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ORIGINAL ARTICLE

Chemical and Functional Characterisation of Cranberry Extract: A Focus on Polyphenolic Content and Evaluation of Stabilisation Strategies

Anisa Veledar-Hamalukić,[1] Emina Pramenković[1]

Abstract

Background/Aim: Cranberry (*Vaccinium macrocarpon*, Aiton) extracts are widely utilised in dietary supplements due to their rich content of polyphenolic compounds, particularly anthocyanins and proanthocyanidins (PACs), which are associated with antioxidative and antimicrobial activities. However, commercial products often lack detailed phytochemical characterisation, raising concerns about efficacy and stability, especially in the presence of additives such as vitamin C. This study aimed to characterise the polyphenolic content of a commercial dry cranberry extract by quantifying its anthocyanins and PACs using both the 4-dimethylaminocinnamaldehyde (DMAC) and modified Bate-Smith assays to explore potential stabilising agents for improved extract stability.

Methods: Anthocyanins and vitamin C were quantified using HPLC-DAD. PACs were quantified using two complementary colorimetric methods: DMAC (with catechin chloride as standard) and a modified Bate-Smith method (with procyanidin B2 standard). Antioxidative activity was assessed using DPPH and ABTS assays. Literature-based evaluation of succinate and glutamate was conducted to assess their potential as polyphenol stabilisers.

Results: Five major anthocyanins were identified, with total content of 9.95 mg/g. PAC content was determined as 53.57 % via DMAC and 36.31 % via Bate-Smith, underscoring the impact of method selection. Antioxidant assays confirmed strong activity (IC50 = 110 µg/mL ABTS, 92.85 µg/mL DPPH). Vitamin C content was low (1.2 mg/g), consistent with extract maturity. Literature suggests that succinate, due to its diacidic nature, may provide enhanced stabilisation compared to other additives.

Conclusion: Analysed cranberry extract was rich in bioactive polyphenols and exhibited significant antioxidant potential. The comparison of analytical methods highlights the need for standard harmonisation. Stabilisation strategies such as succinate addition should be further evaluated to enhance the shelf-life and efficacy of cranberry-based nutraceuticals.

Key words: *Vaccinium macrocarpon*; Anthocyanins; Proanthocyanidins; Polyphenols, stability; Antioxidants.

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Introduction

According to the US News Health Journalism research in 2024, nine cranberry supplements have been identified as the prominent ones.[1] Supplements contain cranberry fruit powder, in addition

to D-mannose and vitamins C and E. Total content is expressed as the amount of the cranberry extract per dosage, however, no characterisation of active ingredients and their quantity is stated. The Food and Drug Association (FDA) requires the supplements to have at least 500 mg of cranberry extract per dosage.[2] However, in Europe, the European Food Safety Authority (EFSA) requires extracts to be standardised to contain approximately 55–60 % proanthocyanidins (PACs), as measured by the OSC-DMAC method.[3] Also, the European Medicines Agency (EMA) recognises the traditional use of *Vaccinium macrocarpon* Ait for relieving mild, recurrent lower urinary tract infection symptoms in women, such as burning sensations and frequent urination, following the exclusion of serious conditions. Consequently, there is a significant need and opportunity to characterise the extracts used in supplement formulations to enhance both health benefits and regulatory standards.

Cranberries (*Vaccinium macrocarpon* Ait) belong to the Plantae kingdom and are recognised for their rich phytochemical composition. Among their bioactive compounds, flavonoids are crucial in determining their biological activity. These secondary metabolites, including anthocyanins, flavonols proanthocyanidins, contribute to cranberry extracts' antioxidative, anti-inflammatory antimicrobial properties, which are of particular interest in nutritional and pharmaceutical research. Flavonoids are naturally occurring phenolic compounds found in plants. Basic flavonoid structure is a fifteen-carbon skeleton, consisting of two benzene rings, A and B, which are linked by heterocyclic pyrane ring C (Figure 1).[4]

Anthocyanins (of the Greek *anthos* = flower and *kianos* = blue) are water soluble vacuole pigments present in fruits, flowers and vegetative organs. They have a role in pollination and protecting plants from UV-ray induced damage by absorbing light and from cold stress. Anthocyanins are glycoside forms of anthocyanidins. Basic structure of anthocyanidins is a type of oxonium ion, called the flavylium cation (Figure 2), that gives rise to different anthocyanidins by hydroxylation in different positions, primarily on carbon C3, C5, C6, C7 and C3', C4' and C5'.[5]

Flavylium cation is responsible for the colour of the anthocyanins. The positive charge of the flavylium ion is delocalised of the heterocyclic pyrane ring (C) and can even be distributed over both benzene rings (A and B). This delocalisation is responsible for the anthocyanin colour. This delocalisation causes an energy difference between the separate molecular orbitals that falls within the range of the visible spectrum. Once visible light hits the chromophore, it can be absorbed by the electron that is excited from the ground to the excited state, what leads to a conformational change of the molecule. The colour that is seen by human eyes is the one that is not absorbed by the reflecting object within a certain wavelength spectrum of the visible light.

If water molecules are conjugated to the heterocycle and if the pH is increased via nucleophilic attack mechanism, positive charge of the flavylium ion will be neutralised.[6] This property of flavylium cation enables usage of spectrophotometric methods for the determination of the content and antioxidative capacity. At low pH values, anthocyanidins are present in their flavylium form and have red or orange colour. With the change of pH to neutral conditions (pH 6-7), uncharged quinones are formed the colour changes to violet. Further increase of pH leads to the formation

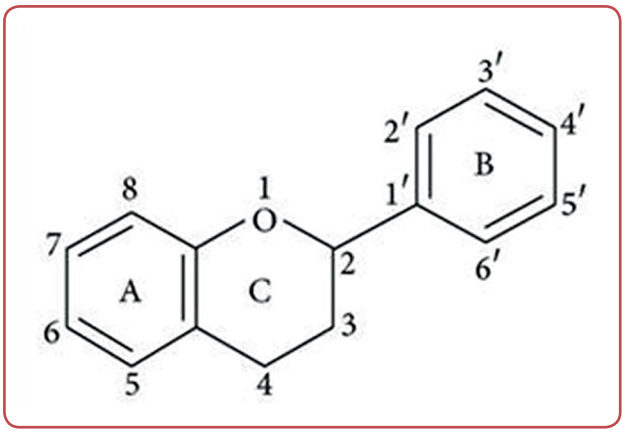

Figure 1: A basic flavonoid structure

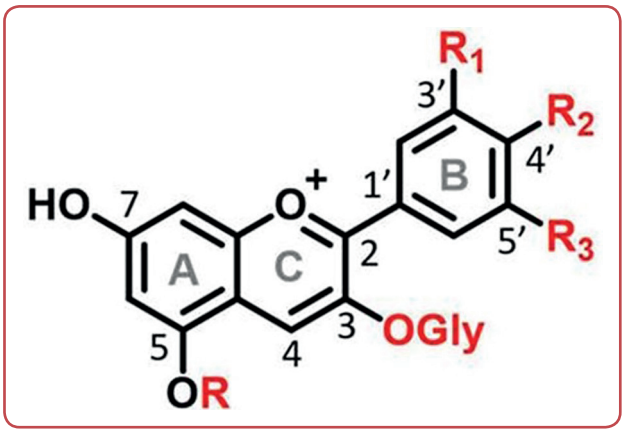

Figure 2: Basic structure of anthocyanidins

of slightly stable anionic quinone (pH 7-8) which has blue colour. Increase of pH above 11 leads to the formation of unstable dianionic form which has blue-green colour.[5]

Stability of the anthocyanidins is not only dependent of the pH, but also on storage, light, oxygen, solvents, presence of enzymes, their chemical structure, presence of the proteins and metallic ions. Relative instability and low extraction percentages limit their pharmaceutical potential.[7]

The substitution of the B ring by hydroxy or methoxy groups increases the reactivity of the aglycon in the neutral media. On the other hand, monoglycosides and diglycosides are much more stable in pH neutral media because the sugar molecules prevent degradation of the anthocyanin to aldehyde and phenolic acid (Figure 3).[7] Moreover, sugar molecules can undergo acylation with aromatic or aliphatic acids (Figure 4), what further increases their stability.

Proanthocyanidins (PAC) are plant-derived flavonoids composed of oligomeric and polymeric structures formed by catechin or epicatechin units, often linked with gallic acid esters. Catechin belongs to the flavan-3-ol subclass of flavonoids and features a molecular structure composed of two benzene rings (A and B) and a dihydropyran heterocycle (C ring), with a hydroxyl group positioned at C3 (Figure 5).

Carbon C2 and C3 are saturated and therefore do not possess the characteristics of flavylium cation. Also, C2 and C3 are chiral centres. There are four diastereoisomers of catechin, two isomers have trans configuration (catechin) other two have cis configuration (epicatechin).[8] Depending on the repeating unit, PAC can be homopolymers and heteropolymers. Same repeating units form homopolymers *vice versa*.[9]

Proanthocyanidins include proanthocyanins, prodelphinidins and propelargonidins. Prodelphinidins are polymers of gallocatechin propelargonidins are polymers of epiafzelechin. In *Vaccinium sp*, the most present is proanthocyanin. Therefore, proanthocyanidins are often referred as proanthocyanins.

The connection method is what differentiates proanthocyanidins type A and B (Figure 6). Type A has at least one double linkage consisting of a C-C bond and an additional ether bond. The most common A-type compounds are A1 and A2. Type B consists of a single interflavan bond between carbon-4 of the B-ring and either C-8 or C-6 of the C-ring. They are the most abundant, with types B1, B2, B3 and B4 occurring most frequently.[10]

Most food exclusively contains proanthocyanidin type B. In contrast to this statement, the profile of cranberry is distinct from that of other berry fruit, being rich in A-type proanthocyanidins. Cranberry has proanthocyanidin consisting of (+)-catechin and (–)-epicatechin such proanthocyanidin form is called procyanidin.

Polymers of proanthocyanidins (PACs) exhibit common chemical characteristics. A and B benzene rings are rich with hydroxyl (OH) groups, which number increases with the level of polymerisation. OH groups are available for electrostatic and hydrogen bonding. They also possess strong electron-donating abilities and, as such, act as strong antioxidants.[11]

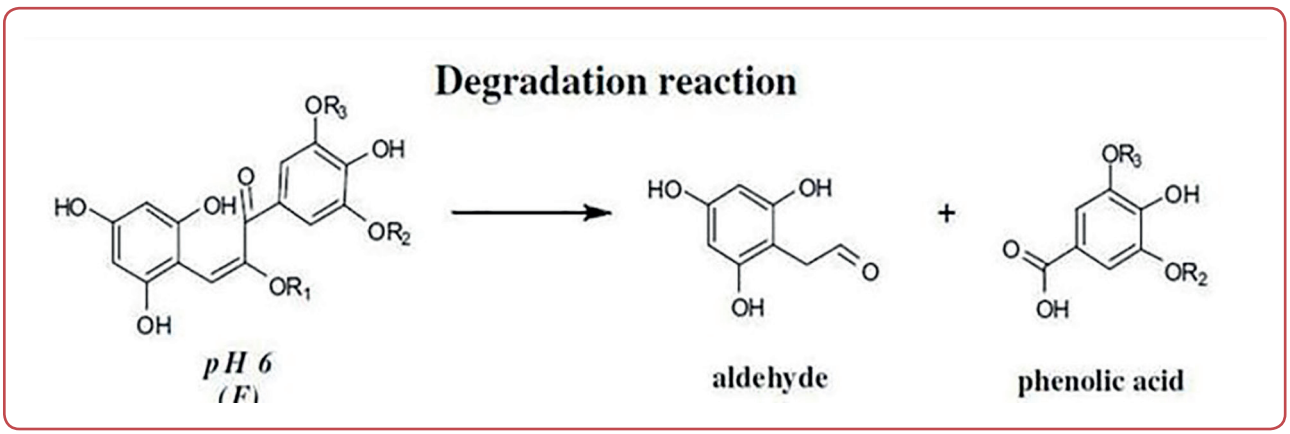

Figure 3: Degradation of the anthocyanin to aldehyde and phenolic acid

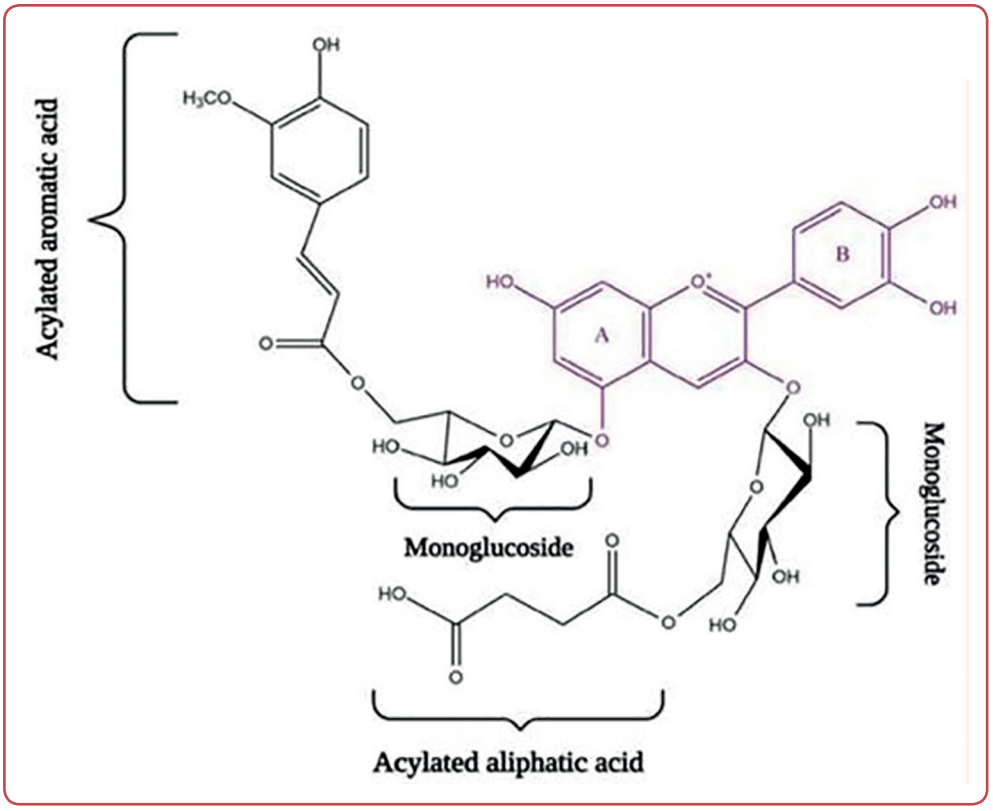

Figure 4: *Complex structure and binding of anthocyanins*

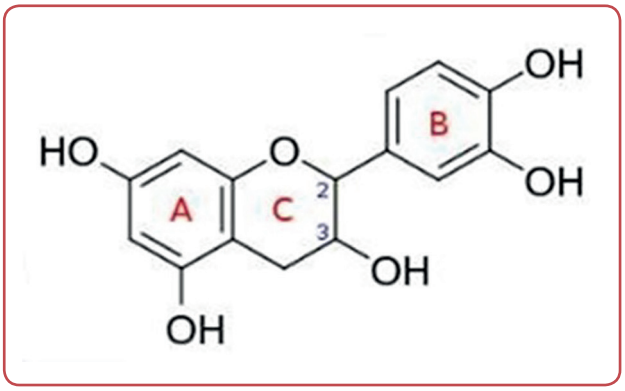

Figure 5: *Chemical structure of catechin*

Hydroxyl groups at the A ring make the molecule susceptible to electrophilic aromatic substitution. The hydroxyl group of the phenol acts as an electron donor in the basic medium, it increases the electron density of the ring, making it nucleophilic. Proanthocyanidins show typical reactions of activated condensed benzene systems, such as alkylation and condensation with aldehydes. Nucleophilic properties of proanthocyanidins increase with the increase of pH, while in acidic medium, reactivity is significantly reduced due to limited ionisation.[11]

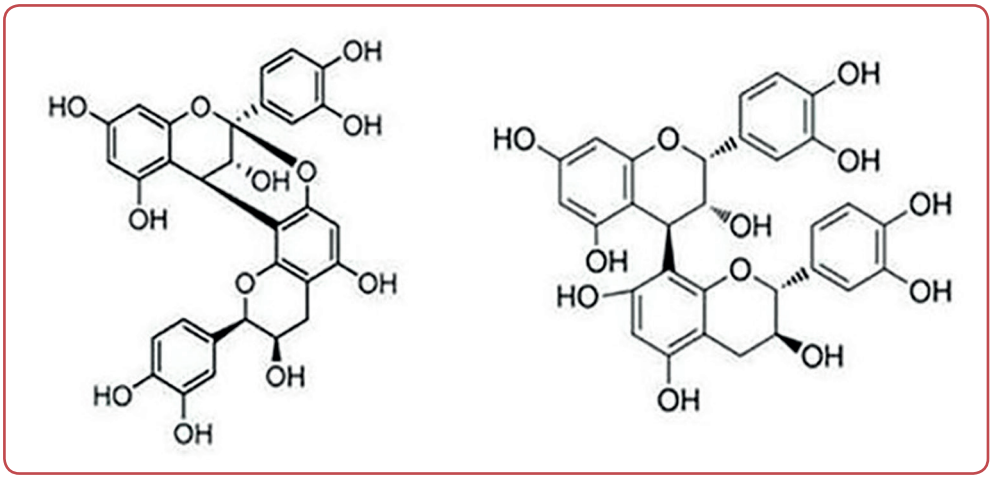

Figure 6: *Structure of proanthocyanidins (PACs): PAC A (left), PAC B (right)*

This study aimed to characterise the origin of a commercially available cranberry sample by analysing its individual anthocyanin profile, total procyanidin content antioxidative properties using two complementary methods. Additionally, the research included the identification of organic acids and the quantitative assessment of vitamin C content to provide a comprehensive evaluation of the sample's phytochemical composition.

Methods

Biological materials, standards, reagents and chemicals

A commercially available dry extract of *Vaccinium macrocarpon* Aiton fruit was used in the study. The sample was purchased from *NexiraTM* (Exocyan cranberry extract).

Standards used in methodology:

1. Cyanidin-3-O-Galactoside Chloride (C3Ga), *LGC,* Teddington, UK
2. Cyanidin-3-O-glucoside (C3Gl), phyproof® Reference Substance, *Sigma Aldrich*, St Louis, USA
3. Cyanidin-3-O-arabinoside (C3Ar), phyproof® Reference Substance, *Sigma Aldrich,* St Louis, USA
4. Peonidin-3-O-galactoside (P3Ga), *LGC,* Teddington, UK
5. Peonidin-3-O-arabinoside (P3Ar), *Extrasynthese,* Genay, France
6. Procyanidin B2 (phyproof® Reference Substance, *Sigma Aldrich,* St Louis, USA)
7. BQC kit (*Bioquochem*, Asturias, Spain, KB03017)
8. Vitamin C (Ascorbic Acid), USP standard

Reagents: ABTS, DPPH
Chemicals: n-butanol, 2 mL of 2N HCl, 0.2 mL of $Fe_2(SO_4)_3$ solution, ethanol, $K_2S_2O_8$,

Identification and quantification of anthocyanins via HPLC-DAD

To confirm if the purchased extract was indeed derived from the *V macrocarpon*, the HPLC method by Brown and Shipley was adjusted and used for screening of the anthocyanins profile.[12] Standards, samples, mobile phases and solvents have been used as described in the method by Brown and Shipley. The optimisation includes different autosampler temperature, about 25 °C different column - Zorbax C18 5 µm 150 x 4.6 mm. Individual content of each anthocyanin was quantified via calibration curve of prepared standards.

Spectrophotometric determination of total PAC with modified Bate-Smith method

An assay of total PACs was performed by modified Bate-Smith method using UV-VIS spectrophotometer Cary 100, Agilent, SAD. Reagent: 6 mL of n-butanol, 2 mL of 2N HCl 0.2 mL of $Fe_2(SO_4)_3$ solution. The reagent is light-sensitive and should be covered with aluminium paper.

Sample procedure

Two tests were performed for each sample. A total of 30 mg of the sample was weighed into a 100 mL vial and dissolved in Milli-Q water. Tube A contained 2 mL of the prepared sample solution and 6 mL of reagent medium, which was mixed thoroughly. Half of this mixture was transferred into screw-cap test tube B. Tube B was heated in a Bain-Marie bath at 100 °C for 30 minutes and then cooled for 15 minutes in the dark. The remaining solution in tube A was kept in the dark. Blank procedure: 2 mL of distilled water was mixed with 6 mL of reagent and prepared the same way as the sample.

Standard solution procedure

Procyanidin B2 (phyproof® Reference Substance, Sigma Aldrich) was used as the standard. Solutions of varying concentrations (0.01, 0.02, 0.1, 0.15 0.2 mg/mL) were following the same procedure as the sample preparation.

Result interpretation

Absorbance values were measured for both tube A (unheated sample) and tube B (heated sample) at 550 nm against the reagent blank. The increase in absorbance after heating (tube B minus tube A) corresponds to the formation of coloured anthocyanidins from PACs. This difference in absorbance (ΔA) was used to quantify the PAC content by comparison to a standard calibration curve constructed using procyanidin B2. The PAC concentration in the sample was then calculated using the linear regression equation derived from the standard curve (y = ax + b, Figure 7).

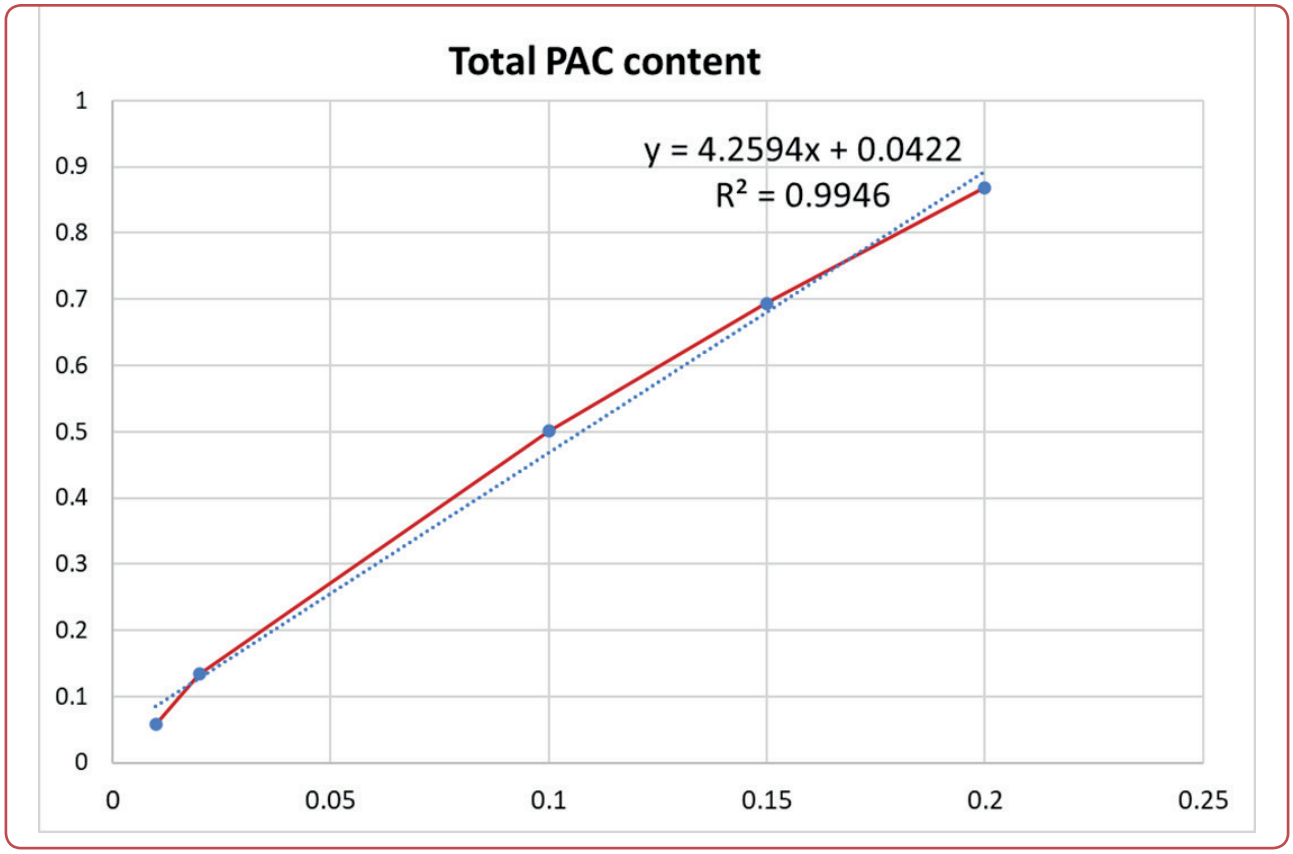

Figure 7: Calibration curve of procyanidin standard

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Spectrophotometric determination of total soluble PACs with4-dimethylaminocinnamaldehyde (DMAC) method

The DMAC assay is a colorimetric method specifically designed to detect flavan-3-ol units within proanthocyanidins (PACs). The assay relies on the electrophilic reaction of DMAC with the C6 or C8 position of the A-ring in flavan-3-ols (eg, catechin, epicatechin) under acidic conditions.

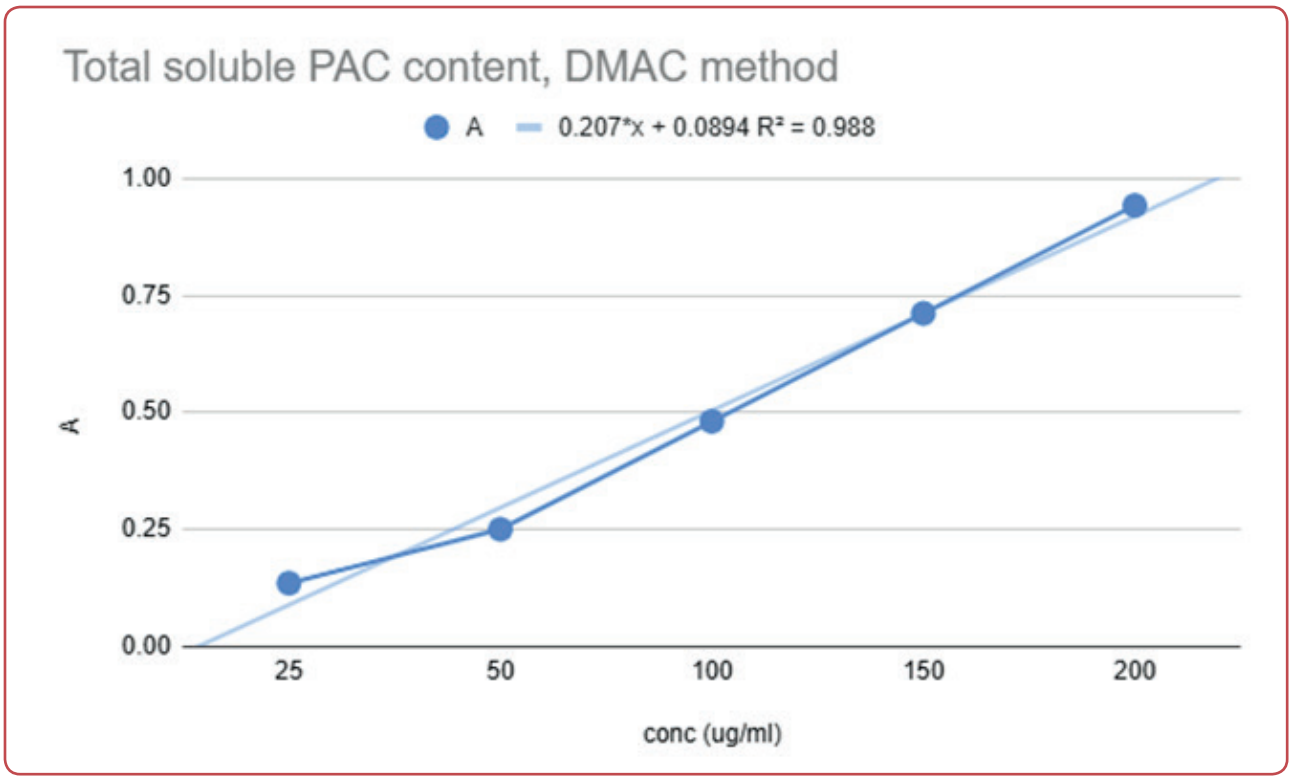

Figure 8: Calibration curve of catechin chloride standard via BQC kit (Bioquochem, Asturias, Spain)

PAC content was determined using BQC kit, according to manufacturer's instructions *(Bioquochem*, Asturias, Spain). Calibration curve is presented in Figure 8.

Sample preparation

Ten mg of dry cranberry extract was dissolved in 100 mL of ethanol, sonificated for 45 minutes, followed by centrifuge at 4500 rpm for 10 min. Then, 1.0 mL of supernatant was transferred in 100 mL ethanol. Following, 10 µL of sample, 230 µL of reagent A and 10µl of DMAC reagent were added in 96-well bottom plates. Plates were left for agitation for 15 min.

The reaction yields a green-blue chromophore with maximum absorbance at λ = 640 nm, which can be quantitatively measured using Agilent BioTek Epoch Microplate Spectrophotometer.

Determination of the antioxidative capacity of dry cranberry extract using the ABTS method

The antioxidative capacity of dry cranberry extract was measured using the ABTS method. First, an ABTS solution was prepared by dissolving ABTS powder in ethanol at a concentration of 7–10 mM. To generate the ABTS radical cation (ABTS•+), potassium persulfate ($K_2S_2O_8$) was added to the solution, ensuring a final concentration of approximately 2.45 mM. The solution was then left in the dark at room temperature for 12–16 hours. During this period, the solution turned dark blue green, indicating the formation of ABTS•+.

After incubation, the ABTS•+ solution was diluted with the same solvent until its absorbance reached approximately 0.8 at 734 nm. The exact dilution depended on the initial concentration and incubation time.

Different concentrations of the dry cranberry extract were prepared at 0.025, 0.05, 0.1, 0.15 0.2 mg/mL (Figure 9) A set amount of each sample was mixed with the diluted ABTS•+ solution. The antioxidants in the extract reacted with ABTS•+, reducing it to a colourless form. The decrease in absorbance at 734nm was measured using a spectrophotometer. A greater decrease in absorbance indicated a higher antioxidative capacity of the extract.

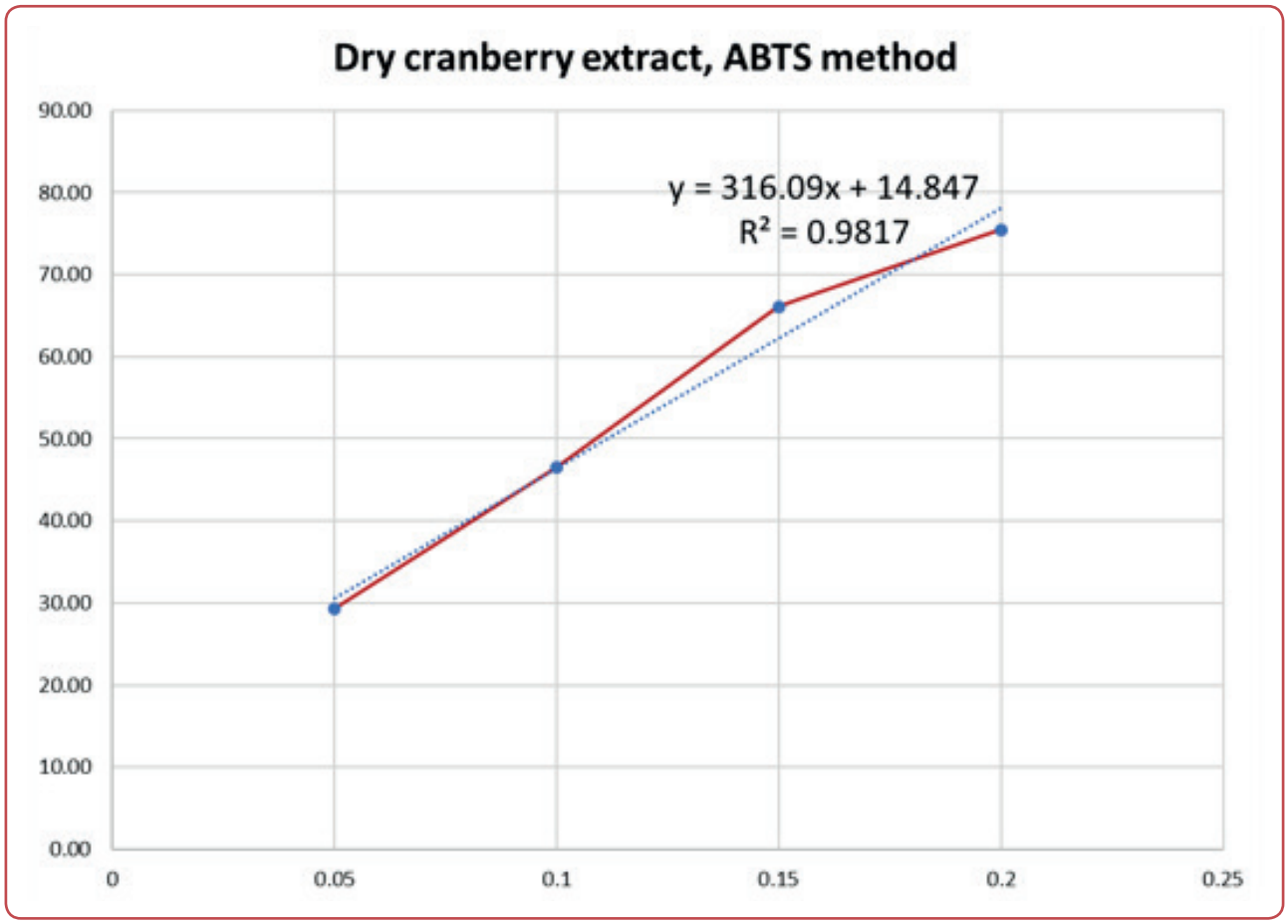

Figure 9: Calibration curve of the antioxidative property of the cranberry sample, ABTS method

For comparison, the antioxidative property was also assessed using vitamin C as a reference standard. Vitamin C solutions were prepared at the following concentrations: 0.003, 0.005, 0.01, 0.015 0.02 mg/mL, the same method was applied. Results were expressed as IC_{50} values, representing the concentration required to inhibit 50 % of the ABTS•+ radical activity.

Determination of the antioxidative capacity of the dry cranberry extract with DPPH method

The antioxidative activity was assessed using the DPPH radical scavenging assay. Initially, DPPH powder (between 10 and 100 mg) was accurately weighed and dissolved in an appropriate solvent, such as methanol or ethanol, to obtain a DPPH solution with a concentration typically ranging from 0.1 to 0.3 mM. This solution was then allowed to incubate in the dark at room temperature for 30 minutes until it developed a deep purple colour, indicative of the formation of the DPPH radical.

A series of test sample solutions was prepared at concentrations of 0.025, 0.05, 0.1, 0.15 0.2 mg/mL (Figure 10) while vitamin C, used as a positive control, was prepared at concentrations of 0.003, 0.005, 0.01, 0.015 0.02 mg/mL.

Equal volumes of the DPPH solution and each sample or vitamin C solution were mixed and then incubated in the dark at room temperature for an additional 30 minutes to allow sufficient interaction between the antioxidants and the DPPH radical. The reduction in absorbance, which reflects the scavenging of the DPPH radical, was measured at 517 nm using a UV-visible spectrophotometer.

The results were expressed as IC_{50} values, which represent the concentration of the antioxidant required to achieve a 50 % reduction in the DPPH radical. These IC_{50} values were then compared between the test samples and vitamin C to assess their relative antioxidative activities.

Identification of organic acids and quantitative determination of vitamin C using HPLC-DAD method

In order to identify organic acids and quantify vitamin C in the sample, an official USP method has been used.[13]

Mobile Phase A (MPA) comprised 20 mM potassium phosphate monobasic, with the pH adjusted to 2.4 using phosphoric acid, while Mobile Phase B (MPB) was acetonitrile. Chromatographic separation was performed using a gradient elution program with

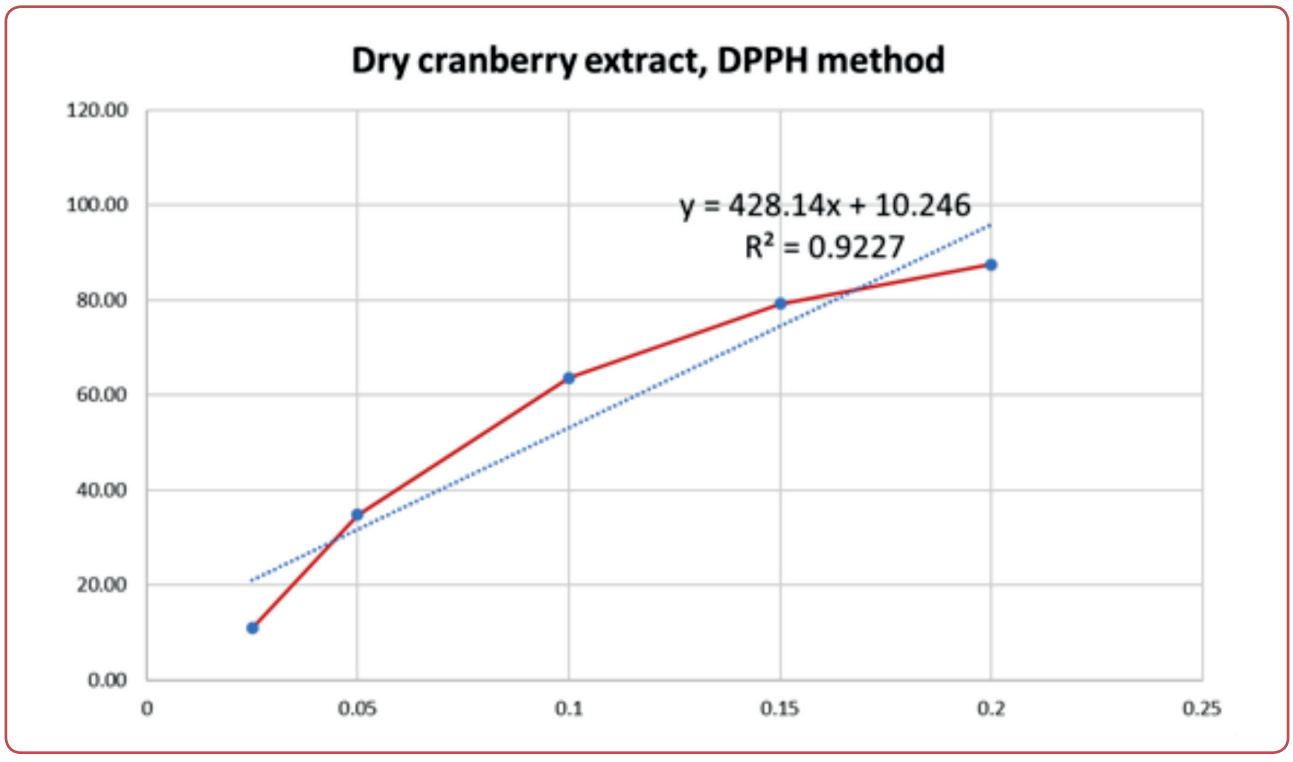

Figure 10: Calibration curve of the antioxidative property of the cranberry sample, DPPH method

the following profile (time, min/% MPB): 0 min, 10 %; 0–11 min, 60 %; 16 min, 60 %; 17 min, 0 %; and 22 min, 0 %. The solvent for sample preparation was 0.3 % hydrochloric acid in water. A standard solution of ascorbic acid was prepared by accurately weighing 10.0 mg of USP-grade Citric Acid RS into a 10 mL dark glass volumetric flask and dissolving it in the solvent. For identification purposes, a tartaric acid solution (purchased) was similarly prepared at a concentration of 1 mg/mL. The sample was prepared by weighing 250 mg of dry cranberry extract into a 25 mL dark glass volumetric flask, to which approximately 20 mL of solvent was added; the mixture was mechanically shaken for 20 minutes and then diluted to the final volume with the solvent. The resulting solution was thoroughly mixed, centrifuged at 4500 rpm for 10 minutes filtered through a 0.45 µm membrane filter. Detection was carried out at 214 nm using a Zorbax C18 column (150 × 4.6 mm, 5 µm particle size) at a flow rate of 0.45 mL/min, with an injection volume of 5 µL. Quantitative calculations were performed using the following equation:

$$\text{Result} = \frac{Ru \times Cs}{Rs} \times \frac{V}{W} \times 100$$

Ru – peak area of the relevant analyte from the sample solution
Rs – peak area of the relevant analyte from the standard solution
Cs – concentration of the standard (citric acid, mg/mL)
V – volume of the sample solution (mL)
W – weight of the sample (mg)

Results

Identification and quantification of anthocyanins via HPLC-DAD

Anthocyanins characteristics of the cranberry profile have been identified and quantified. Cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, peonidin-3-*O*-galactoside and peonidin-3-*O*-arabinoside have been detected, respectively (Figure 11).

Table 1: Anthocyanin content (mg) per 1 g of extract

Anthocyanins	C3Ga	C3Gl	C3Ar	P3Ga	P3Ar
Content assay (mg/1 g extract)	4.72	2.39	1.90	0.50	0.44

C3GA: Cyanidin-3-O-galactoside chloride; C3Gl: Cyanidin-3-O-glucoside (C3Gl); C3Ar: 3. Cyanidin-3-O-arabinoside; P3Ga: 4. Peonidin-3-O-galactoside; P3Ar: Peonidin-3-O-arabinoside;

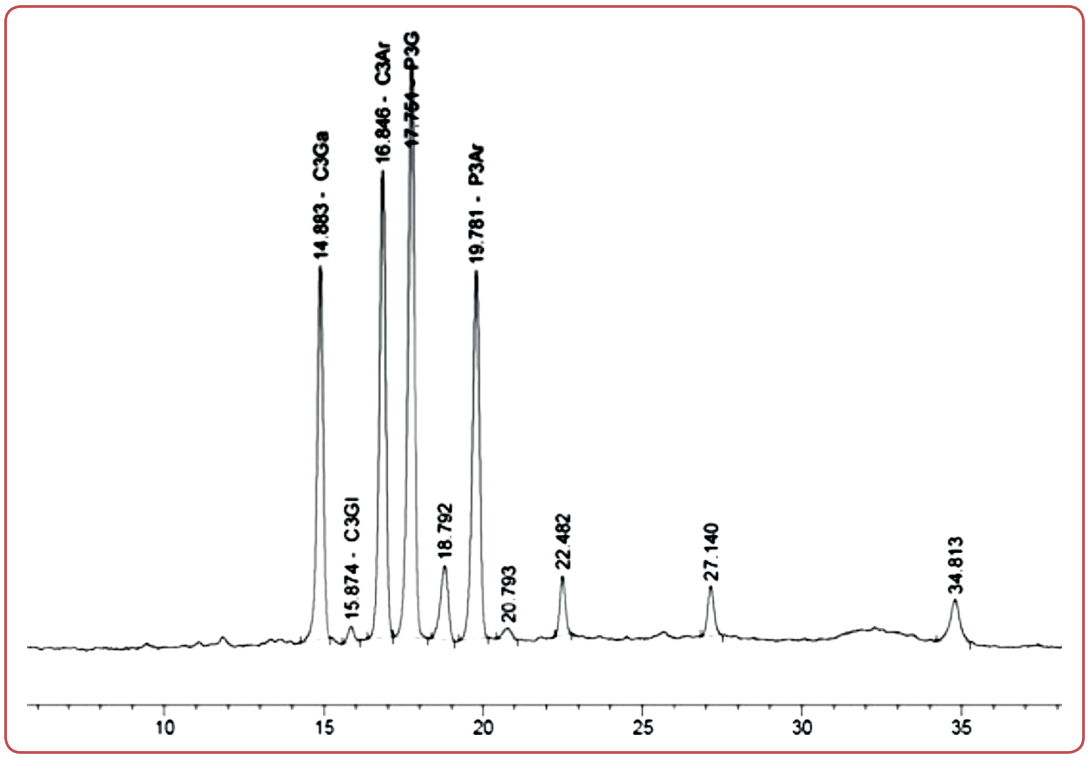

Figure 11: Calibration curve of the antioxidative property of the cranberry sample, DPPH method

The calculation has been performed by the Brown and Shipley method presented in Table 1.

Results of spectrophotometric determination of total PAC with modified Bate-Smith method

The test has been performed in triplicate results are shown in Table 2.

Table 2: Total proanthocyanidins (PACs) content has been expressed as mg of total PAC per 1g of extract

Samples	g total PAC / 100 g of extract (% v/v)
1	37.24
2	32.70
3	39.00
Average	36.31
SD	3.25
RSD	8.95
Average ± 1σ	33.06-39.56
Average ± 2σ	29.81-42.81
Average ± 3σ	26.56-46.07

SD: standard deviation; RSD: relative standard deviation;

Results of spectrophotometric determination of total soluble PACs with DMAC method

Total soluble PAC content by DMAC method is calculated via calibration curve using catechin chloride as standards, per manufacturer's instructions (*Bioquochem*, Asturias, Spain).

The test has been performed in triplicate results are shown in Table 3.

Table 3: Total soluble proanthocyanidins (PACs) content by 4-dimethylaminocinnamaldehyde (DMAC) method

Samples	CE (µg catechin hydrate / mL)	g PAC / 100 g of extract (%)
1	0.5456	54.56
2	0.5314	53.14
3	0.5300	53.00
Average		53.57
SD		0.86
RSD		1.61
Average ± 1σ	/	52.70 - 54.43
Average ± 2σ	/	51.84 - 55.29
Average ± 3σ	/	50.98 - 56.16

SD: standard deviation; RSD: relative standard deviation;

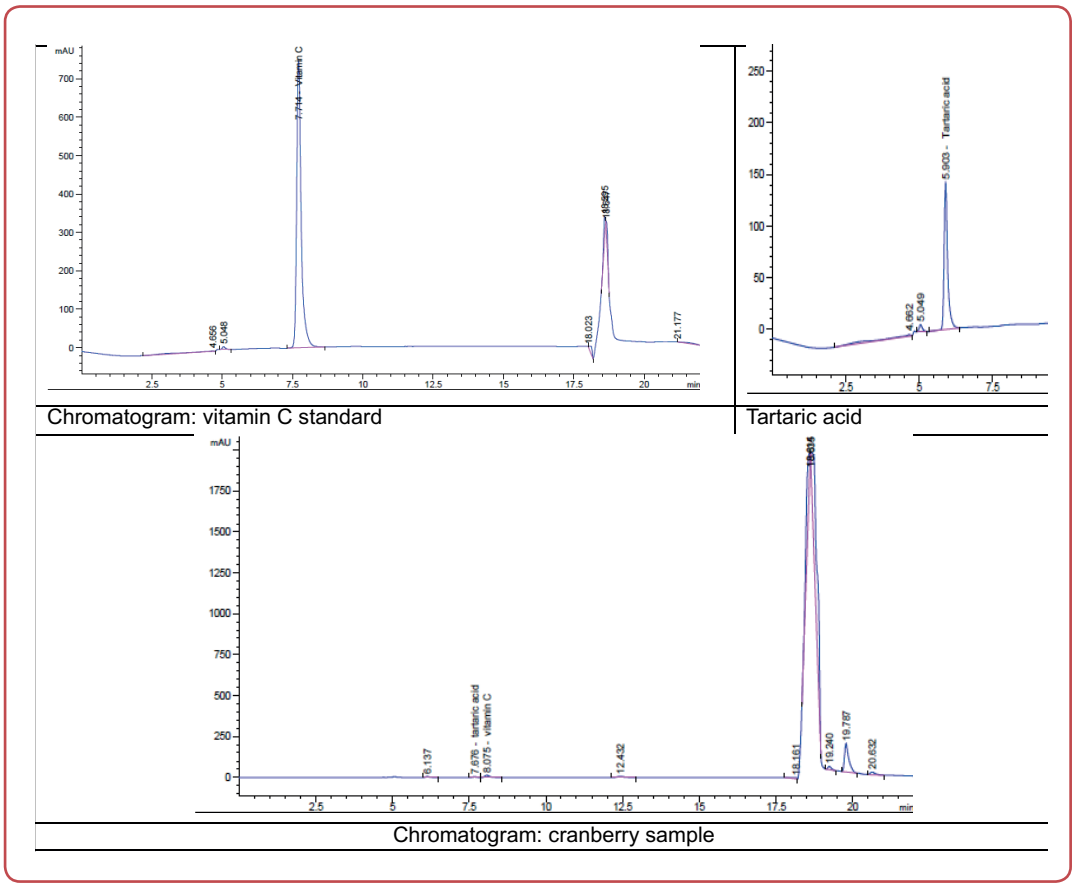

Figure 12: Chromatograms of organic acid content of dry cranberry sample

Results of the antioxidative capacity of the sample

Figure 12 shows the calibration curve of the antioxidative activity of the cranberry sample using the ABTS method. It has been shown that the cranberry sample has antioxidative properties comparable to the antioxidative property of vitamin C.

IC_{50} value of the sample is 110 µg/mL. At the same time, vitamin C was used as a standard whose antioxidative properties were compared to those of the samples. IC_{50} of vitamin C is 10.75 µg/mL. Similar results have been shown using the DPPH method. IC_{50} value of the sample was 92.85 µg/mL. IC_{50} of vitamin C was 30.70 µg/mL.

Results of identification of organic acids and quantitative determination of vitamin C using HPLC-DAD method

Using the HPLC-DAD method via USP guidelines, chromatograms observing vitamin C as a standard and tartaric acid have been made. The chromatogram of the sample also shows the presence of both vitamin C and tartaric acid (Figure 12).

In the sample, tartaric acid has been identified as one of the organic acids that are usually present in the cranberry extracts. The content of vitamin C has been determined it is 0.12 %, or 1.2 mg of vitamin C per 1 gram of extract.

Discussion

The chemical characterisation of the commercially available dry cranberry extract has provided valuable insights into its phytochemical composition and antioxidative potential. Presented study confirmed the presence of the characteristic anthocyanins—cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinoside, peonidin-3-O-galactoside peonidin-3-O-arabinoside—in concentrations ranging from 0.44 to 4.72 mg per gram of extract. The total anthocyanin content of approximately 9.95 mg/g is in line with previous reports, where cranberry extracts typically exhibit values within the range of 5–30 mg/g, depending on the cultivar, extraction method storage conditions.[14-16]

The determination of total PACs by the modified Bate-Smith method revealed an average content of 36.31 %. The method was partially validated in terms of linearity and precision. Linearity was assessed using standard solutions of procyanidin B2 in the range of 0.01-0.2 mg/mL, with a correlation coefficient (R^2) of 0.9946. Precision was evaluated by analysing replicate samples (n = 3), yielding a relative standard deviation (RSD) of 8.95 %, which is acceptable for colorimetric methods involving complex plant matrices. Two of the replicates (37.24 % and 39.00 %) fall within the range of the mean ± one standard deviation (avg ± 1σ), while the third replicate (32.70 %) lies within the broader range of the mean ± two standard deviations (avg ± 2σ). This distribution indicates that the majority of measurements cluster closely around the mean, demonstrating good precision. The single replicate falling within the ± 2σ range suggests a slightly higher deviation but remains within acceptable variability limits for most analytical methods. Overall, the replicate results indicate a consistent and reliable measurement process with no significant outliers.

For comparative purposes, the DMAC method was additionally conducted using a commercially available PAC analysis kit (*Bioquochem*, Asturias, Spain), following the manufacturer's instructions. The resulting calibration curve demonstrated excellent linearity (R^2 = 0.988). The analysis was carried out in triplicate; two replicate values (53.14 % and 53.00 %) fell within the range of the mean ± 1σ, while the third (54.56 %) fell within the range of the mean ± 2σ. The average PAC content was determined to be 53.57 %, with an RSD of 1.61 %, reflecting a high level of precision and reproducibility of the method. The obtained PAC content of 53.57 % is in accordance with EFSA guidelines, which recommend a total PAC concentration in cranberry extracts of approximately 55–60 % when determined using the DMAC method.

Differences in results are due to different standards and nature of reactions. The DMAC assay is a colorimetric method specifically designed to detect flavan-3-ol units within PACs. The assay relies on the electrophilic reaction of DMAC with the C6 or C8 position of the A-ring in flavan-3-ols (eg, catechin, epicatechin) under acidic conditions. The reaction yields a green-blue chromophore with maximum absorbance at λ = 640 nm, which can be quantitatively measured in a UV-Vis

spectrophotometer. The reaction is highly selective for terminal flavanol units (ie, the non-condensed unit at the end of the PAC chain) the DMAC reagent does not significantly react with phenolic acids (eg, gallic acid), anthocyanins non-flavonoid polyphenols. In the Bioquochem PAC assay kit (KB03017), catechin chloride is used to construct the standard calibration curve. Catechin is a monomeric flavan-3-ol, meaning each molecule represents a single reactive terminal unit. Because the DMAC reagent reacts selectively with terminal flavan-3-ol units, polymeric PACs, which contain only a single terminal unit per molecule regardless of chain length, yield disproportionately lower absorbance per unit mass than monomers or dimers. Moreover, the assay only detects PACs that are soluble in the extraction solvent, meaning that insoluble or highly polymerised PACs are not represented in the measurement. As a result, the DMAC assay does not reflect total PAC content, but rather the concentration of soluble, terminal-unit-containing PACs, which often correspond to the lower molecular weight, bioavailable fraction of the sample.

In contrast, the Bate-Smith method cleaves all interflavan bonds under acidic and high-temperature conditions, converting all flavan-3-ol units (terminal and extension) into anthocyanidins, which can be measured spectrophotometrically. This method provides a more accurate representation of total PAC content, particularly for highly polymerised, polymeric PACs that are abundant in many plant-derived matrices.

Traditionally, the Bate-Smith method used cyanidin chloride as a calibration standard. However, this introduces a structural mismatch and the potential for overestimation due to the high molar absorptivity of cyanidin. Replacing cyanidin with procyanidin B2, a naturally occurring B-type PAC dimer, offers several advantages. It improves structural relevance, as B2 more accurately reflects the PACs found in food and plant materials. Additionally, it enables molecular weight correction, since B2 has a defined dimeric structure that allows for mass-based quantification aligned with actual PAC content. Using procyanidin B2 also reduces the likelihood of overestimation related to extinction coefficient differences. Finally, it enhances cross-method comparability, as procyanidin B2 serves as a useful reference point between depolymerisation-based colorimetric methods and chromatographic techniques such as UHPLC-MS used for PAC dimer analysis.

Also, results between Bate-Smith and UHPLC-MS method are more comparable because it involves acid-catalysed depolymerisation of proanthocyanidin polymers into their monomeric anthocyanidin units, thereby providing a comprehensive measure of total PAC content across varying degrees of polymerisation, whereas methods such as DMAC exhibit preferential reactivity toward low-degree oligomers, resulting in disproportionate quantification. Although the Bate-Smith method typically yields higher PAC values than the DMAC assay, due to its ability to depolymerise and quantify all flavanol units, including those in high-molecular-weight polymers, the results of this study showed the opposite trend.

Specifically, PAC content was 36.31 % by Bate-Smith and 53.57 % by DMAC, suggesting that in this case, the extract may be enriched in lower molecular weight PACs or terminal units, to which the DMAC assay is more sensitive. These findings highlight that the relative performance of the two methods can vary depending on the structural composition of PACs in the sample. This result is consistent with the fact that commercial cranberry extracts are often enriched in low-degree polymerisation A-type PACs, which not only exhibit preferential solubility and greater stability during extraction and purification but also react more strongly with the DMAC reagent than with the Bate-Smith method, resulting in higher PAC values measured by DMAC.[17] The antioxidant activity of dry cranberry extract was assessed using ABTS and DPPH radical scavenging assays, with IC_{50} values serving as indicators of efficacy. The extract demonstrated moderate antioxidant capacity, with IC_{50} values of 110 µg/mL (ABTS) and 92.85 µg/mL (DPPH), in comparison to vitamin C, which exhibited significantly stronger activity (IC_{50} = 10.75 µg/mL and 30.70 µg/mL, respectively). The relatively higher IC_{50} values of the extract reflect its lower potency compared to pure vitamin C; however, its notable activity, particularly in the DPPH assay, suggests the presence of effective antioxidant constituents. These differences between assays may be attributed to their distinct sensitivities to hydrophilic and lipophilic compounds. The extract's antioxidant potential is likely due to its complex phytochemical profile, including A-type proanthocyanidins, flavonols phenolic acids, supporting its relevance as a natural source of antioxidant compounds in functional food or nutraceutical applications.

Notably, HPLC-DAD analysis revealed a vitamin

C content of 0.12 % (1.2 mg/g extract), which is considerably lower than the amounts typically found in raw cranberries. In early developmental stages, raw cranberries generally contain higher levels of vitamin C; however, as the fruit matures, there is a natural decline in organic acids—including vitamin C—while the accumulation of anthocyanins and PAC increases.[18] This pattern suggests that the extract analysed in presented study was likely produced from mature fruit, which is desirable from a nutraceutical standpoint because higher anthocyanin and PAC levels are associated with improved antioxidative and health-promoting properties.

A discrepancy arises when considering that many dietary formulations include vitamin C as an additive, despite evidence that vitamin C can catalyse the degradation of anthocyanins and PAC. Several studies have demonstrated that the addition of vitamin C to anthocyanin-rich products can lead to accelerated degradation through redox reactions and pH-dependent mechanisms.[19] This poses a formulation challenge: while vitamin C is valued for its own antioxidant properties, its interaction with cranberry polyphenols may compromise the stability and efficacy of the bioactive compounds.

To overcome these challenges, alternative stabilising agents should be considered. Recent research has investigated the potential of various co-pigments and stabilisers for anthocyanins. For example, glutamate and succinate have been examined as potential candidates for stabilisation.

Glutamate possesses both amino and carboxyl groups that enable it to form hydrogen bonds and ionic interactions with anthocyanins, potentially reducing degradation by stabilising the flavylium cation structure.[20] Its zwitterionic nature may help maintain a more favourable microenvironment around the anthocyanin molecules, although the evidence is still emerging. On the other hand, succinate is a dicarboxylic acid that can lower the local pH and foster stronger hydrogen-bond networks with anthocyanins and PAC molecules. This diacidic structure promotes the formation of stable co-pigmented complexes, which can help shield the anthocyanins from nucleophilic attacks and oxidation. Some studies suggest that succinate might offer superior stabilisation compared to single-carboxylate compounds due to its ability to interact with multiple sites on the anthocyanin molecule simultaneously.

Additional stabilisers have been proposed in the literature to preserve the integrity of anthocyanins and PACs in cranberry extracts. Phenolic acids (eg, ferulic, caffeic gallic acid) have been shown to engage in co-pigmentation with anthocyanins, forming non-covalent complexes that stabilise the flavylium ion structure. This interaction not only enhances colour intensity but also provides protection against pH-induced degradation and oxidation.[21] Cyclodextrins, cyclic oligosaccharides capable of encapsulating hydrophobic molecules within their central cavity, form inclusion complexes that shield anthocyanins from environmental factors such as light, oxygen pH fluctuations, thereby enhancing their stability.[22] Biopolymers, including pectin and gum Arabic, can create a protective matrix around anthocyanins and PACs. This encapsulation effect reduces exposure to degradative conditions and may decelerate the rate of anthocyanin degradation during storage.[23] Proteins, such as whey protein isolate, also contribute to stabilisation through hydrogen bonding and hydrophobic interactions. Whey proteins have been demonstrated to bind anthocyanins and form a protective layer that reduces their degradation.[24] Citric acid and other organic acids are frequently employed to maintain an acidic environment that preserves the flavylium cation form of anthocyanins. Citric acid not only lowers the pH but may also interact directly with anthocyanins to enhance their stability.

In comparing glutamate and succinate as potential stabilisers, glutamate's amino and carboxyl groups allow it to engage in multiple hydrogen bonds and ionic interactions with anthocyanins, creating a favourable microenvironment that protects the anthocyanin structure. In contrast, succinate, as a dicarboxylic acid, possesses two carboxyl groups capable of forming robust hydrogen bonds with the hydroxyl groups of anthocyanins. The bifunctional nature of succinate may enable simultaneous interactions at different sites on anthocyanin molecules, potentially establishing a more effective stabilising network. Preliminary studies suggest that the dual carboxylate functionality of succinate might offer superior stabilisation compared to single-carboxylate compounds, although further research is required to conclusively determine its efficacy.[25] The mechanisms by which these stabilisers operate include co-pigmentation, wherein both phenolic acids and certain biopolymers form complexes with anthocyanins that reduce hydration and subsequent degradation of the flavylium

cation. In addition, cyclodextrins facilitate inclusion complex formation by physically entrapping anthocyanin molecules and shielding them from oxidative agents. Matrix encapsulation provided by biopolymers and proteins serves as a physical barrier, mitigating exposure to light, oxygen pH variations, thereby preserving the stability of both anthocyanins and PACs.

Collectively, these alternative stabilisers offer promising avenues for maintaining the bioactivity of cranberry extracts while circumventing the deleterious effects associated with additives such as vitamin C, which has been shown to accelerate the degradation of anthocyanins and PACs under certain conditions.

In summary, the results obtained in our study are consistent with literature findings regarding the composition of cranberry extracts. The lower vitamin C content, when compared with raw cranberries, supports the conclusion that the extract was derived from mature fruit—a stage at which anthocyanins and PACs accumulate, while vitamin C and other organic acids diminish. Given the known destabilising effects of vitamin C on these compounds, the common practice of adding vitamin C in dietary formulations appears contradictory. Future work should explore alternative stabilising agents, with preliminary evidence favouring succinate over glutamate, to improve the stability of anthocyanins and PACs in cranberry products.

Conclusion

This study confirms that the dry cranberry extract is derived from mature Vaccinium macrocarpon fruit, as indicated by its low vitamin C (0.12 %) coupled with high levels of anthocyanins (~9.95 mg/g) and proanthocyanidins (~36 %). While the extract demonstrates significant antioxidative activity via both ABTS and DPPH assays, its potency remains lower than that of pure vitamin C—a finding that aligns with the known properties of complex polyphenolic mixtures. Moreover, the common formulation practice of adding vitamin C may undermine the stability of anthocyanins and PACs. Alternative stabilisers such as phenolic acids, cyclodextrins, biopolymers, proteins, glutamate succinate offer promising strategies for protecting these bioactives against degradation. Future work should focus on optimising these stabilisation methods to ensure the sustained efficacy of cranberry extracts in nutraceutical and pharmaceutical applications.

Ethics

This study did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Conflicts of interest

The author declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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References

1. Vanessa Caceres. Best Cranberry Pills and Supplements. OTC Medicine & Health Products [Internet]. 2025 [Cited:3-Apr-2025]. Available from: https://health.usnews.com/otc/rankings/cranberry-supplements.
2. FDA. FDA announces qualified health claim for certain cranberry products and urinary tract infections [Internet]. 2020 Jul 21. [Cited:3-Apr-2025]. Available from: https://www.fda.gov/food/hfp-constituent-updates/fda-announces-qualified-health-claim-certain-cranberry-products-and-urinary-tract-infections.
3. The European Food Safety Authority (EFSA). EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Safety of cranberry extract powder as a novel food ingredient pursuant to Regulation (EC) No 258/97. 2010;8.
4. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013;2013:1–16. doi: 10.1155/2013/162750.
5. Mattioli R, Francioso A, Mosca L, Silva P. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. Molecules. 2020 Aug 21;25(17):3809. doi: 10.3390/molecules25173809.
6. Lichota A, Gwozdzinski L, Gwozdzinski K. Therapeutic potential of natural compounds in inflammation and chronic venous insufficiency. Eur J Med Chem. 2019 Aug;176:68–91. doi: 10.1016/j.ejmech.2019.04.075.
7. Castañeda-Ovando A, Pacheco-Hernández MaDL, Páez-Hernández MaE, Rodríguez JA, Galán-Vidal CA. Chemical studies of anthocyanins: A review. Food Chem. 2009 Apr;113(4):859–71. doi: 10.1016/j.foodchem.2008.09.001.
8. Enaru B, Drețcanu G, Pop TD, Stănilă A, Diaconeasa Z. Anthocyanins: factors affecting their stability and degradation. Antioxidants. 2021 Dec 9;10(12):1967. doi: 10.3390/antiox10121967.
9. Rehan M, Ahmed F, Khan MI, Ansari HR, Shakil S, El-Araby ME, et al. Computational insights into the stereo-selectivity of catechins for the inhibition of the cancer therapeutic target EGFR kinase. Front Pharmacol. 2024 Jan 11;14:1231671. doi: 10.3389/fphar.2023.1231671.
10. Rue EA, Rush MD, van Breemen RB. Procyanidins: a comprehensive review encompassing structure elucidation via mass spectrometry. Phytochem Rev Proc Phytochem Soc Eur. 2018 Feb;17(1):1–16. doi: 10.1007/s11101-017-9507-3.
11. McGraw GW. Reactions at the A-ring of Proanthocyanidins. In: Hemingway RW, Karchesy JJ, Branham SJ, editors. Chemistry and significance of condensed tannins. Boston, MA: Springer US; 1989. p. 227–48.
12. Brown PN, Shipley PR. Determination of anthocyanins in cranberry fruit and cranberry fruit products by high-performance liquid chromatography with ultraviolet detection: single-laboratory validation. J AOAC Int. 2011;94(2):459–66. doi: 10.1093/jaoac/94.2.459.
13. United States Pharmacopeial Convention. Cranberry Fruit Dry Juice. In: United States Pharmacopeia and National Formulary (USP 46–NF 41). Rockville (MD): United States Pharmacopeial Convention; 2023. doi: 10.31003/USPNF_M12276_03_01.
14. Stebbins Nathan Blaine. Characterization and Mechanisms of Anthocyanin Degradation and Stabilization. University of Arkansas, Fayetteville; 2017.
15. Urbstaite R, Raudone L, Janulis V. Phytogenotypic anthocyanin profiles and antioxidant activity variation in fruit samples of the american cranberry (Vaccinium macrocarpon Aiton). Antioxid Basel Switz. 2022 Jan 27;11(2):250. doi: 10.3390/antiox11020250.
16. Lee J. Anthocyanin analyses of Vaccinium fruit dietary supplements. Food Sci Nutr. 2016 Sep;4(5):742–52. doi: 10.1002/fsn3.339.
17. Gardana C, Simonetti P. Evaluation of the degree of polymerization of the proanthocyanidins in cranberry by molecular sieving and characterization of the low molecular weight fractions by UHPLC-orbitrap mass spectrometry. Molecules. 2019 Apr 17;24(8):1504. doi: 10.3390/molecules24081504.
18. Guo X, Wang D, Shakeel M. Transcriptome analysis reveals light-induced anthocyanin synthesis candidate genes in rabbiteye blueberry (Vaccinium ashei: Reade). Biotechnol Biotechnol Equip. 2021 Jan 1;35(1):747–58. doi: 10.1080/13102818.2021.1924078.
19. Hosoda Y, Okahara F, Mori T, Deguchi J, Ota N, Osaki N, et al. Dietary steamed wheat bran increases postprandial fat oxidation in association with a reduced blood glucose-dependent insulinotropic polypeptide response in mice. Food Nutr Res. 2017 Jan;61(1):1361778. doi: 10.1080/16546628.2017.1361778.
20. Minakawa AFK, Faria-Tischer PCS, Mali S. Simple ultrasound method to obtain starch micro- and nanoparticles from cassava, corn and yam starches. Food Chem. 2019 Jun;283:11–8. doi: 10.1016/j.foodchem.2019.01.015.
21. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017 Jan;61(1):1361779. doi: 10.1002/fsn3.374.
22. Xiong Y, Chang M, Shi ZW, Li YY, An SY, Ren DF. Encapsulation of rose anthocyanins with β-cyclodextrin for enhanced stability: Preparation, characterization, and its application in rose juice. Food Biosci. 2025 Feb;64:105875. doi: 10.1016/j.fbio.2025.105875.
23. González De Llano D, Moreno-Arribas MV, Bartolomé B. Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. Molecules. 2020 Aug 1;25(15):3523. doi: 10.1007/s00289-020-03292-3.
24. Chung C, Rojanasasithara T, Mutilangi W, McClements DJ. Stability improvement of natural food colors: Impact of amino acid and peptide addition on anthocyanin stability in model beverages. Food Chem. 2017 Mar;218:277–84. doi: 10.1016/j.foodchem.2016.09.087.
25. Fourati Z, Sauguet L, Delarue M. Structural evidence for the binding of monocarboxylates and dicarboxylates at pharmacologically relevant extracellular sites of a pentameric ligand-gated ion channel. Acta Crystallogr Sect Struct Biol. 2020 Jul 1;76(7):668–75. doi: 10.1107/S205979832000772X.