The Polyphenols – Valuable Bioactive Compounds

Andrea-Maria Malanca, Eniko Miheleș, Magdalena Roșu, Patricia Cristina Tarkanyi, Monica Dragomirescu*

University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului, 119, 300645, Romania

Abstract

Polyphenols are molecules with biological activity, present in many foods such as fruits and vegetables, tea and coffee, but they are also found in wine and chocolate. Polyphenols are known for their potentially positive effects on health. Their bioactivity is exhibited by antioxidant and anti-inflammatory activity, cardiovascular and neurodegenerative protection, anticancer properties, modulating effects on the gut health, improving memory, reducing the risk of diabetes, Alzheimer's, etc. The biological activity of polyphenols is correlated with their complex structure. As the name implies, polyphenols are molecules with several phenolic groups. The phenolic groups are hydroxyl groups linked to the aromatic rings and have the ability to donate protons or electrons and reduce the reactive oxygen containing species. The polyphenols have the ability to form complex molecules with metals, thus helping to reduce the oxidative stress. The structural characteristics of polyphenols make them to interact with free radicals, proteins, but also with enzymes, highly selective protein biocatalysts, essential in the functioning of living organisms. The polyphenol content and the antioxidant activity can be assayed, in the laboratory, by spectrophotometric methods.

Keywords: bioactive compounds, polyphenol-protein complex, chemical structure, health benefits, side effects

1. Introduction

Nature is a rich source of valuable compounds, many of which have a significant impact on human health and well-being [1]. One of the most notable groups of such compounds are polyphenols. These natural molecules are widely recognized for their powerful antioxidant and anti-inflammatory properties, which can positively influence a wide range of body functions. Polyphenols, over 8,000 to date, are found in a variety of plant-based foods, and their benefits play a critical role in maintaining good health, preventing chronic diseases, and supporting overall longevity [2]. This vast diversity in polyphenols makes them an intriguing area of study, as each type may offer unique health benefits depending on its structure and concentration in different foods. For these reasons too, we have chosen to study more about polyphenols, as we believe that understanding their mechanisms of action and potential applications could lead to significant advances in improving public health and preventing disease.

2. Polyphenols in plants

Polyphenols are a group of secondary metabolites that are predominantly found in plants, including a wide variety of crops, fruits, vegetables, and even edible flowers like roses [3]. These compounds provide plants with a range of important benefits, which contribute to their survival and adaptation to their environment. Chemically speaking, polyphenols are organic substances with one or more -OH substituents attached to at least one aromatic nucleus. This unique structure is what allows polyphenols to function as powerful antioxidants, as the hydroxyl groups enable them to capture and neutralize free radicals—unstable molecules that can cause cellular damage [4].

* Corresponding author: Monica Dragomirescu, mdragomirescu@animalsci-tm.ro

Also, polyphenols are capable to interact with various proteins within the plant. Factors such as the molecular weight of the polyphenols and their hydrophobicity—meaning how well they repel / counter water—play an important role in these interactions. Larger or more hydrophobic polyphenolic compounds may bind more strongly with proteins, affecting the plant's responses to different stresses and influencing its growth. The association of these two structural components is achieved through covalent and/or non-covalent bonds, with the specific category of polyphenol being crucial to this process [5].

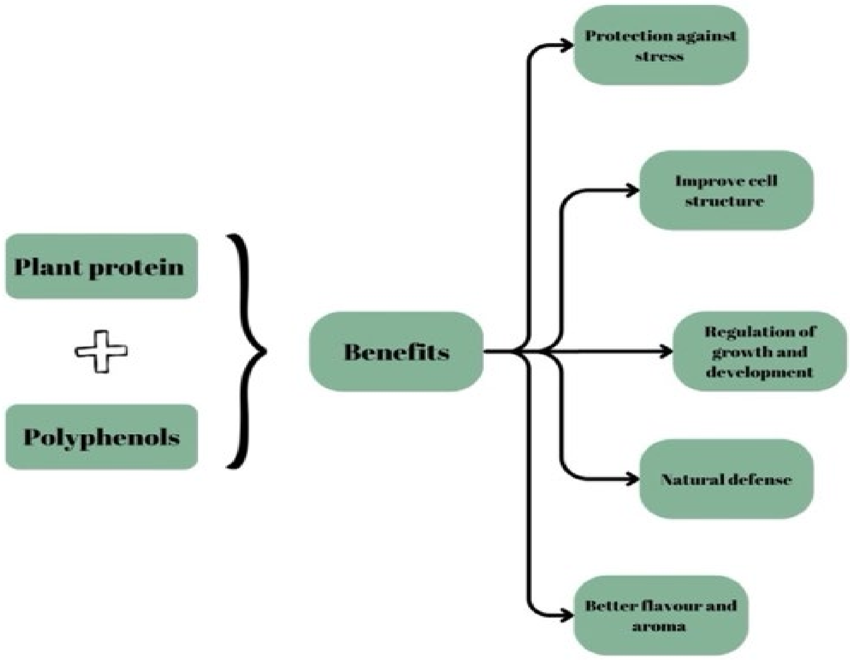

Figure 1. Benefits of polyphenol-protein complex

These interactions between polyphenols and plant proteins are vital for the plant's ability to protect itself from both biotic and abiotic stresses. Biotic stresses come from living organisms, such as pests, pathogens, or competing plants, while abiotic stresses are caused by non-living factors, like extreme temperatures, drought, or exposure to harmful UV rays. Additionally, polyphenols help enhance the structural integrity of plant cells, can regulate growth and development (Figure 1). Also, polyphenols (catechins, flavonoids) can contribute to the plant, by giving aroma and flavour [6].

3. Sources of polyphenols

Polyphenols are naturally occurring compounds found abundantly in plants and are widely distributed across various food sources. These compounds, known for their antioxidant properties, play an important role in plant defense mechanisms and offer numerous health benefits to humans (Figure 2, Table 1).

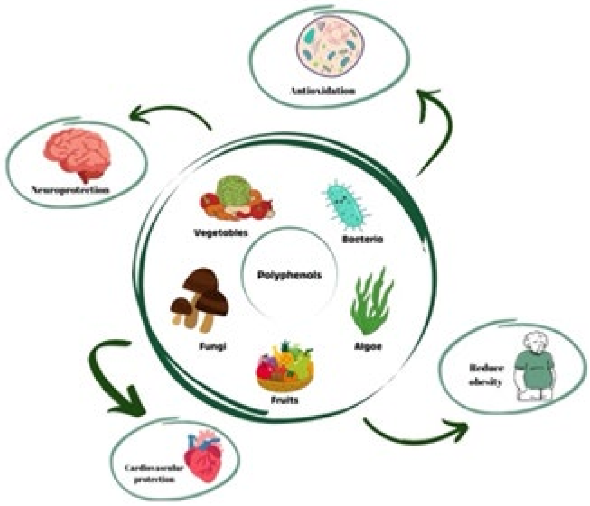

Figure 2. Sources and benefits of polyphenols

Table 1. Sources and examples of polyphenols

Polyphenols	Example	Sources
Flavonoid	Cyanidin	Berries, plums, red onion, apples
Non-flavonoids	Resveratrol	Grapes, berries, peanuts, pine tree
Phenolic acids	Caffeic acid	Barley, brewed coffee, thyme, apricots

Polyphenols are abundant in fruits like berries, apples, and citrus, which contain compounds such as anthocyanins, flavonoids, and phenolic acids. Vegetables like spinach and broccoli are also rich in flavonoids and phenolic acids, contributing to their antioxidant properties. Algae, such as brown and red algae, provide unique polyphenols, which have shown potential health benefits, such as better controllability of hypertension, diabetes and reducing obesity [2, 3]. Mushrooms are also rich in phenolic acids, including cinnamic, gallic, salicylic, syringic, caffeic acid, playing an essential role in mushroom`s biological activity and human health, because of their antioxidant capacity [1, 7]. Bacteria can not produce polyphenols, but at least it is possible the interaction with them in ways that contribute to their bioavailability or beneficial effects [8].

4. Chemical classification of polyphenols

Polyphenols can be classified, based on their chemical structure, into flavonoids, non-flavonoids, and phenolic acids.

4.1 Flavonoids

Flavonoids represent the main group present in plants, consisting of two benzene rings (C6) connected through a heterocyclic pyrene ring (C6-C3-C6) [8, 2]. The subgroups of this type of polyphenol include anthocyanins and anthoxanthins, which, in turn, encompass flavones, isoflavones, flavanols, flavonols, and flavanones (Figure 3) [2].

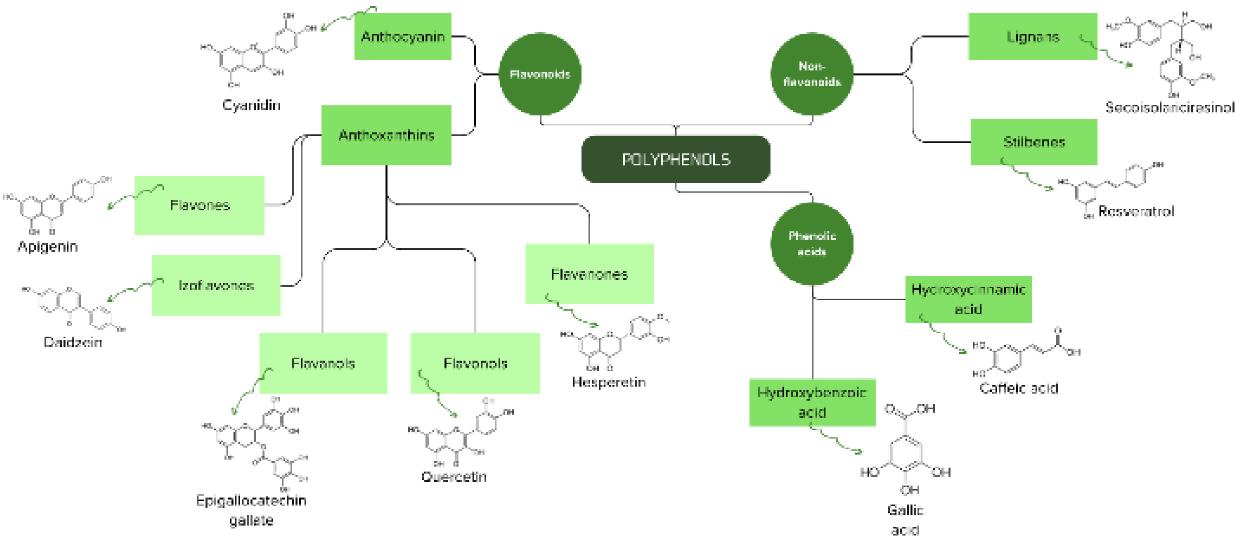

Figure 3. Chemical classification of polyphenols

4.2 Lignans and stilbenes are part of the non-flavonoid group, with the most notable examples being magnolol (from the Magnolia plant) and resveratrol (found in grapes and red wine).

4.3. Phenolic acids are also a major class of phenolic compounds, usually found in conjugated forms. They consist of a phenyl group attached to a carboxyl group and one or more hydroxyl groups. There are two main types based on their carbon backbone: hydroxycinnamic acids (C6–C3), which include caffeic, ferulic, p-coumaric, and sinapic acids and hydroxybenzoic acids (C6–C1), such as vanillic, syringic, ellagic, p-hydroxybenzoic, and protocatechuic acids. Their differences come from various substitutions, such as methoxy and hydroxyl groups [9, 10].

5. Polyphenols properties

Polyphenol's chemical structures give them unique biological activities, including antioxidant, anti-inflammatory and antimicrobial effects. These properties make polyphenols important for preventing chronic diseases, supporting overall health and even for removing bacteria from biological contaminated water [11]. Firstly, one example of benefits could be those from consuming grapes, which are rich in polyphenols responsible for biological processes like antioxidation, gut-microbiota regulation, cardiovascular protection, antidiabetic and anticancer properties [12]. Regarding the polyphenols contained by apples, they have the potential to protect against aging, infections, hypertension, cancer, diabtes, asthma, osteoporosis and cognitive disorders as well [13]. Besides, these natural compounds are abundant in tea and coffee, which exhibit health-promoting properties, they can also take part in the treatment of covid-19 and, additionally, they have effects on fertility by improving the sperm quality cand in vitro fertilizations [14] Not to mention that, in addition to the polyphenols from tea leaves, there are fermentation processes which enriches their polyphenol content and improves their effects on the human body [15].

6. Limitation for polyphenols usage

Even if polyphenols are known for their benefits on human and animal health, it has been shown that, by consuming these natural compounds (trough what we eat or by supplements), are potentially toxic, having adverse effects. Some of these were reported in the case of quercetin (a plant flavonol from the flavonoid group of polyphenols), their consumption (intravenous administration) leading to gastrointestinal issues such as, nausea, vomiting and mild headache [2]. Also, their bioavailability is low, and their functional performance can be easily affected by oxidation or food processing technologies [3]. Another limitation is that these compounds have poor absorption and water solubility [16].

7. Determination of Antioxidant Activity and Polyphenol Content

To evaluate the polyphenol content and antioxidant activity in a sample, two common analytical methods are used: the Folin-Ciocalteu method for quantifying polyphenols and the CUPRAC method for determining antioxidant activity. Both methods are spectrophotometric techniques.

The determination of the antioxidant capacity in vitro can be done by applying numerous methods which, depending on the technique on which it is based, are divided into spectrometric, electrochemical and chromatographic methods. The most used are the spectrometric methods and differ from each other by the principle underlying the method. In the ORAC and HORAC methods, the fluorescein fluorescein fluorescence loss is measured. In the TRAP method is measured Chemiluminescence quenching. The most applications have recorded by the colorimetric methods CUPRAC, FRAP, PFRAP, ABTS and DPPH. The CUPRAC method has the advantage that it is suitable for both hydrophilic and lipophilic antioxidants [17].

Polyphenols have a strong antioxidant activity and the experimental studies revealed a strong positive correlation between total polyphenol content (TPC) and antioxidant capacity. The Folin-Ciocalteu method is widely used to quantify polyphenols in plant derived extracts [18].

The Folin-Ciocalteu method and CUPRAC assays are often applied to characterize bioactive properties of natural products.

7.1. Determination of the Polyphenol Content

The Folin-Ciocalteu method is based on the reaction of polyphenols (PolyPhs) with the Folin-Ciocalteu reagent (FC). This reaction generates a blue coloured complex, which has an absorption of 750-760 nanometers, also oxidized polyphenols are formed (OxPolyPhs).

$$\text{PolyPhs} + \text{FC} \rightarrow \text{Blue complex} + \text{OxPolyPhs}$$

For the quantification of polyphenols, the results obtained are compared with a standard curve, built based on the concentrations of gallic acid, a reference polyphenol. The blue color of the resulting solution intensifies proportionally to the increase in the concentration of polyphenols [17, 18].

7.2. Determination of Antioxidant Capacity by the CUPRAC Method

The CUPRAC (copper reducing antioxidant capacity) method is a technique used to evaluate the ability of an antioxidant to reduce copper ions Cu^{2+} to Cu^{+}. This chemical reaction results in a color change of the solution, which can be quantified using a spectrophotometer. The experimental protocol involves adding an antioxidant extract to the reaction mix containing copper ions, ammonia and a complexing agent. After a reaction period of 10-25 minutes, the light absorption is measured at a wavelength of 445-450 nm.

Cu^{2+} + antioxidant → Cu^{+} + oxidized antioxidant

Cu(I) + neocuproine → Cu(I)-neocuproine (orange/yellow complex)

The antioxidant capacity of the sample is determined by comparing the absorption obtained with that of a standard solution of Trolox, an analogue of vitamin E. The result is expressed in units of μmol Trolox equivalent (μmol TE), providing a quantitative measure of the antioxidant efficiency of the analyzed sample. This method is commonly used in research into nutrients and bioactive compounds in foods and natural extracts, helping to highlight the health benefits associated with their consumption [17].

8. Research perspectives

Polyphenols continue to increase interest these days because of their properties and their presence almost everywhere in nature. That's why, there are many researches on this subject, including identification of new polyphenols and methods for their extraction and determination, which should be part of green-chemistry (the usage of green solvents, such as deep eutectic solvents) [19, 9]. Other aspects to be studied could be the interaction between polyphenols and gut microbiota, proteins in plants and dietary fibers endogenous in cereals. This final interaction has been tought to have synergistic effects on the human body [9]. Also, in the future it could be therefore interesting to improve the bioavailability of phenolic compounds, to better understand the synergistic and additive effects of the combination of polyphenols with nutraceuticals and between different polyphenols [4]. Finally, but not least, there should be studies to discover how to increase the bioavailability of polyphenols, such as galloylation (particularly common in tannins) do [20].

9. Conclusions

Polyphenols are a group of naturally compounds found in plant-based foods such as fruits, vegetables, tea, coffee, and whole grains, but not only. These compounds are known for their powerful antioxidant and anti-inflammatory properties, which contribute to various health benefits, including reducing the risk of chronic diseases. The main subclasses of polyphenols include flavonoids, phenolic acids, and lignans, each with unique chemical structures that influence their biological activity. While polyphenols offer numerous health benefits, their effectiveness can be limited by challenges such as low bioavailability, instability during processing, and variability in content across different food sources. Also, ongoing research aims to improve the bioavailability of polyphenols, explore their interactions with the gut microbiome, and develop better delivery systems to maximize their therapeutic potential. As our understanding grows, polyphenols could play an even more significant role in promoting health and preventing disease.

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