



## Ease of Phytochemical Extraction and Analysis from Plants?

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## Bitkilerden Fitokimyasal Özütleme ve Analizinin Kolaylığı?

**Abstract:** Plants are vital and sustainable resources for our world. From the ancient times, they are not only supplier of oxygen but also the important part of the food pyramid. Besides, they are sources of thousands of bioactive phytochemicals. With emerging technologies, human being can made synthetic drugs based on phytochemicals to improve their life quality. However, nowadays, Humans have started the search again for natural sources because of emerging new diseases and side effects of drugs. Although lots of chemicals identified and purified from plant materials, it is obvious that there is still more phytochemicals than we discovered especially for foods. Determining full potential nutraceutical value of foods may lead classical breeding or biotechnological studies to more promising area. In concordance, aim of this review is to briefly indicate the bottleneck of analysis of plant phytochemicals by pointing the critical parts of the whole analysis process.

**Key words:** Phytochemicals, Phenolics, Extraction, Antioxidant Capacity

**Özet:** Bitkiler, dünyamız için hayati ve sürdürülebilir kaynaklardır. Eski çağlardan beri sadece oksijen tedarikçisi değil aynı zamanda gıda piramidinin önemli bir parçasıdır. Ayrıca, binlerce biyoaktif fitokimyasal kaynağıdır. Gelişmekte olan teknolojilerle, insanoğlu fitokimyasallara dayalı sentetik ilaçlar üretebiliyorlar. Ancak günümüzde insanlar ortaya çıkan yeni hastalıklar ve ilaçların yan etkilerinden dolayı doğal kaynakları araştırmak için tekrar araya girdiler. Bitki materyalinden tanımlanan ve arıtılmış birçok kimyasal madde olmasına rağmen, özellikle gıdalar için keşfettiklerimizden çok daha fazla fitokimyasal bulunduğu açıktır. Gıdaların potansiyel nutrisyonel değerinin belirlenmesi, klasik ıslah veya biyoteknolojik çalışmaların daha umut verici bir alana gelmesine neden olabilir. Bu amaçla, bu incelemenin amacı, bütün analiz sürecinin kritik kısımlarına işaret ederek bitki fitokimyasal analizinin darboğazını kısaca göstermektir.

**Anahtar Kelimeler:** Fitokimyasallar, Fenolikler, Özütleme, Antioksidan kapasite

### 1. Introduction

In addition to being one of the main food sources for humans and animals, plants have been used as the main source of treatment for many diseases throughout history (Cheynier, 2012; Oroian and Escriche, 2015; Shahidi and Ambigaipalan, 2015). The metabolites that the plants produce to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to treat various diseases (Bhattacharya et al., 2010; Elmastas et al., 2017; Galindo et al., 2017; Liu et al., 2017). In ancient times, the treatment is carried out by direct usage or brewing of this plant, on the other hand, currently, the active ingredients are purified and synthetically produced with new technologies (Cheynier et al., 2013; Pezeshkpour et al., 2018).

From past to present day, plants used as natural medicines in the treatment of many diseases. Among these diseases, we can give an example of a wide range from simple injuries to stomach aches to feverish diseases to aging (Bahense et al., 2017; Dai and Mumper, 2010; Ferhat et al., 2017; Kolakul and Sripandkulchai, 2017; Li et al., 2018; Mabeku et al., 2017; Marques et al., 2017). In the past years, plants were being used directly and the information about how their mechanisms and dosage were decided by means of trial and error. Moreover the information flow from one generation to next is by means of hearsay. However, with today's technology, active substances can now be identified and purified and effects and dosage can be determined individually (Azmir et al., 2013; Barba et al., 2016; Bhat and Riar, 2017; Dominguez-Rodriguez et al., 2017; Nadpala et al., 2018; Wang and Zhu, 2017).

The importance of dosage of any substances is indicated by "*Sola dosis facit venenum*" in Latin which means "the dose makes the poison" in English. The phrase is the condensed form of Paracelsus statement "All things are poison and nothing is without poison; only the dose makes a thing not a poison" (Siddiqui et al., 2003; Stall et al., 2008). In addition, in 1907, Paul Ehrlich introduced the chemotherapy concept using Arspenamine, the first synthetic drug, in medical therapy (Siddiqui et al., 2003). With the concept of chemotherapy, dosing was become widespread around the world. We can illustrate the significance of the dose through vitamin C, which everyone knows very well. Everyone can easily mention the benefits of vitamin C, but the less known part is the reversal of the antioxidant properties, especially when taken at high doses, where it acts like free radicals (Oroian and Escriche, 2015). Similar situations apply to all medicines we actually use. In synthetic drugs, the dose concept is determined by bio-accessibility as measuring the absorption level, the serum value and the excretion of the drug. However, it is not possible to carry out a similar research method to measure the bio-accessibility of direct usage of plants. Firstly, the plant has many bioactive substances. Secondly, the absorption of bioactive substances depends on the material of the plant, the way of use and the interactions of bioactive chemicals. Phytochemicals in the plants can exhibit synergistic effect and increase the effect or show antagonistic interaction and reduce the efficiency (Acosta-Estrada et al., 2014; Burgos-Edwards et al., 2017; Celep et al., 2017). Therefore, the usage of plants and/or their extracts, and the phytochemical combinations should be considered carefully.

With the added concern of antibiotic multiple drug resistance and the side effects of synthetic drugs, research has turned to finding new and natural resources. The high potential of plants as having bioactive phytochemicals, decrease in the cost and time of the research for finding, and assessing antioxidant substances have been resulted in focusing on finding and determining the phytochemicals in plants and their usage as natural sources.

Antioxidants prevent damage to lipids, proteins and DNA by means of preventing radicals initiation, breaking chain propagation or suppressing formation of them. Thus, they can prevent deteriorating disease (i.e. cancer) and slowing down the aging process (Bahense et al., 2017; Iseri et al., 2011; Kada et al., 2017; Nunes et al., 2017; Oke-Altuntas et al., 2017; Pisoschi and Pop, 2015; Sadi and Sadi, 2010). The phytochemicals having antioxidant activity can be grouped in 3: vitamins, carotenoids and phenolics (Oroian and Escriche, 2015).

Plants are used for food and medical treatment from ancient years. Although, thousands of plant bioactive compound have been determined, purified and used as drug substance, there is still need for further investigation to reveal the full potential of plants especially for foods. Therefore, the aim of this paper was to briefly cover the bottleneck of the discovering new chemicals from plants by focusing on phenolics and their extraction methods and analysis procedures.

## 2. Phenolics in Plants

Although the phenolic and polyphenolic terms are used as synonymously, the definition of polyphenolic should be as the phenolics that contains at least 2 phenol rings. The definition of polyphenol was first used in 1962 and was later revised in 1994 and 2011 and accepted as “term polyphenol should be used to define the plant secondary metabolites derived from the shikimate derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression” (Cheynier, 2012) (Figure 1).

Although the secondary metabolites are not considered as vital metabolites, they help the organism in its survival. Plants produce many phenolic substances as secondary metabolites. However, the type and amount of phenolic substances varies according to plant material, the biotic and abiotic stresses that the plant is experiencing with regard to the physical and biological interaction of the plant with the soil microorganisms, season, and environment (Demir et al., 2014; Elmastas et al., 2017; Galindo et al., 2017; Majdoub et al., 2017; Martini et al., 2017; Mirto et al., 2018; Ojeda-Amador et al., 2018; Petropoulos et al., 2017; Petropoulos et al., 2018; Silva and Sirasa, 2018; Sutay Kocabas et al., 2015; Tupec et al., 2017). Some phenolic species are common and widespread among plants, while some are species-specific. Because, these substances are the metabolites of plants and their production is related to the metabolic activity, thus, their genetic background (Cheynier, 2012; Cheynier et al., 2013) (Figure 2).

The phenolic substance identified from the plants has exceeded 8000 and increasing in number every day (Cheynier, 2012). Although the extraction and analysis of phenolic substances from plants seems to be very easy, it

is not true that they can be obtained by a single process or by a single method. As mentioned, the quantity and type of phenolic substances are influenced by many factors, so it is not possible to obtain them by a single method (Acosta-Estrada et al., 2014; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017; Pezeshkpour et al., 2018). Similarly, antioxidant capacity assays cannot be assessed through a single method since they can be water soluble or bound to cell materials such as cell membrane. It was also indicated in literature that bound phenolics have higher antioxidant capacity than water soluble ones (Acosta-Estrada et al., 2014; Dominguez-Rodriguez et al., 2017; Shahidi and Zhong, 2015). Therefore, both for extraction and analysis at least more than one method or combination of methods should be used to precisely determine phenolics.

## 3. Extraction Methods

Along with the anxiety caused by the side effects of synthetic drugs, recent research has focused on the nutraceutical properties of plants and foods. The most prominent features of these properties are the antioxidant capacities and the most studied and investigated molecules among the antioxidants are phenolic molecules (Acosta-Estrada et al., 2014; Cheynier, 2012; Cheynier et al., 2013).

Obtaining a molecule from a plant takes place in five steps: i) macroscopic matrix pretreatment, ii) molecule separation, iii) molecule extraction, iv) purification, and v) product formation (Galanakis, 2012; Oroian and Escriche, 2015). It should be noted that the most important step is the extraction. Because being easy and cheap at this stage, the organic solvent extraction method is the most cited method in the literature. In addition, pulsed electric fields, ultrasound, high pressure processing methods are cited as more technological and effective processes to increase the yield (Azmir et al., 2013; Barba et al., 2016; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017; Pezeshkpour et al., 2018). Therefore, it will be the best to select the combination of methods and process depending on the plant material and desired molecules.

### 3.1 Organic Solvent Extraction

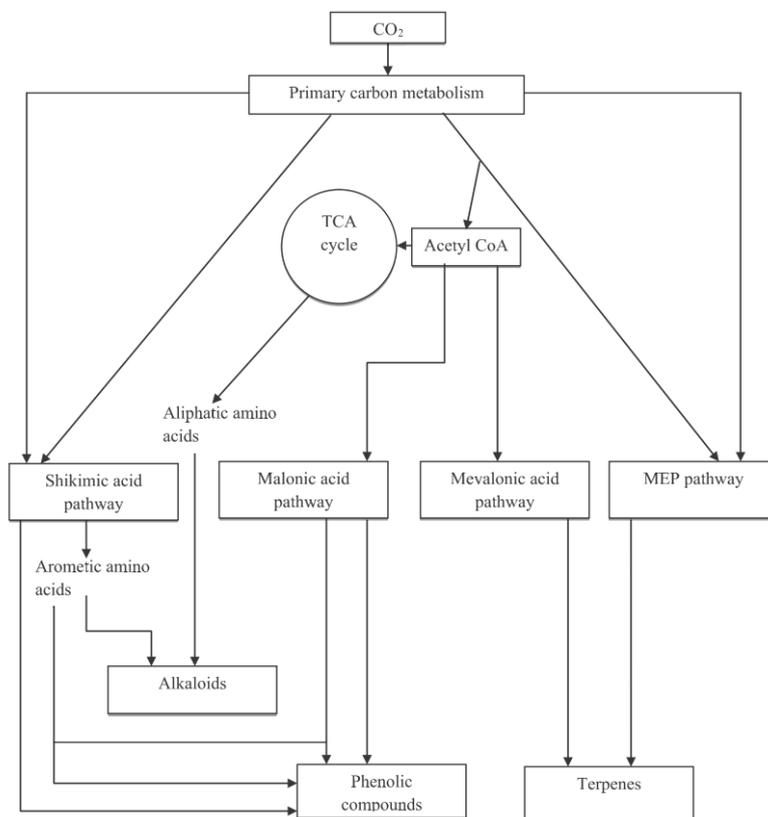
The organic solvent extraction phase uses either a single solvent (water, ethanol, ethyl acetate and hexane) as solid-liquid extraction or an aqueous form of these solvents as a two phase extraction method. In general, ethanol is preferred because at the point of consumption of the molecules obtained, ethanol is considered as "Generally Accepted As Safe (GRAS)" according to American Food and Drug Administration (Azmir et al., 2013; Nunes et al., 2017; Oroian and Escriche, 2015). On the other hand, the studies showed that addition of water to the extraction solvent resulted in increase in the yield of phenolic compound extraction. Moreover, it should be indicated here that for carotenoid extraction, acetone or ethyl acetate had higher yield than ethanol (Oroian and Escriche, 2015). Furthermore, acid addition to organic solvent is recommended for anthocyanin extraction. Another important point for extraction is extraction time and temperature. Especially for longer time, the yield decreases because of the oxidation of the phenolics (Dai and Mumper, 2010). Therefore, although aqueous ethanol extraction is widely used and accepted method, it is wise to consider that the interaction between solute and solvent system determines the solubility. Besides conventional

solvent extraction, special extraction methods can be performed.

#### Super Critical Fluid extraction

Super critical fluid extraction is the usage of the fluid at their super critical stage. Accepted as GRAS, CO<sub>2</sub> was the

generally used fluid in this method. Although it requires a special equipment, it is fast process and requires less amount of sample, and solvent than conventional solvent extraction (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).



**Figure 1.** Pathways for production of three major groups of plant bioactive compounds (Azmir et al., 2013).

#### Subcritical water extraction

It is performed by keeping water in its liquid form at higher degrees of temperature such as 100 – 300 °C by controlling pressure. Therefore, this process reduces water polarity and provides the solubilization of both hydrophobic and hydrophilic molecules. This method also need specialized equipment (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

### 3.2 Advanced Extraction Techniques

These techniques are generally used to assist the solvent extraction by disrupting the plant materials to increase the yield of phenolics.

#### Microwave assisted extraction

With controlled pressure and temperature, usage of microwave helps to reduce the time needed for extraction and also decrease the amount of solvent to be used. By this way, it increases the yield of phenolic compounds obtained from plant material (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

#### Ultrasonic extraction

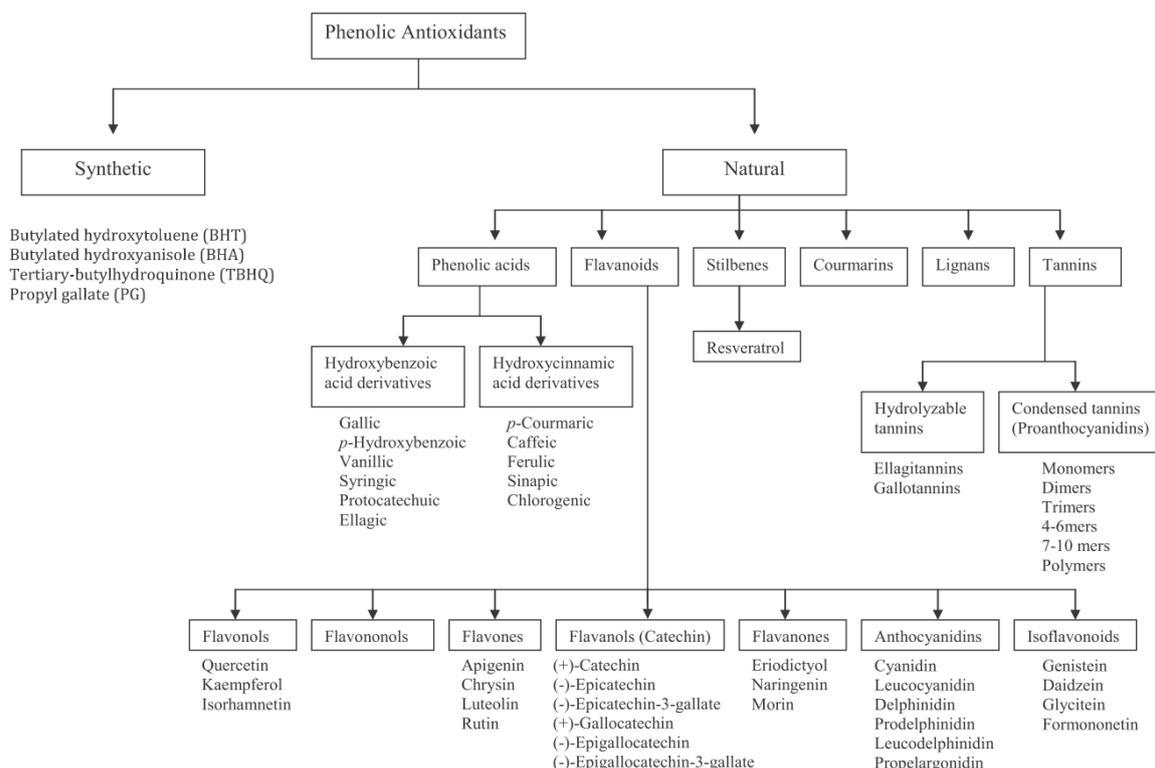
The process depends on the production of local pressures by making bubbles with ultrasonication. When bubbles exploit, they disrupt the plant cell and cell wall. Therefore, it increases the amount of released phenolic compound (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

#### Pulsed electric field extraction

It is based on the same principle with electroporation, which give electro-shock to the plant materials. Thus it makes the cell soft and influences cell infrastructure, which resulted in higher yield by increasing the release of phytochemicals (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

#### Enzyme assisted extraction

This is the most specialized method that uses special enzymes especially for degradation of cell wall. Hence, it causes the release of cell wall bound phytochemicals and reduces the amount of solvent usage (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).



**Figure 2.** Classification of phenolic antioxidants (Shahidi and Ambigaipalan, 2015)

#### 4. Analysis of phenolic compounds

Analysis on phenolics is performed in three main framework: I) Determination of total phenolic content, II) Determination of antioxidant capacity and III) Optional analysis which can include antimicrobial, anticancer, anti-inflammatory and cytotoxic effects etc.

Although reducing sugars and amino acids (especially ascorbic acid) can react with the Folin-Ciocalteu reagent, the Folin-Ciocalteu assay is widely used and accepted method for total phenolic content determination (Silva and Sirasa, 2018). Besides that, HPLC coupled with the reverse phase C18 is also preferred technique for phenolic content determination and its combination with mass spectroscopy provides chemical analysis of the phytochemicals as both in qualitative and quantitative characterization (Acosta-Estrada et al., 2014; Dominguez-Rodriguez et al., 2017).

On the other hand, for the antioxidant capacity measurement, there are more than one equivalent methods. Conventional methods includes DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging activity, ferric reducing/antioxidant power (FRAP), hydroxyl radical-scavenging capacity (HRSCA), Trolox equivalent capacity (TEAC), oxygen radical absorbance capacity (ORAC), cupric ion reducing antioxidant capacity (CUPRAC) assays (Dai and Mumper, 2010; Oroian and Escriche, 2015; Silva and Sirasa, 2018). Although all these five methods are cheap, fast and easy to handle, DPPH and FRAP assays are the most cited and widely used assays for antioxidant capacity measurement. Besides these methods, cyclic

voltammetry (especially for beverages), biosensors, chemiluminescent assay, and electron spin resonance spectroscopy are the other methods used for antioxidant capacity determination (Oroian and Escriche, 2015). Although the latter ones are expensive, they are more sensitive than classic methods. It should be noted that all the methods by itself are enough to evaluate the antioxidant effect. Whereas, it should be considered to use more than one method for the precise assessment of to the antioxidant capacity and noted that each analysis has its own standard method.

#### 5. Conclusion

Phytochemical composition of plants can vary depending on the situation that plant experiences at that moment. As a living thing, plants together with their biological conditions react to environmental stimuli. In other words, each factor either autochthonous (biotic) or resulted from environment (abiotic) may cause metabolic changes in the plant. Therefore, development stages of the plant, climatic changes, interaction with microorganisms (either symbiotic or pathogen), injuries, and *etc.* can affect the metabolic composition of a plant. Moreover, since these metabolites have different functions and differ in structure, their location and behavior against a solvent should be distinctive. Thus, pretreatment of plant materials and usage of more than one method based on features of molecules of interest for extraction could help the revealing of plants full potential. Besides, investigation of new methods is required.

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