBC correlation spectra data, which is used to confirm the binding sites between backbones and indirectly towards hydroxyls, alkoxy, or other substituents in the structure. Since sensitivity becomes an issue in HMBC spectra measurement, the

quantity and duration of scanning should be sufficient. Thus, it is recommended to have at least a 5-mg sample and adequate scanning time for obtaining sufficient HMBC spectra data. Some chiral carbons may be present in polyphenol structures, particularly in flavonoids, lignans and oligomer stilbenoids, which results in certain spatial moiety orientations. To determine their relative stereochemistry, besides using chemical modeling, nuclear overhauser effect (NOE) by irradiating deduced proxymite hydrogens accordingly. Enhanced signals of counterpart hydrogens make it evident that they are at the same spatial orientation, otherwise in the opposite direction. Sometimes, the stereochemistry data are obtained by using ROESY or NOESY in some complex structures. However, care should be taken because the adjacent hydrogens in NOESY and ROESY correlations contain COSY correlations, which result in biased spatial proximity.

Benzoic and caffeic acid derivatives typical signals

Because simple polyketides with one benzyl ring are very rare, the conceptual understanding of 1H and ^{13}C NMR and 2D NMR spectra data is based on the simplest polyphenol backbones of shikimic acid derivatives, such as benzoic and caffeic acid types. Further steps on the other polyphenol types spectra data will be conveniently estimated. Caffeic acid and benzoic acid derivatives are produced from chorismate in the shikimate pathway. Benzoic acid is rationally proposed from cinnamic acid rearrangement [21]. Well-known caffeic acid derivatives are ferulic, sinapic, coumaric, salvianic, and trihydroxycinnamic acid, while benzoic acid derivatives are gallic, vanillic, syringic, and protocatechuic acid [22]. Since they are produced from the same pathways, they share the same aromatic hydrogen and carbon signal characters in 1D NMR spectra, such as their hydrogen signals being recognized between δ_H 6.5–8 ppm and their carbon signals at δ_C 128–160 ppm [23]. Depending on the ornamenting hydroxyls or alkoxy attached, some carbon signal positions are noticeably shifted between δ_H 140 and 160 ppm. Since the aryl of hydroxybenzoic acid is a symmetric ring (Figure 3.A), two doublet signals ($J = 9$ Hz) are recognized in the 1H NMR spectrum with an integration value of 2. On the other hand, ABX splitting patterns result in aromatic ferulic acid of H-1, H-2, and H-3 with doublet of doublet (dd) signal shapes with corresponding J values of 6–10 and 1–3 Hz, respectively. Finally, the other important identity of this group is the three-carbon side chain, whether consisting of alkenyls (δ_C 105–125 ppm) or just alkyls (δ_C 30–65 ppm). The presence of carbonyl carbon can be recognized around δ_C 169 ppm. The bold lines in Figure 3.B indicates that connectivity between hydrogens in COSY spectra is the primary use of 2D NMR in the determination of benzoic and caffeic acid derivatives. Moreover, key HMBC correlations for identifying hydroxyl and alkoxy attachment sites based on selected aryl hydrogen correlations with the nearest carbon within 1 to 3 adjacent carbons, especially shifted carbon signals at δ_C 140–160 ppm, give a clear location of these functional groups in the benzyl ring. In the case of the alkoxy binding site near the benzyl ring, the hydrogen alkyl is the first basis correlation toward direct shifted aryl carbon signals (δ_C 140–160 ppm). It is also supported by the later

correlations among the aryl hydrogens to the nearest oxygenated carbons.

Coumarin skeleton typical signals

Coumarin is distributed in the families Compositae, Leguminosae, Umbelliferae, Rutaceae, Mignonette, Mulberry, and Thyme. Ascleutin, scopoletin, umbelliferone, and daphnetin are frequently identified as the major coumarins [24]. The reported significant biological activities of coumarins are anti-inflammatory, anticoagulant, anticancer, antifungal, and antiviral [25]. Coumarin is made up of a phenylpropanoid skeleton, and the propanoid side chain goes through cyclization to form the lactone bridge to the aromatic ring (Figure 3.C.1). Thus, the ^1H and ^{13}C signals of coumarin benzyl are similar to those of the model compound in Subsection of benzoic acid and caffeic acid derivatives typical signals. In total, nine carbons with distinct chemical shifts are at carbon numbers 2, 3, and 4 around δ_{C} 160, 113, and 140 ppm, respectively [26,27]. Those three signals are assumed to be the typical identity of the coumarin skeleton, in particular its lactone carbon. Although there is still limited data, it is possible to combine coumarins to produce a dimer or oligomer. As explained in Subsection of benzoic acid, the main use of 2D NMR is to use its key HMBC correlations to figure out where the alkoxyis are in the aromatic ring. Very often, coumarin is modified with isoprenyls in many plant species. This case is featured in Subsection polyphenol combination.

Diarylheptanoid skeleton typical signals

The most popular diarylheptanoid type is curcuminoids, which are present in some rhizomes of the Zingiberaceae genus and have been used as food condiments and traditional remedies for centuries. They have been demonstrated to have anti-inflammatory, anti-tumor, anti-estrogen, hepatoprotective, anti-leishmanial, and neuroprotective effects in both in vitro and clinical tests [28]. There are three types of diarylheptanoids: linear, cyclic, and cyclic ether-type [29]. Two phenylpropanoids (C9) connected by one carbon make up a diarylheptanoid (Figure 3.2). Their hydrogen and carbon signals are recognizable at δ_{H} 6.5–8 ppm and δ_{C} 115–160 ppm, respectively. Depending on the ornamenting hydroxyls or alkoxyis attached to the carbon, their signal positions are shifted between δ_{C} 140 and 160 ppm. For aliphatic curcuminoid chains, vinylic hydrogen signals are between δ_{H} 6.0 and 7.5 ppm, and carbon signals are between δ_{C} 120 and 140 ppm. Finally, their ketone signals have characteristically short peaks at δ_{C} 180–185 ppm [30]. The contribution of 2D NMR here is the key HMBC correlation for identifying the hydroxyl binding site. Based on some aryl hydrogen correlations with 3.2 adjacent carbons at δ_{C} 140–160 ppm, it is clear that this group is in the benzyl ring. In the case of alkoxyis, their alkyl hydrogen is the first point of correlation toward certain aryl carbons. It also supports correlations between the aryl hydrogens that surround aryl carbons. Similar patterns can be seen in the covalent bond between two aryls in a cyclic diarylheptanoid and in the ether bridge in a cyclic ether-type diarylheptanoid polyphenol.

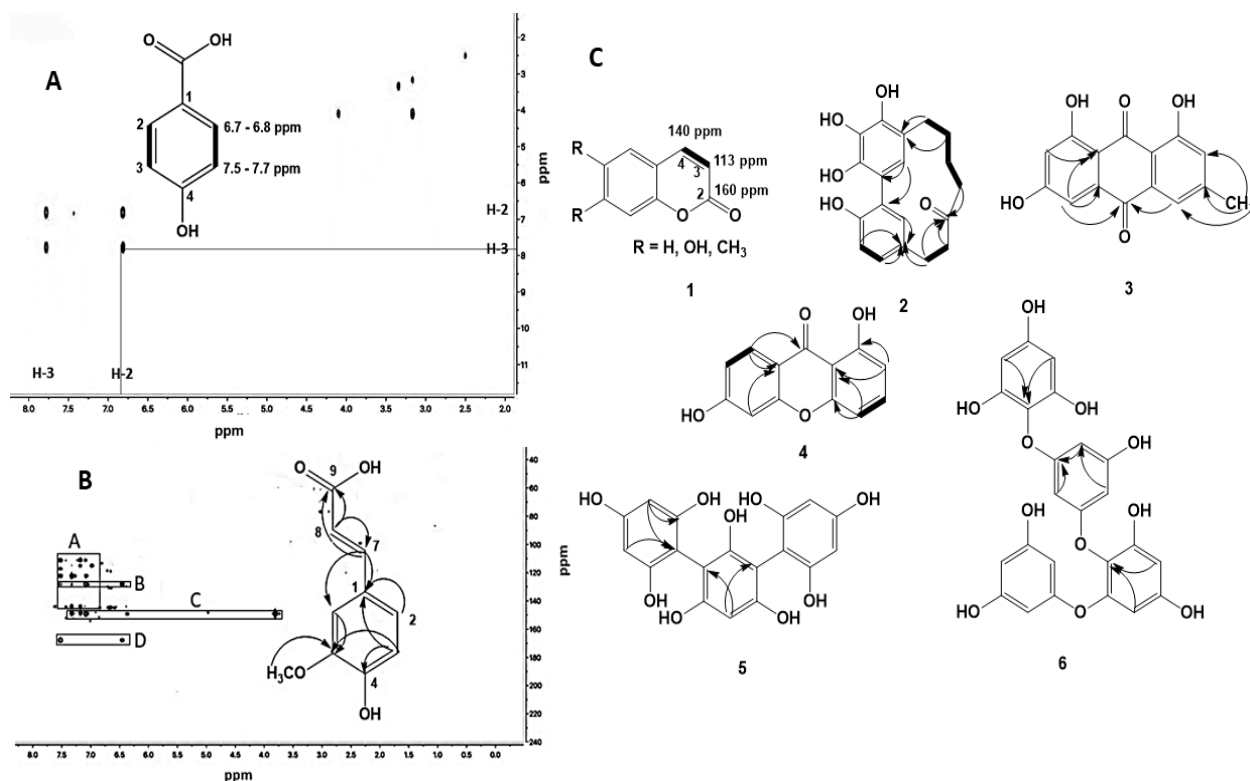


Figure 3. Connectivity in aromatic ring (bold line) based on COSY spectrum of hydroxy benzoic acid (A) and key HMBC correlation between hydrogens and carbons of ferulic acid (B). These concepts on simple structure are applicable to coumarin (1), diarylheptanoid (2), anthraquinone (3), and xanthone (4). The lack of hydrogens in phloroglucinol groups (5 and 6) the structural determination can be assisted with molecular models

Anthraquinone, phloroglucinol, and xanthonoid polyphenol skeleton typical signals

Unlike phenylpropanoids, simple polyketide polyphenols are rarely found in nature. Polyketide polyphenols like anthraquinone, phloroglucinols, and xanthonoid hydroxyls have been found in fairly large numbers. Anthraquinones are promising to have antimicrobial, neuromodulatory [31] and anticancer potential [32]. On the other hand, hydroxylated phloroglucinols have been found to be promising as PTP1B inhibitors [33], antivirals [34], and antioxidants [35]. Hydroxylated xanthenes exhibited potent α -amylase and glucosidase inhibitors [36], antioxidants [37], and EGFR-Tyrosine Kinase inhibitors [38]. As featured in the section of basic phenolic, if the hydroxyl or alkoxy number available is more than two, they tend to be in meta position. As a consequence, a double of doublet (dd) splitting pattern with a small J value (1-3 Hz) will result. For known compounds, resolving this typical coupling constant even in unpurified samples will provide an initial clue to their presence. Specifically, aromatic methyl is frequently present in the anthraquinone and phloroglucinol groups. To identify its presence, the key HMBC correlations must appear between the methyl hydrogen and 2-3 adjacent carbons (Figure 3.C.3). This mode may be followed for the more complex structures of anthraquinone, phloroglucinol, and xanthonoid. While the COSY contribution is not significant since adjoining hydrogen is rarely present in these polyphenol types. Therefore, besides 1D and 2D NMR spectra data, by helping of chemical models the structure of these compound type may be established.

Flavonoid skeleton typical signals

The flavonoid group is very popular and has gained two decades of popularity in the studies of natural product dietaries. It consists of more than 6,000 reported flavonoids [39]. It has a wide range of bioactivities, but it is urgently linked to preventive and health-promoting areas, such as the prevention of atherosclerosis [40], erectile dysfunction [41], the risk of death in cancer patients [42], Alzheimer's disease, and dementia [43]. Biosynthetically, flavonoid skeletons are constructed from the combination of polyketide and phenylpropanoid backbones from the shikimate pathway. As a result, flavonoids have four main skeleton types: flavon, flavan, antocyanin, and isoflavan. The only difference between the first three is where the double bond on the pyrone is and whether or not there is a ketone group. The last one is made when the phenyl moves to the C-3 position. Basically, these four subgroup skeletons can be deduced based on a fifteen-carbon number, including twelve in the aromatic area (δ_C 140–160 ppm). The further chemical shifts of aromatic hydrogen and carbon in rings A and B are similar in many cases to those featured in section of basic aromatic phenol for rings B. Therefore, the ring C character becomes the focus of the rest of the determination. A carbonyl carbon signal around δ_C 170 ppm and a short singlet hydrogen around δ_H 7 ppm or no hydrogen signal are indication of ring C flavon (Figure 4.1). Indicators of ring C of flavan are the presence of ketone groups around δ_C 178–180 ppm or their absence, a doublet or singlet of hydrogen short signal at δ_H 4.5 ppm, and an alkyl hydrogen signal at δ_H 1.5–2.5 ppm. While a

distinctive hydrogen singlet signal or doublet at δ_H 8.6–8.8 ppm and two carbon signals at δ_C 155–162 ppm and without a ketone signal indicate ring C of antocyanidin type [44] (Figure 4.3).

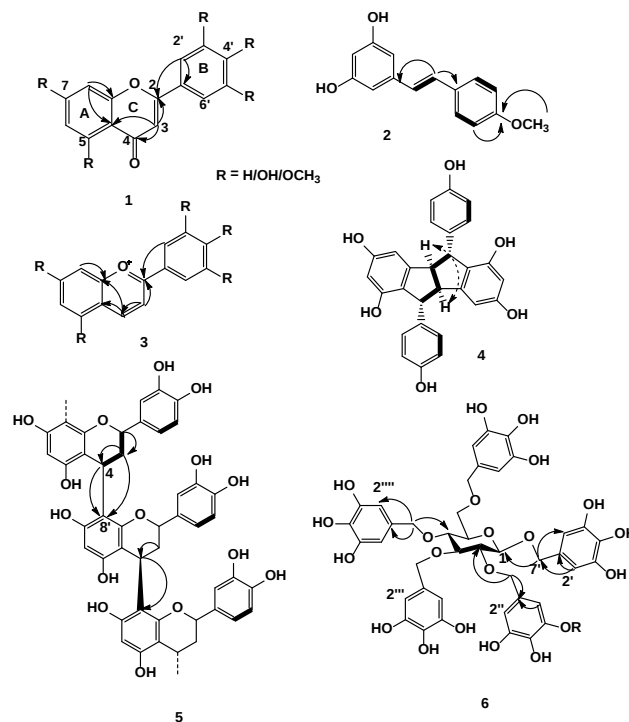


Figure 4. Selected key HMBC correlations in model of flavon (1), antosian (2), nonhydrolysable tannin (5), and hydrolysable tannin (6). COSY correlations in stilbenoid models (3-4)

On the other hand, the correlated hydrogen signals at δ_H 4.0–4.6 ppm and carbon-bearing oxygen at δ_C 80–84 ppm in the HMQC spectrum are attributable to isoflavan C ring structure. The adjacent hydrogens at the pyrone ring can be examined on the COSY spectrum, besides the splitting pattern supporting data between them. Otherwise, it is absent as isolated hydrogen. As featured in Benzoic and caffeic acid derivatives, the basic application of key HMBC correlations in flavonoid structure is to identify the alkoxy location in the benzyl rings and to establish ring C type. Meanwhile, ketone group presence at C-4 can be examined with H-5 signal correlations. The attachment of the B ring toward the pyrone ring can be examined based on key HMBC correlations between H-2' or 6' and C-1. The specificity of anthocyanidin can be examined by key HMBC correlations between H-4 (δ_H 8.6–8.8 ppm) and C-6 (δ_H 155–158 ppm).

Stilbenoid skeleton typical signals

The stilbenoid is constructed from a polyketide benzyl and a carbon that seems to be missing from the phenylpropanoid side chain. Because its aromatic parts come from similar biosynthetic pathways with the flavonoid group, their characters have similar fashion. The center of difference is in the two-membered alkyl bridge. For simple stilbenoid, beside recognizing their aromatic chemical shifts, two alkenyl hydrogen signals are noticeable at δ_H 6.87–6.91 and 7.0–7.04 ppm with a typical trans coupling constant (J) of 16.2 Hz [45]. The connectivity among alkenyl or cyclic alkyl hydrogens can be observed based on COSY spectra. The key HMBC

correlations of alkenyl hydrogens with 1-3 adjacent carbons will in the meantime determine the positions of aromatic carbons. Because some chiral carbons construct stilbenoids that result in three-dimensional structures. Their stereochemistry can be elucidated by using chemical modeling and NOE (Nuclear Overhauser Effect) spectrometry, as depicted by the double arrow (Figure 4.4). The 2D technique ROESY or NOESY can also be applied for stereochemistry determination; however, the COSY contribution becomes a drawback in both methods.

Non hydrolysable tannin skeleton typical signals

Understanding the flavonoid skeleton and specific chemical shift patterns in both ^1H and ^{13}C NMR becomes the basic concept for the typical unhydrolysable tannin skeleton signal since non-hydrolysable tannins are constructed from flavonoid, in particular flavan, attached covalently between C-4 and C-8 with other flavan (Figure 4.5). Therefore, the numbering order of this tannin is the same as that of flavonoids. This tannin type is also called condensed tannin. The important aspect of this group structure is to determine the attachment site between flavonoid monomers. While the HMBC correlations between H-4 (δ_{H} 4.5 ppm) and C-8 (δ_{C} 110 ppm) become the key determinants for this structure [46,47]. In the case of chiral carbon present in the structure, the relative stereochemistry is determined based on NOE, NOESY, or ROESY spectrometry.

Hydrolysable tannin skeleton typical signals

These tannin types are well known as the main constituents in tea leaves. The gallic acid unit, which results from the shikimate pathway, is responsible for creating its fundamental structure. These units are further conjugated with a monosaccharide ring through ether bridges (Figure 4.6). In the opposite direction, tannin that can be hydrolyzed goes through a hydrolysis reaction at its ether bridges, which makes sugar and gallic acid units. The specific chemical shifts of H-1,2,3,4,6 of glucopyranosyl are recognized between δ 4.4 to 6.23 ppm with each J values around 9.5 Hz [48]. The key HMBC correlations between H-7 each galloyl to selected C-1, 2, 3, 4, 6 of sugar are used to identify the connection site [49]. The simpler or more complex hydrolysable tannins, such as sanguin H-6 and others, may follow that elucidation pattern. However, a direct covalent bond between benzyl rings may occur, such as in ascorgeraniin. The determination of their binding site may involve HMBC correlations, a chemical structure model, and MS spectrometry measurements since there is no hydrogen benzyl within at most 3 adjacent carbon distances of the counterpart benzyl ring. Finally, both ^1H and ^{13}C NMR characteristic signals of the benzyl ring have similar fashion to those of the general phenylpropanoid except due to the frequently present ortho tri-hydroxyl functional group, which results in a singlet shape signal of H-2 and H-5 with an integration value of 2. Therefore, by following these fundamental patterns, approaches can be applied for other hydrolysable tannin identifications.

Lignan skeleton typical signals

Although lignan is distributed relatively limitedly in the Phyllanthaceae, Piperale, and Magnoliace plant families, it becomes an interest in the current study of polyphenols

since lignan clinically offers prevention of numerous metabolic syndromes [50,51]. Lignan metabolite products such as secoisolaricresinol, enterolactone, and enterodiol, the bioactives from flaxseeds, are reported to significantly reduce blood lipids ranging from cholesterol, triglycerides, low density lipoprotein cholesterol, and lipid peroxidation products [52]. Structurally, lignans consist of two phenylpropoid units linked through β,β -bonds (Figure 5.1). However, more complex lignans consisting of more than three propanoid groups have been reported too [53]. Hence, understanding NMR signals in the aliphatic part becomes the fundamental factor in determining the lignan structures. While the aromatic parts have similar characteristics to previous phenolic phenylpropanoid derivatives. Since oxygenated carbon is often found in the propanoid part, the hydrogen signals come from around δ_{H} 3.5–4 ppm, while the rest are usually at δ_{H} 1–2.4 ppm in the alkyl area. In cases where methoxy is present, they are recognized with tall singlet peaks with an integration value of 3. It should be noted here that some lignans have symmetric structures, so the integration value is accordingly relative. Therefore, it should be confirmed by mass spectra for the deduced novel compound, while apparently known compound spectral comparisons with reported data will be sufficient. The application of 2D NMR is specifically in the propanoid to identify the linking pattern and functional groups in particular aromatic alkoxy groups. Their propanoid parts can be characterized based on the splitting pattern of hydrogen signals, COSY, and key HMBC correlations. Based on key HBMC correlations of selected hydrogen toward 1-3 adjacent oxygenated and aromatic carbons, the propanoid part characteristics can be distinguished from one another.

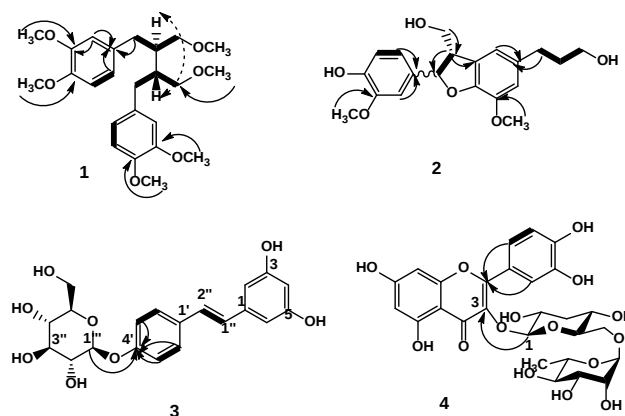


Figure 5. COSY (bold lines) and selected key HMBC correlations (arrow) in lignan (1-2), stilbene, and stilbenoid models (3-4). NOE correlation (dashed double arrow) is present in chiral carbons of lignan

Polyphenol glycoside typical signals

The term of glycoside refers to the conjugation between any organic compound and a sugar group. Therefore, to elucidate the polyphenol glycosides, comprehending very well all previous polyphenol unit characteristics is a fundamental requirement. As structurally featured in Subsection hydrolysable tannins, the sugar binds to polyphenol units through ether bridges. The presence of sugar units is easily recognized in ^1H and ^{13}C NMR spectra with chemical shift signals between δ_{H} 3–4 ppm and δ_{C} 70–90 ppm, respectively. Most of their hydrogen

signal integration values are 1. In cases where the hexosyl is present as a sugar unit greater than 1, an integration value of 2 is present, representing H-7 (Figure 5.3-4). Meanwhile, the rest of the signals corresponding to each polyphenol type can be referred to in the above-mentioned sections. The key HMBC correlations are used to identify the conjugation site between the sugar and aglycon units, as depicted by the correlation between H-1 and C3, which indicates the attachment sites.

Combined polyphenols with other skeletons

Not only can different kinds of polyketide and shikimic polyphenol combine with each other, but each kind can also combine with terpenoid groups that come from the mevalonic or deoxyxylulose pathways. Usually, the isoprene building block becomes the main combinator such as in cannabinoid group. For the information, cannabinoid source *Cannabis sativa* now is legalized even for food condiment in Thailand [54]. Terpenoid skeletons can be found in a compound's structure by observing at its hydrogen and carbon signals at δ_H 0.5–2.5 ppm and δ_C 10–55 ppm. These signals stand out because their integration ratios are proportionally coherent to those of the aromatic area. Since the specific identity of terpenoid is branching methyls, frequently as high signals, either singlet or doublet, result in an integration value of 3 at δ_H 0.5–2.0 ppm. Figure A of Supplemental information shows how key HMBC correlations are used to find the bonding sites between a terpenoid's hydrogen and the closest aromatic carbons.

4. Metabolomic Analysis of Polyphenols in Mixtures

Metabolomic analysis of any compounds in the natural sample can be conducted without exhausting fractionation and purification steps. For targeted compounds, NMR solvent choice is the determinant to enable maximum solubility during the measurement. Since polyphenol's first identity is its aromatic ring, its hydrogen characteristics become the focus of analysis. Their characteristic finger printing in 1H NMR spectra can be recognized based on the chemical shift, splitting patterns, and coupling constant. Since overlapping signals are a potential problem, J value resolves them to determine targeted compound entities [55,56]. For known targeted compounds, detailed analysis can be performed to match the presence of functional group signal identity. However, since NMR sensitivity is an issue, long scanning is mandatory for producing sufficient signal acquisition data in the metabolomic/nomic analysis. Usually, overnight scanning is required to obtain all those parameters. Moreover, at least three independent experiments must be conducted to ensure repeatability. On the other hand, determining novel polyphenol compounds in a natural or fermented sample is also possible by comparing the blank or untreated sample for certain distinguished appearance signals. Sometimes, partition steps of samples are necessary to provide strong NMR signals.

Spectroscopy is a reliable technique that can quickly examine mixtures at the molecular level without the need for separation or purification processes. It is particularly well-suited for use in food science applications. Although

NMR is gaining popularity among food scientists, it remains underused in this field mostly because to its expensive cost, relatively low sensitivity, and the lack of NMR competence among many food scientists [57,58]. The use of food and beverage final products is now restricted to certain areas, such as wine products [59] and goods containing vanillin [60]. Nevertheless, the ongoing advancement of this technique remains arduous in meeting the demands of everyday industrial requirements.

5. Conclusion

In this review, we have made it easier to understand the structure of polyphenols based on a biosynthetic approach. We have also shown how 1H and ^{13}C NMR can be used in a very basic way. The two most fundamental natural phenols, ferulic acid and hydroxybenzoic acid, set the typical signal that identifies each type of polyphenol. In polyketide compounds, aromatic hydrogen has a chemical shift signal δ_H 7–8 ppm, and aromatic carbon has a signal at δ_C 140–160 ppm in shikimic derived polyphenol compounds. In cases where *ortho*, *meta*, and *para* of hydrogen positions can be deduced based on J values and corresponding splitting patterns as featured. Methoxyl is frequently present to replace hydroxyl groups, and its position is determined based on the correlation between hydrogen methoxy and typical oxygenated carbon. Usually, hydroxyl or methoxyl *meta* coupling ($J = 1\text{--}4$ Hz) is an indication of polyketide aryls. Apprehending their typical aromatic spectra will facilitate the more complex polyphenol structures. The differentiating elements among polyphenols are their side-carbon chain structure or their combination with sugars or terpenoids. Once the integration value among hydrogens in the spectra is established proportionally, 2D NMR is used to confirm the polyphenol structural environment, in particular through HMBC correlations. The key HMBC correlations between selected hydrogens of the phenylpropanoid side chain (alkyl or alkenyl) and certain carbons of the polyketide aromatic building block are the key data for determining flavonoids, stilbenoids, condensed tannins, diarylheptanoids, and lignans. The presence of HMBC correlations between aromatic methyl hydrogens (δ_H 2.3–2.5 ppm) and two aromatic carbons usually points to anthraquinones. This is supported by some keton carbon signals (δ_C 175–180 ppm). Frequently, phloroglucinol has basic character *meta* coupling of hydrogens ($J = 1\text{--}4$ Hz) and present as oligomers or combine with other skeletons. On the other hand, xanthonoids typical 1D NMR signals can be recognized with a keton carbon signal (δ_H 180–185 ppm) and two oxygenated carbons (δ_C 80–95 ppm) of ring C, together with key HMBC correlations between selected aromatic hydrogens and either of those two carbon types. While the evidence for correlations of aromatic hydrogen to sugar's carbonyl carbon or sugar hydrogens to selected aromatic carbons is an indication of hydrolysable tannins and polyphenol glycosides, On the other hand, selected correlations of alkyl hydrogens to certain aromatic carbons become indications of prenylated polyphenol skeletons. Because optimum HMBC signal appearance depends a lot on the sample quantity, providing sufficient sample weight and scanning durations are mandatory.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence this paper.

ACKNOWLEDGMENT

The authors are grateful to Universitas Muhammadiyah Surakarta, Hokuriku University and Ritsumeikan University for granting this study.

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