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In vitro antioxidant activity of *Physalis peruviana* L. fruits

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Physalis peruviana L. (Cape gooseberry) is recognized as a valuable source of nutrients and antioxidants, and is extensively cultivated nowadays in many countries of the tropical and sub-tropical zone. To the best of our knowledge, there are no previous reports about the antioxidant capacity of *P. peruviana* fruits originating from Bulgaria. We hypothesized that the local environmental, variety, production and other factors would have an impact on the antioxidant properties, the polyphenol and flavonoids content of the fruits. Thus, the aim of the current study was to evaluate the antioxidant activity of different extracts from two genotypes of locally produced *P. peruviana* fruits (BF and BP) and to compare it with that of the imported fruit available on the market (CM). The extracts with acetone (AEPP), water (WEPP), 95% ethanol (EEPP), and 50% methanol (MEPP) were obtained from the whole berries and the pulp fraction. The total phenolic content was the highest in EEPP and AEPP from the pulp fraction, varying from 23.98 to 30.60 GAE/100 g FW (EEPP) and from 14.99 to 26.06 GAE/100 g FW (AEPP). Similarly, EEPP and AEPP from the pulp fraction were with a high total flavonoids content. There was an origin-related differentiation; the fruits from Colombia (CM) were the richest in phenolics and flavonoids, followed by the fruits of Bulgarian origin (BF and BP). All extracts demonstrated antioxidant activity, which generally was well expressed in the EEPP from fruit pulp. DPPH activity was the highest in CM (176.99 mM TE/100 g FW). The same tendency was observed in other assays. The maximal antioxidant activity values were: ABTS - 384.20 mM TE/100 g FW (CM), FRAP - 170.94 mM TE/100 g FW (BP), and CUPRAC - 588.36 mM TE/100 g FW (CM). The results showed positive linear correlations between antioxidant activities and total phenolic and flavonoids content. According to this study, Cape gooseberry from Bulgaria possesses radical scavenging and metal chelating properties that are not inferior to those of the varieties produced worldwide.

Key words: *Physalis peruviana* L., ABTS, FRAP, CUPRAC, DPPH, antioxidant activity, flavonoids, polyphenols.

INTRODUCTION

Physalis peruviana L. is the commercially most important fruit among the over 100 species of the genus *Physalis* (family Solanaceae) [1, 2]. The plant originates from the Andean region (Peruvian and Ecuadorian Andes), spreading throughout South America in pre-Incan and Incan times. Nowadays, its cultivation extends to many countries of the tropics and sub-tropics, Central and South Europe, the United States, and Asia [3]. *P. peruviana*, also known as Cape gooseberry, Inca berry, golden berry or Peruvian ground cherry, is an herbaceous, semi-shrub plant, annual in the temperate zones and perennial in the tropics and sub-tropics, well-adapted to different altitudes, soils and climatic conditions [4]. As in all *Physalis* species, the fruit is a berry completely covered by an inflated balloon or lantern-like protective calyx (husk), formed by the strongly grown sepals. The berries are small, with a diameter between 1.25 and 2.50 cm and weigh between 4 and 10 g, oval, containing approx. 100 to 300 small seeds. The ripe erries are bright yellow to orange in color, shiny, with a tender and juicy texture, rich in flavor (sweet and sour, with a hint of citrus). The berries are consumed mostly fresh, but a substantial part of the annual production is also dehydrated or processed into jams, jellies, juices, dressings and other products [1, 5-7]. The largest producer and exporter of fresh or dehydrated fruit is Colombia, followed by South Africa [2-4].

P. peruviana fruit has a long history of ethnomedical purposes worldwide, as an antimycobacterial, antileukemic, antipyretic and diuretic agent [2] and it was used in the treatment of cancer, hepatitis, asthma, malaria, dermatitis, rheumatism, hyperglycemia, fevers, and many other conditions [3, 8, 9]. The pursuit of functional foods has provoked intensive scientific research on Cape gooseberry fruit in the last two decades, revealing the presence of various classes of metabolites. The fruit contains vitamins [10-13], minerals [5, 6], polysaccharides [6, 10], protein [14], fatty acids and phytosterols [2, 3, 9], polyphenols [6, 7, 12-15], and many other functional nutrients [1, 11, 16-18]. The phytochemical composition of Cape gooseberry fruit outlines its antimicrobial, antiviral, antioxidant, anti-inflammatory, immunomodulatory hepato-protective, anti-diabetic, antitumor and other properties [3, 10, 19-21], making it valuable

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for the nutraceutical and pharmaceutical industries [1, 17, 18].

Many biochemical assays have been introduced to quantify the antioxidant activity (AOA) or antioxidant capacity (AOC) of food and other biological samples, but none of them is currently accepted as a single versatile assay, unanimously applied to different matrices [22]. In general, most of AOA assays are associated with single electron transfer (SET) or hydrogen atom transfer (HAT) reaction kinetics, although there is no distinct boundary between them [22-24].

Along with other exotic fruits, such as goji berry, Acai berry, Maqui berry, lychee or pitaya, Cape gooseberry is recently being promoted as one of the "superfruits" [16]. Cape gooseberry fruit is supported by numerous data for the high levels of antioxidants such as phenolic acids, flavonoids, flavanols, proanthocyanidins, coumarins, tannins, carotenoids, and anthocyanins, and the evidence for its medicinal benefits. Total polyphonic content in plant samples of the same species is affected by factors such as environmental conditions, phenological stage, genotype, etc., but the qualitative polyphenol profiles are more stable and species-specific [7, 13, 25, 26]. Although *P. peruviana* and some of the other cultivated *Physalis* species (*P. philadelphica* Lam., *P. ixocarpa* Brot., *P. pubescens* L.) are gaining popularity worldwide, Cape gooseberry remains considerably unknown in Bulgaria. A brief overview of Cape gooseberry status in Bulgaria reveals that fresh fruit imported from Colombia is occasionally available in some of the biggest supermarkets, while packed dry berries (or mixes with other dry fruits) are distributed by a number of bio food suppliers, often under the label of "exotic superfruits". The most popular use of fresh fruit remains that of an exotic decoration to the various dishes and desserts in gourmet restaurants. There is fragmented public information about some recent endeavors in Cape gooseberry cultivation of a few organic farms in Bulgaria. However, practically no data are available about yields, quality or market success. In the period between 1996 and 2001 the only Bulgarian variety of *P. peruviana* named "Plovdiv" has been selected at the Department of Horticulture at the Agricultural University of Plovdiv, and in 2006 it has been registered in the Official Variety List by the Executive Agency for Variety Testing, Field Inspection and Seed Control [27]. The ripe fruits of the local variety are described as having a typical strawberry-vanilla flavor and a pleasant, sweet to slightly sour taste. They contained 35.45 mg% vitamin C, 10.72% total sugar, 1.03% pectin, 1.03% total acids, and 0.51% flavonoids [27]. Several studies afterwards reported data on the fruit yield, fruit post-harvest ripening dynamics, the possibilities of extended market supply with locally-produced fruit, and on other details of the experimental production of "Plovdiv" variety in Bulgaria [28-34].

To the best of our knowledge, this is the first report about the antioxidant capacity of *P. peruviana* fruits originating from Bulgaria. We hypothesized that local environmental, variety, production and other factors would have an impact on the antioxidant properties, the polyphenol and flavonoid content of the fruit, and that there would be some variation of the data available for fruit of other origin. Therefore, the aim of this study was to evaluate the antioxidant activity of different extracts from locally produced *P. peruviana* fruits and to compare them with that of the imported Colombian fruit available on the Bulgarian market.

EXPERIMENTAL

Plant material

The ripe fruits of Cape gooseberry (*P. peruviana* L.) from three different origins were studied. Two of them represented Cape gooseberry genotypes grown under the environmental conditions in Bulgaria. The first of these genotypes was the only locally selected variety named "Plovdiv" (BP), and was produced in the experimental fields of the Agricultural University, located in Plovdiv, Central Southern Bulgaria. The second sample (BF) consisted of fruit of an introduced Cape gooseberry variety, produced and purchased from a certified organic farm (Versol Bio-farm, Lik village, municipality of Mezdra, North-West Bulgaria). These were compared to the fruits imported from Colombia (produced by C.I. FRUTIREYES S.A.S., Bogotá DC, Colombia; imported by KM Delivery EOOD), purchased from a local supermarket (CM). The fruit calyces were removed. A portion of the berries was further processed to obtain fruit pulp (without the seeds), which was analyzed individually in order to examine the influence of the different parts of the berry. The fruit samples were kept in a refrigerator at a temperature of –18°C until analysis.

Methods

Extraction procedure: The extracts with four different polarity solvents: acetone (AEPP), water (WEPP), 95% ethanol (EEPP), and 50% methanol (MEPP), were obtained from whole berries and the pulp fraction of *P. peruviana*. The extraction procedure was performed in an ultrasonic bath

(SIEL, Gabrovo, Bulgaria), operating at a frequency of 35 kHz and power of 300 W for 20 min, at 75 °C and a solid-to-solvent ratio of 1:10 (w/v). The obtained extracts were filtered, and the extraction was performed in duplicate. The combined extracts were used for further analyses.

Total phenolic compounds: Total phenolic content (TPC) was measured by a slight modification of the Folin-Ciocalteu method. One ml of Folin-Ciocalteu reagent (diluted five times) was mixed with 0.2 ml of the extracts and then 0.8 ml of 7.5% Na_2CO_3 were added. After a reaction time of 20 min at room temperature (20±2°C) the absorbance of the solution was read at 765 nm against the blank. The results were expressed as milligram equivalents of gallic acid (GAE) per gram fresh weight (FW) [35].

Total flavonoids: The total flavonoids (TF) content was analyzed according to the spectrophotometric method with 10% $Al(NO_3)_3$ reagent previously described [36]. The absorbance of the reaction mixture was measured after 40 min at 415 nm against the blank. The results were presented as milligram equivalents of quercetin (QE) per gram fresh weight (FW) [35].

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: A portion of the extracts (0.15 ml) was mixed with 2.85 ml of freshly prepared 0.1 M solution of DPPH in methanol. The sample was incubated for 15 min at 37°C in the dark. The reduction of absorbance was measured at 517 nm, in a parallel to the blank containing methanol [35].

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay: The ABTS radical was generated by mixing aliquot parts of 7.0 mM ABTS in the distilled water and 2.45 mM $K_2S_2O_8$ (Merck) in double-distilled water. The reaction was performed for 16 h at room temperature (20±2°C) in the dark. Before analysis, 2.0 ml of the stock solution with the generated ABTS radicals was diluted with methanol at a proportion of 1:30 (v/v), in order to adjust the absorbance of the working solution to 1.0 ÷ 1.1 at 734 nm. Working solution (2.85 ml) was mixed with 0.15 ml of plant extracts. After incubation for 15 min at 37°C in the dark the absorbance was measured at 734 nm against methanol [35].

Ferric reducing antioxidant power (FRAP) assay: The assay was performed according to [37] with a slight modification. The FRAP reagent was freshly prepared by mixing 10 parts of 0.3 M acetate buffer (pH 3.6), 1 part of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (Fluka) in 40 mM HCl (Merck) and 1 part of 20 mM $FeCl_3.6H_2O$ (Merck) in double-distilled water. FRAP reagent (3.0 ml) was mixed with 0.1 ml of the investigated extracts. After 10 min at 37 °C in darkness the absorbance was measured at 593 nm relative to a reagent blank prepared with solvent instead of an extract.

Cupric reducing antioxidant capacity (CUPRAC) assay: The reaction mixture contained 0.1 ml of the analyzed extract mixed with 1 ml of $CuCl_2.2H_2O$, 1 ml of Neocuproine (7.5 ml in methanol), 1 ml of 0.1 M ammonium acetate buffer and 1 ml of distilled water. The solution was incubated at 50°C for 20 min in darkness and the absorbance was measured at 450 nm [38].

All assays for measuring the antioxidant activity of the extracts were performed in triplicate and the results (mean ± SD) were expressed as mM Trolox equivalents (mM TE) per 100 g by fresh weight.

RESULTS AND DISCUSSION

Data about the total phenolic content and total flavonoids content in the analyzed extracts of Cape gooseberry fruit are presented in Table 1. As hypothesized, the results showed certain trends of variation of the respective chemical indices, depending on the solvent, the genotype and the fraction of Cape gooseberry fruit.

The highest total phenolic content was in the ethanol and acetone extracts, regardless of fruit genotype or berry fraction (whole berries or pulp). Significantly less phenolic compounds were extracted with 50% methanol and water. These results were obviously determined by the extracting potential of the solvent with regard to total phenolic substances. The results were in compliance with the previous findings [39], stating that the yields of polyphenols are strongly influenced by the solvent (the percentage of the organic solvent in the extraction mixture). A partial exception was BP genotype, producing MEPP with higher phenolic content than the EEPP or AEPP - 10.34 GAE/100 g FW and 15.87 GAE/100 g FW, respectively, for MEPP from the whole berries and the pulp fraction. In all of the studied fruit genotypes the total phenolic content was higher in the extracts obtained from the pulp fraction, compared to the respective value in the intact berries. The total phenolic content in this category of extracts varied in the range from 23.98 to 30.60 GAE/100 g FW (EEPP) and from 14.99 to 26.06 GAE/100 g FW (AEPP). The differences were obviously connected to the nature of the studied plant matrices. The isolation of seeds to obtain the pulp fractions resulted in an increase of the total phenolic content relative to the fresh weight of the sample.

Table 1. Total phenolic content and total flavonoid content in extracts from different *P. peruviana* L. fruit

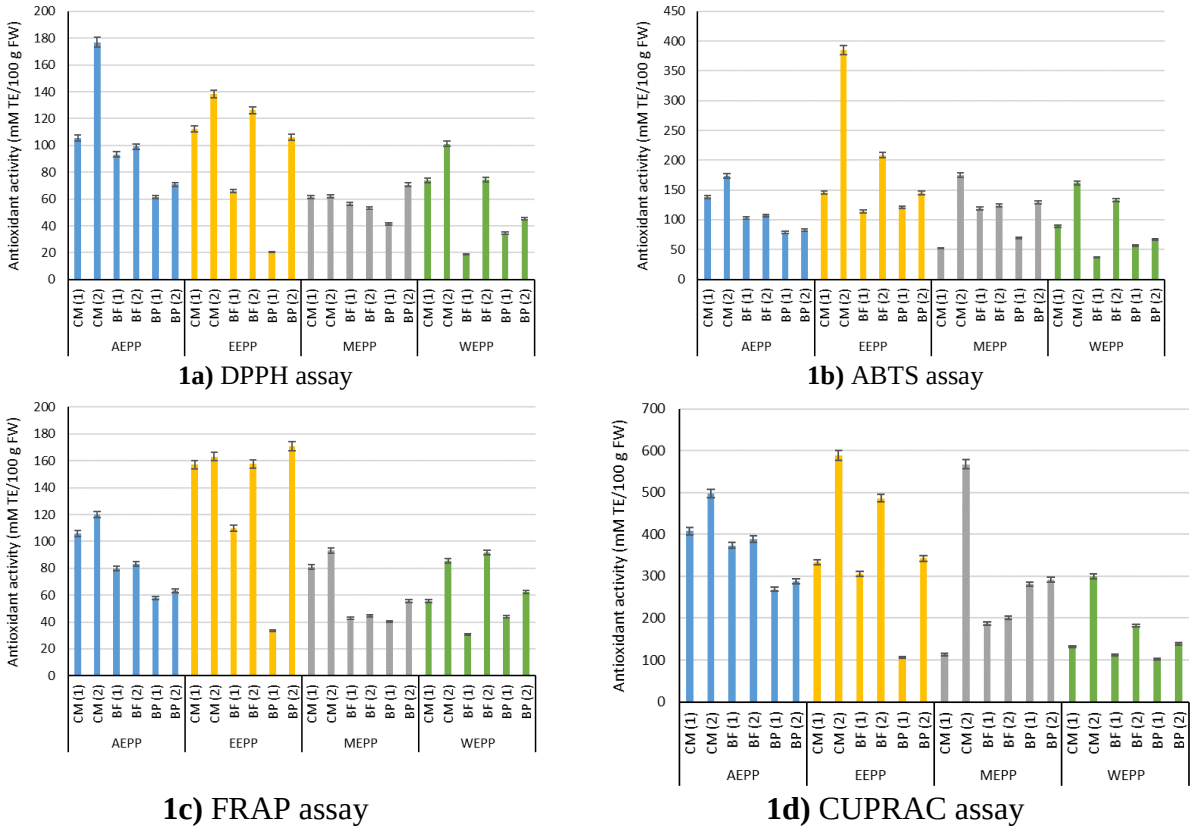
Fruit sample	Extract	Total phenolic content (mg GAE/100 g FW)	Total flavonoids (mg QE/100 g FW)
CM (1) [a]	Acetone	22.59±0.31	17.49±1.31
	95% Ethanol	22.29±0.55	19.63±0.54
	50% Methanol	15.14±0.85	0.73±0.12
	Water	13.76±0.23	nd[d]
CM (2) [a]	Acetone	26.08±0.21	17.88±0.21
	95% Ethanol	25.48±0.45	28.06±0.34
	50% Methanol	16.50±0.32	1.58±0.54
	Water	16.16±1.11	nd
BF (1) [b]	Acetone	18.67±0.85	17.35±0.43
	95% Ethanol	18.15±0.34	10.98±0.11
	50% Methanol	5.15±0.12	1.51±0.30
	Water	3.82±0.81	nd
BF (2) [b]	Acetone	23.06±0.80	18.00±0.13
	95% Ethanol	30.60±0.34	11.08±0.31
	50% Methanol	12.97±0.12	1.22±0.43
	Water	10.16±0.65	nd
BP (1) [c]	Acetone	14.53±0.11	9.48±0.30
	95% Ethanol	5.61±0.12	4.65±0.21
	50% Methanol	10.34±0.81	0.80±0.11
	Water	6.81±0.11	nd
BP (2)[c]	Acetone	14.99±0.21	12.87±0.19
	95% Ethanol	23.98±0.22	4.58±0.28
	50% Methanol	15.87±0.43	1.11±0.35
	Water	13.28±0.54	nd

[a] CM – origin Colombia, as supplied from the market; [b] BF – origin Bulgaria, from Bio-farm "Versol", Lik village; [c] BP – origin Bulgaria, variety "Plovdiv", from the Agricultural University, Plovdiv; [d] nd – not detected; (1) whole berries; (2) berries without seeds

Similarly, the total flavonoids content in the pulp fraction was relatively higher – between 4.58 and 28.08 mg QE/100 g FW (EEPP) and between 12.87 and 18.00 mg QE/100 g FW (AEPP). None of the WEPP extracts were found to contain flavonoids, while MEPP were with very low total flavonoids values (between 0.73 and 1.58 mg QE/100 g FW). As these results suggest, there was a more significant variation between the extracts with regard to their flavonoid content compared to that of total phenolic content.

These results revealed that Cape gooseberry fruits were rich in the phenolic compounds, which are a group of bioactive agents with expressed antioxidant activity. As suggested, there were some interesting origin-related differences, both in total phenolic and in total flavonoid content. In general, the imported Colombian fruits (CM) were the richest in phenolics and flavonoids, followed by the fruits of local origin, the farm (BF) and variety "Plovdiv" (BP) genotypes, although some variation also existed, for example in BF pulp (AEPP and EEPP) and BP pulp (EEPP) extracts. These results were probably due to the effect of genetic, environmental, ripeness stage and other factors on the accumulation of phenolic metabolites and their individual composition. The data suggest that fruit origin and genotype is a significant aspect that has to be considered when the biologically active or beneficial properties of Cape gooseberry are discussed, and provide fields for future studies on the chemical composition of the fruit from the different origins and on the respective influencing factors [40].

The observations in the present study were supported by previous findings, although a direct comparison of data was not always possible, due to the variation of the applied solvents, the extraction procedures and analytical methods used in different studies. The values of total phenolic content were very close to those reported by [40-47] (24.91-77.42 mg GAE/100 g), and those of the total flavonoid content – to the values of other authors [15].



1a) DPPH assay **1b)** ABTS assay

1c) FRAP assay **1d)** CUPRAC assay

Fig. 1. The antioxidant activity of acetone (AEPP), ethanol (EEPP), methanol (MEPP) and water (WEPP) extracts from *P. peruviana* L. fruit: CM – origin Colombia, from the market; BF – origin Bulgaria, Bio-farm; BP – origin Bulgaria, variety "Plovdiv"; (1) whole berries; (2) berry pulp.

All previous studies, as well as the current study, unanimously revealed that Cape gooseberry fruit is a rich source of polyphenols and other biologically active constituents determining the nutritional quality.

The results of the antioxidant activity assays for Cape gooseberry fruit of different origin are presented in Figure 1. All extracts demonstrated antioxidant activity which was generally well expressed in the EEPP from fruit pulp. In these extracts, the DPPH radical scavenging activity was the highest in CM (176.99 mM TE/100 g FW), followed by BF (126.38 mM TE/100 g FW) and BP (106.11 mM TE/100 g FW). The same tendency in the antioxidant potential was observed in other assays. ABTS activity values were 384.20 mM TE/100 g FW for CM, 208.38 mM TE/100 g FW for BF and 145.20 mM TE/100 g FW for BP, respectively. The maximal FRAP values were 163.12 mM TE/100 g FW in CM, 157.58 mM TE/100 g FW in BF and 170.94 mM TE/100 g FW in BP. CUPRAC results were in the ranges from 588.36 mM TE/100 g FW (CM) to 486.60 (BF) and 342.76 mM TE/100 g FW (BP). These results were in accordance with previous findings about Cape gooseberry antioxidant activity determined by different assays [7, 10, 13, 42-44, 46-50]. Although the antioxidant activity of the extracts from locally produced fruit was generally weaker than that of the imported Colombian fruit, the parallel to the available data from the studies cited above revealed that both BF and BP were in no way inferior to those of the varieties produced worldwide. Our results reflected correspondingly the known differences between the respective SET antioxidant activity assays, as well as the correlations between them [51]. Several studies reported that Cape gooseberry antioxidant activity was lower than that of cranberries, blueberries and other small fruit, but higher or close compared with that of apples, pears, cherries, plums, red grape, pitaya, etc. [23, 45, 47, 51]. Our results were in accordance with these studies, as well. One very important aspect in the interpretation of results about the antioxidant properties of plant extracts is their intended use. Their applicability depends on the safety of the solvent [39]. In this context the high values of the antioxidant activity of the ethanol extracts in this study, as well as the radical scavenging and metal chelating activity demonstrated by the water extracts can be assumed as promising results, as these extracts are fully applicable in food, beverage or cosmetics production [47, 52].

The correlations between the values of DPPH, FRAP, ABTS and CUPRAC activity and total phenolic contents were also evaluated (Table 2).

Table 2. Correlation coefficient (r^2) between total phenolic content, total flavonoids and the antioxidant activities (DPPH, ABTS, FRAP and CUPRAC) of *P. peruviana* fruit

Index	DPPH	ABTS	FRAP	CUPRAC
Total phenols	0.8811	0.6006	0.8566	0.8000
Total flavonoids	0.7240	0.5515	0.5690	0.5904

Positive linear correlations between the total antioxidant activities, total phenolic contents and the total flavonoids content were found. Therefore, according to the current study, the phenolic and flavonoid compounds of Cape gooseberry fruit have antioxidant, radical scavenging and metal chelating properties. The correlations between the total antioxidant activities and the total phenolic content were better defined (coefficient of correlation r^2=0.88 and 0.86 for DPPH and FRAP values, respectively), than for the total flavonoid content, suggesting that mainly total phenols in Cape gooseberry provided antioxidant activity. Several studies have indicated a positive correlation between phenolic contents and the antioxidant power of plant extracts. Our results were in good agreement with the findings by [23] about the strong positive correlation of the antioxidant capacity of the different fruits, vegetables and beverages with total phenolic content (r^2=0.946 for ABTS and 0.897 for DPPH, respectively) and the moderate correlation with total flavonoid content (0.718 for ABTS, 0.708 for DPPH, respectively). Similar results on total phenolics basis were obtained by [51] (r^2=0.7569 for DPPH, 0.8447 for FRAP and 0.8025 for ABTS, respectively), [45] (r^2=0.9871 for DPPH), [47] (r^2 values between 0.87 and 0.78 for DPPH, FRAP, ABTS and CUPRAC) and others [53]. It should be considered that the antioxidant power depends not only on the overall quantity of these classes of phytochemicals, but also on their individual composition and proportions, and moreover, on the synergistic effect of other antioxidants of different chemical nature existing in the extracts [7, 10, 13, 18, 25, 46, 47, 50, 52]. Therefore, future studies on the phenolic profiles and on other phytochemicals with antioxidant properties would be relevant in order to characterize in more detail the antioxidant potential of Cape gooseberry genotypes from Bulgaria.

CONCLUSIONS

To the best of our knowledge, this study presented for the first time results about the antioxidant activity (determined by DPPH, ABTS, FRAP, and CUPRAC assays) of *P. peruviana* fruit originating from Bulgaria (in comparison with imported fruit of Colombian origin), and about the total phenolics and total flavonoids content in different extracts obtained from them. The Cape gooseberry fruits were a rich source of total phenols and flavonoids, with the highest concentration of the bioactive compounds achieved in the ethanol extracts of fruit pulp fraction (23.98-30.60 GAE/100 g FW and 4.58-28.08 mg QE/100 g FW, respectively for the total phenolic content and total flavonoid content). All extracts demonstrated antioxidant activity (DPPH, FRAP, ABTS and CUPRAC), which was generally well expressed in the EEPP from fruit pulp. Although the antioxidant activity of the extracts of the locally produced fruit was generally weaker than that of the imported Colombian fruit, the study revealed that the two local genotypes were in no way inferior to those of the varieties produced worldwide. There were positive linear correlations between total antioxidant activities, total phenolic contents and the total flavonoids, therefore, according to the current study, phenolic and flavonoid compounds of Cape gooseberry fruit have antioxidant, radical scavenging and metal-chelating properties. The results of the study make relevant a further investigation on Cape gooseberry fruit originating from Bulgaria, aimed at a more detailed characterization of their composition, health benefits and potential for use.

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