

ANTIOXIDANT CAPACITY OF BLACKBERRIES AND BLUEBERRIES: CORRELATION WITH POLYPHENOLS AND IMPACT OF GROWING CONDITIONS

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REVIEW ARTICLE

Abstract

Since ancient times, the relationship between man and nature has been one of coexistence, nature providing vital resources for survival. Although, at first, man only intuited the importance of this connection, the evolution of knowledge has highlighted the need for a correct and conscious attitude towards the environment, considered an essential source for achieving human happiness and prosperity.

With scientific advances, people are actively searching nature for untapped natural remedies to their full potential, aware of the benefits they can provide. Particular interest is given to polyphenols, the main constituents of plant products. These compounds are found in abundance in medicinal plants, which are a major source of new pharmaceuticals.

The antioxidant properties of plant constituents are the basis of their role in disease prevention and control, being associated in particular with the wide range of polyphenolic compounds (including phytochemicals, pro-vitamins and nutraceuticals).

The aim of this paper was to analyze published scientific articles to evaluate and compare data on: total polyphenol content, total flavonoid content and antioxidant capacity of extracts obtained from berries (wild or cultivated) harvested from various geographical areas globally.

Keywords: natural remedies, polyphenols, flavonoids, medicinal plants, berries

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INTRODUCTION

Polyphenols are natural phytochemical compounds found in plants, fruits, vegetables, whole grains, vegetables, tea, coffee, wine, cocoa and medicinal plants, with more than 8000 polyphenolic compounds being identified, including phenolic acids, flavonoids, stilbenes, lignans and polymeric lignans in plant foods (Pandey & Rizvi, 2009).

Polyphenols are secondary metabolites of plants that act as a barrier against ultraviolet radiation, oxidants and pathogens (Beckman, 2000). Based on these possible therapeutic properties, in recent years numerous researchers have been involved to find new

sources of polyphenols that can have applications in the food industry and the pharmaceutical industry. Studies have estimated that the dietary intake of polyphenols is about 1 g of polyphenols/day (Khoddami et al., 2013). The bioavailability of these bioactive compounds depends on the preparation process of plant products containing polyphenols, the process of gastrointestinal digestion, their absorption and metabolism (Scalbert & Williamson, 2000). During absorption, dietary polyphenols must be hydrolyzed by intestinal enzymes or microflora in the colon and then conjugated in intestinal cells and subsequently in the liver by methylation, sulfation or glucuronidation (Scalbert et al., 2002). So, polyphenols reach and accumulate in the target

tissue and induce biological properties, and polyphenol derivatives are mainly eliminated through bile and urine.

Various studies in the literature have shown that some of the polyphenolic compounds (procyanidins, quercetin, procyanidins, flavanols) have a rapid absorption in plasma, having maximum plasma concentrations 2-3 hours after ingestion (Manach et al., 2004). There are studies attesting to the biological activities and beneficial properties of these compounds, including: antioxidant, antiallergic, anti-inflammatory, antiviral and antimicrobial, anti-proliferative, anti-mutagenic, anti-carcinogenic, free radical blocking, regulation of cell cycle stopping, apoptosis and induction of antioxidant enzymes. Free radicals can damage cells and play an important role in heart disease, cancer, and other diseases. Studies suggest that a rich intake of antioxidants is associated with a lower risk of cancer, cardiovascular disease, Parkinson's disease, and Alzheimer's (Sharma et al., 2013).

1. DEFINITION, CHEMICAL STRUCTURE

Polyphenols are a large and varied group of aromatic benzene ring compounds with one or more hydroxyl groups, produced by plants mainly for protection against oxidative stress, synthesized during plant development (Harborne et al., 1982) or in response to certain adverse conditions (UV radiation, etc.) (Shahidi, 2004).

Over time, polyphenols have been classified into several categories based on the number of phenolic rings and the structural elements that connect these rings to each other (Pietta et al., 2003), this class includes simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins.

Phenolic acids comprise two main branches: derivatives of hydroxybenzoic acid (protocatechic acid, gallic acid, p-hydroxybenzoic acid) and derivatives of hydroxycinnamic acid (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, synapic acid). Caffeic, p-coumaric, vanilla, ferulic and protocatechic acids are acids present in almost all plants.

Flavonoids are the most abundant polyphenols in the human diet and more than 4000 types have been identified. These compounds have at least two phenol subunits, and compounds that possess three or more

phenol subunits are called tannins (hydrolyzable and non-hydrolyzable). Flavonoids are planar molecules, ubiquitous in plants, consisting of the aromatic amino acid, phenylalanine, tyrosine and malonate (Harborne et al., 1986). The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6). There are six subclasses of flavonoids including anthocyanins, flavonols, flavanols, flavanones, flavones, and isoflavones; Anthocyanins (cyanidin, pelargonidin, delphinidin, malvidine) which are found in the family of berries, red wine, red cabbage, cherry, black grapes and strawberries. Flavonols, including quercetin, campferol, and myricetin, have been detected in onions, leeks, broccoli, and blueberries. Isoflavones are the most important dietary flavonoids that include daidzein, genistein, and glycitain. Stilbenes occur in the human diet in small quantities; Resveratrol, one of the well-studied compounds in these groups, is largely detected in grapes and red wine (Pandey & Rizvi, 2009).

2. CLASSIFICATION OF POLYPHENOLS

Polyphenols in food can be classified according to the number of phenolic rings and the structural elements to which the phenolic nucleus attaches, into four broad categories:

- **Phenolic acids** - found in abundance in food and are divided into two subclasses: benzoic acid derivatives and cinnamic acid derivatives. The content of hydroxybenzoic acids in edible plants is generally low. Exceptions are certain red fruits, black radish and onions, where they can have concentrations of several tens of mg/kg of fresh product. Hydroxycinnamic acids are more common than hydroxybenzoic acids and consist mainly of p-coumaric, caffeic, ferulic and synapic acids.

- **Flavonoids** are the most studied group of polyphenols. Flavonoids have a common basic structure consisting of two aromatic rings that are linked together by three carbon atoms that form a heterocycle with oxygen. More than 4,000 types of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruits, and leaves. The classification by the heterocycle involved, divides flavonoids into six subclasses: flavonols (kaempferol, quercetin, myricetin), flavones (apigenin, luteolin), flavanones (naringenin, ariodictiol, hisperidine), flavanols (quercetin, catechin/epicatechin, gallo catechin, tannins or proanthocyanidins,

procyanidins, prodelfinidins, etc.), anthocyanins (pelargonidin, cyanidin, delphinidin, petunidin, malvidin) and isoflavones (glycein, formononetin, genistein). Quercetin, myricetin, catechins, etc., are some of the most common flavonoids.

- **Stilbene** – contain two linked phenyl through a methylene bridge. The occurrence of stilbenes in the human diet is quite low. Most plant stilbenes act as antifungal phytoalexins, compounds that are only synthesized in response to infection or injury. One of the best-studied, naturally occurring polyphenol stilbene is resveratrol (3, 4', 5-trihydroxystilbene), which is mostly found in grapes, in red wine.

- **Lignins/lignans** - are diphenolic compounds containing a 2,3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans (secoisolariciresinol), are considered phytoestrogens. The richest dietary source is flaxseed, which contains secoisolariciresinol (up to 3.7 g/kg dry product) and small amounts of matairesinol (Pietta, Gardana & 2003).

3. EXTRACTION, ESTIMATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS

3.1. Extraction of phenolic compounds

Phenolic compounds are very important components for plants and for human health, so it is very useful to know their concentration and their biological activity, which may indicate a potential use of them as therapeutic agents (Benedec et al., 2013).

In general, the extraction of phenolic compounds from the plant product is influenced by their chemical nature, sample size, extraction time and storage conditions, as well as the presence of interfering substances. The most widely used method of extracting polyphenolic compounds from plants is the one that uses solvents, being an easy, efficient method that can be applied on a very large scale. Chemical extraction depends on the type of solvent being used, the polarity of the solvent used, the extraction time and temperature, as well as the chemical composition and physical characteristics of the samples being analyzed.

Among the most used solvents we can mention: water, acetone, methanol, ethanol, N, N-dimethylformamide (DMF) or their mixtures with water, the mixtures being more efficient at extraction, because the solvents are of different polarities and thus, they can solubilize several components of plant products (Rajbhar et al.,

2015)]. Hydrophilic polyphenols, including aglycones, glycosides and oligomers, are extracted using water, polar organic solvents such as methanol, ethanol, acetonitrile and acetone, or their mixture with water. Liquid extracts are sometimes divided with solvents such as ethyl acetate, depending on the solubility of the target polyphenols. Polyphenols are more stable at a low pH because the acidic environment helps polyphenols remain neutral, so they can be easily extracted into organic solvents (Khoddami, Wilkes & 2013). The use of an alcoholic solution ensures a satisfactory extraction. Methanol, acetone, and water are inefficient solvents for the total extraction of phenols from plants in powder form, as polyphenols are associated with other biomolecules such as proteins, polysaccharides, terpenes, chlorophyll, lipids, and inorganic compounds. However, methanol extracts have been shown to be better for extracting catechins, epicatechin, and epigallocatechin (Koffi et al., 2013). Aqueous acetone mixtures are good solvents for polar polyphenols, but unwanted residues remain in the extracts. The low solubility of polyphenols in absolute organic solvents is due to the strength of the hydrogen bonds between polyphenols and proteins. The increase in solubility by adding water to organic solvents is due to the weakening of hydrogen bonds in aqueous solutions.

3.2. Estimation and quantification of phenolic compounds

For the estimation and quantification of phenolic compounds, the Folin-Ciocalteu test is used. The test is a method of quantifying the total polyphenol content using different solvents (e.g. methanol) to demonstrate their extraction efficiency. The test uses the Folin-Ciocalteu reagent, a reagent containing molybdenum in a superior state of oxidation (+6), which has a yellow color. Polyphenolic compounds lead to the reduction of Mo6+ from a higher oxidation state to one of the lower oxidation states (Mo4+, Mo5+) which have a blue color and can be monitored at a wavelength of 765 nm (Folin & Ciocalteu, 1927).

Polyphenol estimation can be done by different techniques, for example: nuclear magnetic resonance spectroscopy, near-infrared reflection spectroscopy, high-performance thin-layer chromatography (HPTLC), liquid chromatography coupled with mass spectroscopy (LC-MS), high-performance capillary electrophoresis (HPCE) and high-

performance liquid chromatography (HPLC). In addition to organic solvent extraction, qualitative and quantitative analysis techniques can be applied, as well as isolation and purification procedures for more specific results. Chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and subsequently capillary electrophoresis (CE), column chromatography (CC) over Sephadex LH-20 are used for final purification, due to the clear solutions that are obtained, without residues (Rajbhar, Dawda & 2015).

3.3. Total flavonoid estimation

The results reported in the literature for the total flavonoid content were obtained using the $AlCl_3$ colorimetric method. The principle on which the colorimetric method is based is that $AlCl_3$ forms stable complexes with the carbonyl groups (keto-C-4 and/or with the hydroxyl C-3 or C-5 group) of flavones and flavonols. Such complexes also form $AlCl_3$ with the ortho-dihydroxyl groups in the A or B ring of flavonoids, whose absorbance can be measured with the help of a spectrophotometer at an appropriate wavelength.

3.4. Evaluation of antioxidant capacity

There is a growing demand to evaluate the antioxidant properties of plant extracts, and in recent years, attention has focused on antioxidant products from natural sources (De Judicibus, 2011).

Much research is focused on antioxidant activity, flavonoid content of medicinal plants, and total polyphenol content, but for other medicinal plants there is no data on their antioxidant action (Nikolova et al., 2013).

The methods used to determine total antioxidant capacity can be divided into two major groups: methods based on electron transfer (SET-single electron transfer) and hydrogen atom transfer reaction (HAT). SET methods include ABTS/TEAC analysis (Trolox equivalent antioxidant capacity), FRAP (iron reduction ability), CUPRAC (copper reduction) test, and DPPH (2,2-diphenylpyrylhydrazil radical capture capacity); HAPX test. The hydrogen atom transfer reaction (HAT) method includes ORAC (oxygen radical absorption capacity) and TRAP (total radical-trapping antioxidant parameter) analysis. These tests are frequently used to measure the total antioxidant capacity of extracts, but a single test will not reflect all the antioxidants present in the system (De Judicibus, 2011).

Using the data provided by the literature, we will list the most common tests to check the antioxidant capacity of extracts.

3.5. DPPH Test

DPPH test is a simple, fast, inexpensive analysis that determines antioxidant capacity *in vitro* and is based on the evaluation of the free radical scavenging activity of plant extracts using a radical reagent of 1,1-diphenyl-2-picrylhydrazil. DPPH is an intense purple color that, after being reduced by an antioxidant (the flavonoids in the plant extract), turns into a pale yellow or colorless product. It measures the ability of compounds extracted from different plant products to fight free radicals or compounds that can release hydrogen ions. The turn can be seen with the naked eye (Güzel et al., 2019).

The absorbance after the neutralization reaction of the DPPH reagent by the flavonoids contained in the plant extract can be read with the help of a UV-VIS spectrophotometer at the wavelength of 515 nm.

3.6. FRAP Test

The FRAP test is a method by which the antioxidant capacity of compounds from plant extracts can be evaluated by evaluating the reduction power of the Fe^{3+} ion to Fe^{2+} , which is chelated by TPTZ to form the Fe^{2+} - TPTZ complex, with a maximum absorption at 593 nm, using a spectrophotometer to measure the absorbance of the colored product (Benzie & Strain, 1996).

III.7. CUPRAC test – reduction of Cu^{2+} ions

The FRAP test is based on the evaluation of the capacity to reduce copper ions (Cu^{2+}) evidenced by changing the color from light blue to yellow-orange of a complex: $Cu(II)$ -neocuproine (2,9-dimethyl-1,10-phenanthroline) (Benzie & Strain, 1996).

III.8. Oxygen Radical Absorbing Activity (ORAC)

By this method, the activity of peroxy radical absorption is measured using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as standard (Huang et al., 2002).

III.9. ABTS Radical Cation Discoloration Test

The ABTS analysis is based on the ability of an ABTS radical cation capture sample (ABTS+), a green-blue chromophore that absorbs at 734 nm, the method being compared to the Trolox standard (Arnao et al., 2001).

1. TOTAL DETERMINATION OF POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT CAPACITY OF BERRIES

In one study, three varieties of blackberries were analyzed: prickly, thorny and sweet tomatoes, harvested at three different stages of maturity, maturity appreciated by their color. The study found that red blackberries have the highest polyphenol content (104 ± 1 , 31 ± 1 , 20.0 ± 0.5 mg GAE/g DW), followed by blackberries (93 ± 1 , 25.3 ± 0.2 , 17.6 ± 0.1 mg GAE/g DW) and then sweet blackberries (83 ± 2 , 20.3 ± 0.2 , 13.5 ± 0.3 mg GAE/g DW).

The ORAC test (expressed as mmol Trolox equivalent/g dry mass, *mmol TE/g DW*), showed that antioxidant activity decreases from green, pink, and ripe fruits from prickly berries (2805 ± 25 , 1543 ± 71 , 994 ± 9 mmol TE/g DW), to prickly berries (2131 ± 15 , 1230 ± 12 , 767 ± 5 mmol TE/g DW) and then to sweet blackberries (1781 ± 28 , 866 ± 14 , 551 ± 10 mmol TE/g DW). The results are similar to the total polyphenol content found in the first Folin-Ciocalteu method. As for the anthocyanin content determined on the three types of blackberries, reported as mg cyanidin-3-glucoside equivalent/g dry sample, increased as the fruit ripened, in order: prickly blackberries (1.38 ± 0.06 , 3.3 ± 0.2 , 15.4 ± 0.3 mg cyanidin-3-glycoside equivalent), prickly blackberries (0.92 ± 0.01 , 2.37 ± 0.06 , 11.3 ± 0.1 mg cyanidin-3-glycoside equivalent), blackberry (0.71 ± 0.05 , 2.02 ± 0.06 , 9.0 ± 0.4 mg cyanidin-3-glycoside equivalent) (Van de Velde et al., 2019).

In another study carried out on blueberry (*Vaccinium myrtillus*) and sea buckthorn (*Hippophaë rhamnoides*) fruits, from Romania, it was observed that the total polyphenol content determined with the Folin-Ciocalteu reagent of the freeze-dried extract of blueberry fruits (343.93 ± 0.01 mg GAE/100g DW) is lower compared to that of the alcoholic extract of the same fruit (812.13 ± 0.02 mg GAE/100g DW). For sea buckthorn fruits, higher content of total polyphenols was found in the freeze-dried extract (251.47 ± 0.02 mg GAE/100g DW), compared to the alcoholic extract (211.91 ± 0.02 mg GAE/100g DW). The total flavonoid content for blueberry fruits was higher for the alcoholic extract (125.82 ± 8.91 mg QE/100 g DW) than for the freeze-dried extract (72.80 ± 7.61 mg QE/100g DW), and for sea buckthorn fruits, the flavonoid content was higher for the freeze-dried extract (145.44 ± 8.72 mg QE/100g DW) compared to the alcoholic extract (43.14 ± 2.42 mg QE/100g DW).

The antioxidant capacity of the two extracts was carried out by three established methods: DPPH, ABTS and FRAP, and the results obtained for the blueberry fruits were:

- in the case of lyophilized extract - DPPH 8.51 ± 1.11 %, ABTS 326.95 ± 5.98 $\mu\text{mol TE/g DW}$, FRAP 73.05 ± 0.00 $\mu\text{mol TE/g DW}$,
- in the case of alcoholic extract - DPPH 34.02 ± 2.01 %, ABTS 280.91 ± 1.12 $\mu\text{mol TE/g DW}$, FRAP 201.91 ± 0.05 $\mu\text{mol TE/g DW}$. For sea buckthorn fruits, the results were:
- for freeze-dried extract - DPPH 4.12 ± 0.11 %, ABTS 48.67 ± 0.31 $\mu\text{mol TE/g DW}$, FRAP 44.41 ± 0.01 $\mu\text{mol TE/g DW}$,
- for alcoholic extract - DPPH 47.41 ± 3.22 %, ABTS 56.85 ± 3.25 $\mu\text{mol TE/g DW}$, FRAP 52.78 ± 0.01 $\mu\text{mol TE/g DW}$ (Jurca, Vicas, et al., 2016).

Another plant product analyzed was the blueberry fruit, *Myrtillum vaccinum* (blueberry), collected from Bihor County, Apuseni Mountains. The harvested blueberries (a1) were analyzed compared to four other types of samples: (a2) - blueberry tea, (a3) - blueberry tincture, (a4) - blueberry capsules, (a5) - cranberry alcoholic extract, on the Romanian market.

After harvesting, the cranberry fruits were dried, and ground to obtain a powder, samples A2 and A4 were extracted with 70% ethanol solution (1:10 g/v) using a magnetic mixer for 20 min and then sonicated for 5 minutes, and samples A5 and A3 were centrifuged and supernatants were evaporated in vacuum and then frozen and freeze-dried.

The comparative data on bioactive compounds such as total polyphenols, total flavonoids and antioxidant capacity of the five blueberry preparations are different.

- Total polyphenol content, expressed in mg GAE/100 g DW, was: a1- 343.90 ± 7.32 , a2 - 344.60 ± 4.96 , a3 - 811.26 ± 14.53 , a4 - 341.51 ± 9.11 , a5 - 813.42 ± 15.55 .
- Total flavonoid content, expressed in mg QE/100 g DW, was: a1- 1182.95 ± 6.46 , a2 - 1180.55 ± 6.47 , a3 - 1426.00 ± 1.80 , a4 - 1179.19 ± 7.90 , a5 - 1425.44 ± 1.94 .
- The antioxidant capacity of the five The samples analyzed were carried out with the help of three methods, and the results obtained were:
 - ✓ DPPH (a1- 82.38 ± 17.56 , a2 - 86.36 ± 13.67 , a3 - 3.12 ± 0.33 , a4 - 78.50 ± 16.85 , a5 - 3.24 ± 0.40 %);
 - ✓ ABTS (a1- 326.36 ± 3.88 , a2 -

327.63±3.42, a3 - 275.55±21.10, a4 - 324.66±7.41, a5 - 283.51±19.59 μmol TE/g DW);

✓ FRAP (a1- 73.16±1.86, a2 - 74.78±4.86, a3 - 3.39±0.16, a4 - 69.12±3.19, a5 - 3.46±0.22 μmol TE/g) (Jurca, Vicaș, et al., 2016).

The objectives of another study were to investigate and compare the nutritional substances and antioxidant potential of three blackberry species (*Rubus ellipticus*, *Rubus niveus*, *Rubus ulmifolius*) that are the most consumed by Himalayan communities. Fresh and ripe fruits were collected from different localities in the Himalayan Mountains, Pakistan. In addition to the antioxidant effect, they also analyzed the content of fats, saccharides, proteins, fibers and essential minerals: Ca, P, Mg, K, Na, Cl, S, Mn, Zn, Fe, Cu, Se, Co, Ni.

The fruits were air-dried at a temperature of 65°C for 48 hours. To analyze the antioxidant capacity, the dried fruits are ground and extracted with methanol. Methanol extracts of fruits of *Rubus* species were tested with the DPPH test, using an extract with a concentration of 1 mg crude extract/mL. The extracts show a high antioxidant capacity (>50%).

The DPPH analysis performed for the three types of blackberry (*Rubus ellipticus*, *Rubus niveus*, *Rubus ulmifolius*), of four concentrations showed different results: at 50 μg/mL - *R. ulmifolius* (80.28%) > *R. niveus* (68.30%) > *R. Ellipticus* (45.97%), at 100 μg/mL - *R. ulmifolius* (87.62%) > *R. niveus* (74.54%) > *R. ellipticus* (54.82%), at 200 μg/mL - *R. ulmifolius* (94.46%) > *R. niveus* (80.38%) > *R. Ellipticus* (72.23%), at 400 μg/mL - *R. ulmifolius* (98.89%) > *R. niveus* (91.64%) > *R. Ellipticus* (84.00%). It was also observed that with the increase in the concentration of the extract used, the antioxidant capacity increased. The authors' conclusion was that due to the nutritional content of *Rubus* fruits, they may constitute an alternative source of edible wild fruits (Ahmad et al., 2015).

Another study looked at fresh blackberries, blueberries, black raspberries, and red raspberries purchased from a local market in Texas. To obtain the extracts, the fresh fruits were frozen and ground, then 95% ethanol (1: 4 w/v) was added over them, at room temperature for 2 days, then centrifuged at 3500 revolutions/minute for 20 minutes. The supernatants were filtered and stored at -20 °C.

The total determination of polyphenols (expressed in mg GAE/g FW), flavonoids (expressed in mg QE/g FW) and proanthocyanidins (mg GAE/g FW) led to various results:

- blueberries: 443.6, 151.7, 1589.6;
- Blackberry: 269.5, 56.7, 763.2;
- black raspberry: 965.4, 186.4, 2677.0;
- Red raspberries: 434.3, 114.5, 946.9.

The highest concentration of polyphenols, flavonoids and proanthocyanidins was found in black raspberries, followed by blueberries, red raspberries, blackberries.

Determination of antioxidant capacity using the FRAP method (expressed in mmol Fe²⁺/kg FW, showed a variation in the order: red raspberries > blackberries > black raspberries and blueberries. The *in vitro* antioxidant capacity in fruit extracts was determined with the DPPH and ABTS assays/methods. For blueberry, black raspberry and red extracts, very high values were obtained, which increased with increasing extract concentration (20–100 μg/mL): blueberries 38.5% to 87.9%, black raspberries 64.2% to 89%, blackberries 40.5% to 77.8% and red raspberries 37.6% to 87%. Comparing fruit extracts of the highest concentration, the descending order of antioxidant capacity is: black raspberries > blueberries > blackberries > red raspberries. The ABTS analysis to determine the antioxidant capacity led to the obtaining of different values, values that increased with the increase in the concentration of the extracts: for blueberries 11.1-23.1%, for blackberries 18.2-25.3%, for black raspberries 20.1-21.3% and for red raspberries 20.9-31.1% (Basu & Maier, 2016).

In order to compare the quality characteristics, as well as the content of polyphenols and anthocyanins and antioxidant activity, six fruits were analyzed, including black currant (*A. Melanocarpa*), blueberry (*Vaccinium ugliedinosum*), cherries, cranberries (*Vaccinium macrocarpom*), raspberries and strawberries. These fruits have been evaluated for potential use in the development of functional drinks. The fruit comes from the store and has been frozen. For the preparation of the samples, 5 g of frozen fruit was ground and then mixed with 45 mL ethanol of 94.5% concentration. The mixtures were stirred for 24 hours, at a temperature of 24°C, at 100 revolutions/minute, then centrifuged at 4°C, for 20 minutes at 8000 revolutions/minute and then filtered.

The highest total polyphenol content was determined in black squirrels (6001.3 mg GAE/100 g FW), followed by blueberries (2322.3 mg GAE/100 g FW), cranberries (1561.0 mg GAE/100 g FW), raspberries (1225.5 mg GAE/100 g FW), strawberries (1082.0 mg GAE/100 g FW) and cherries (693.3 mg GAE/100 g FW).

The anthocyanin content of the fruits analyzed in the study ranged from 0.27 to 1.74 mg anthocyanin/g FW: cranberries (1.74), raspberries (1.50), black carrots (1.04), followed by blueberries (0.80), cherries (0.27) and strawberries (0.13).

The antioxidant capacity of these fruits was determined by using the DPPH test and showed that cranberries have the highest antioxidant capacity (52.4%), followed by raspberries (39.8%), black scabs (35.9%), blueberries (33.5%), cherries (18.5%) (Kim et al., 2018).

CONCLUSIONS

This review shows that blackberries and blueberries are outstanding natural sources of antioxidants (polyphenols and anthocyanins), their antioxidant capacity being closely correlated with these components. It is observed that wild varieties are generally superior to those grown in terms of antioxidant content, and blackberries demonstrate a higher antioxidant capacity than blueberries. The study shows that in blackberries, the green ripening phase contains the highest concentration of polyphenols and has the highest antioxidant activity, while anthocyanins increase as the fruit ripens. Extraction is most effective using 95% ethanol, and most of the antioxidants in blueberries are found in the skin of the fruit. This data is valuable for both researchers looking for new sources of antioxidants and growers targeting strains with increased health benefits.

REFERENCES

- Ahmad M., Masood S., Sultana S., Hadda T.B., Bader A. & Zafar M. (2015). Antioxidant and nutraceutical value of wild medicinal Rubus berries. *Pak J Pharm Sci*, 28(1), 241-247.
- Arnao M.B., Cano A. & Acosta M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food chemistry*, 73(2), 239-244.
- Basu P. & Maier C. (2016). In vitro Antioxidant Activities and Polyphenol Contents of Seven Commercially Available Fruits. *Pharmacognosy Res*, 8(4), 258-264. <https://doi.org/10.4103/0974-8490.188875>
- Beckman C.H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and Molecular Plant Pathology*, 57(3), 101-110. <https://doi.org/https://doi.org/10.1006/pmpp.2000.0287>
- Benedec D., Vlase L., Oniga I., Mot A.C., Damian G., Hanganu D., Duma M. & Silaghi-Dumitrescu R. (2013). Polyphenolic Composition, Antioxidant and Antibacterial Activities for Two Romanian Subspecies of *Achillea distans* Waldst. et Kit. ex Willd. *Molecules*, 18(8), 8725-8739.
- Benzie I.F.F. & Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70-76.
- De Judicibus M. (2011). *Botanical notebook*. UoM Custom Book Centre.
- Folin O. & Ciocalteu V. (1927). On tyrosine and tryptophane determinations in proteins. *J. biol. Chem*, 73(2), 627-650.
- Güzel A., Akyüz M. & Şanda M.A. (2019). Determination of Antioxidant activity of *Hypericum perforatum*. *Bütünleyici ve Anadolu Tıbbi Dergisi*, 1(1), 9-18.
- Harborne J.B., Tomás-Barberán F.A., Williams C.A. & Gil M.I. (1986). A chemotaxonomic study of flavonoids from European *Teucrium* species. *Phytochemistry*, 25(12), 2811-2816.
- Harborne J.B., Williams C.A. & Wilson K.L. (1982). Flavonoids in leaves and inflorescences of Australian *Cyperus* species. *Phytochemistry*, 21(10), 2491-2507.
- Huang D., Ou B., Hampsch-Woodill M., Flanagan J.A. & Prior R.L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of agricultural and food chemistry*, 50(16), 4437-4444.
- Jurca T., Vicas L., Marian E., Vicas S. & Muresan M. (2016). A new natural antioxidant supplement-design and development. *Farmacia*, 64(1), 135-142.
- Jurca T., Vicaş L., Toth I., Braun M., Marian E., Teusdea A., Vicaş S. & Mureşan M. (2016). Mineral elements profile, bioactive compounds and antioxidant capacity of wild blueberry and of pharmaceutical preparations from blueberry (*Vaccinium myrtillus*). *Farmacia*, 64(4), 581-587.
- Khoddami A., Wilkes M.A. & Roberts T.H. (2013). Techniques for Analysis of Plant Phenolic Compounds. *Molecules*, 18(2), 2328-2375.
- Kim M.K., Kim M.Y. & Lee K.G. (2018). Categorization of fruits according to their content of polyphenols and vitamin C, antiradical activity, and quality parameters. *Journal of Food Processing and Preservation*, 42(2), e13421. <https://doi.org/https://doi.org/10.1111/jfpp.13421>
- Koffi E.N., Le Guernevé C., Lozano P.R., Meudec E., Adjé F.A., Bekro Y.A. & Lozano Y.F. (2013). Polyphenol extraction and characterization of *Justicia secunda* Vahl leaves for traditional medicinal uses. *Industrial Crops and Products*, 49, 682-689.

-
- Manach C., Scalbert A., Morand C., Rémésy C. & Jiménez L. (2004). Polyphenols: food sources and bioavailability. *Am J Clin Nutr*, 79(5), 727-747. <https://doi.org/10.1093/ajcn/79.5.727>
- Nikolova M., Petrova M., Zayova E., Vitkova A. & Evstatieva L. (2013). Comparative study of in vitro, ex vitro and in vivo grown plants of *Arnica montana*–polyphenols and free radical scavenging activity. *Acta Botanica Croatica*, 72(1), 13-22.
- Pandey K.B. & Rizvi S.I. (2009). Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity*, 2(5), 497-498. [https://doi.org/https://doi.org/10.4161/oxim.2.5.9498](https://doi.org/10.4161/oxim.2.5.9498)
- Pietta P., Gardana C. & Pietta A. (2003). Flavonoids in herbs. *Oxidative Stress and Disease*, 9, 43-70.
- Rajbhar K., Dawda H. & Mukundan U. (2015). Polyphenols: Methods of extraction. *Sci. Revs. Chem. Commun*, 5(1), 1-6.
- Scalbert A., Morand C., Manach C. & Rémésy C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed Pharmacother*, 56(6), 276-282. [https://doi.org/10.1016/s0753-3322\(02\)00205-6](https://doi.org/10.1016/s0753-3322(02)00205-6)
- Scalbert A. & Williamson G. (2000). Dietary intake and bioavailability of polyphenols. *J Nutr*, 130(8S Suppl), 2073s-2085s. <https://doi.org/10.1093/jn/130.8.2073S>
- Shahidi F. (2004). Functional Foods: Their Role in Health Promotion and Disease Prevention. *Journal of Food Science*, 69(5), R146-R149. <https://doi.org/https://doi.org/10.1111/j.1365-2621.2004.tb10727.x>
- Sharma S.K., Singh L. & Singh S. (2013). A review on medicinal plants having antioxidant potential. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(3), 404.
- Van de Velde F., Esposito D., Grace M.H., Pirovani M.E. & Lila M.A. (2019). Anti-inflammatory and wound healing properties of polyphenolic extracts from strawberry and blackberry fruits. *Food research international*, 121, 453-462.