

DETERMINATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN SELECTED CUCURBITACEAE FRUITS

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Abstract

This study investigates the use of the photochemiluminescence (PCL) assay in two different systems - hydrophilic (ACW) and lipophilic (ACL) - along with the DPPH method to evaluate the antioxidant potential of three fruits from the Cucurbitaceae family: watermelon, yellow watermelon, and melon. The results showed that the analyzed fruits contain phenolic compounds ranging from 7.40 to 15.43 mg of Gallic Acid Equivalent (GAE) per 100 g of fresh weight (FW). Regarding flavonoid content, concentrations were approximately 1 mg Rutin Equivalent (RE) per 100 g FW, while anthocyanin content was below 1 mg cyanidin-3-glucoside equivalent (CGE) per 100 g FW. Notably, melon exhibited the highest antioxidant capacity among the fruits in both ACW and ACL systems, followed by watermelon. A strong positive correlation was found between antioxidant capacity assessed with the ACL method and the DPPH assay, with a correlation coefficient (r) of 0.9932. Furthermore, a significant correlation was also observed between the ACW system and the DPPH assay, with a correlation coefficient of 0.9752.

Key words: watermelon, yellow watermelon, melon, phenolics, antioxidant capacity.

INTRODUCTION

Recent statistical studies indicate that chronic disorders are a predominant health challenge in the modern world, accounting for over 40 million deaths annually. Notably, 74% of global deaths result from cardiovascular diseases (CVD), cancer, chronic respiratory diseases, and diabetes-related kidney disease. Chronic inflammation, closely linked to oxidative stress and thrombo-inflammatory processes, plays a crucial role in the progression of these conditions (Lu et al., 2022; Manikandan et al., 2023). However, adopting healthier dietary habits has been shown to exert significant anti-inflammatory effects, contributing to the prevention of chronic diseases and an overall improvement in quality of life (Tsoupras, Lordan, & Zabetakis, 2018; Zabetakis, Lordan, & Tsoupras, 2019).

Fruits and vegetables are essential components of a healthy diet, as they provide a diverse range of micronutrients with antioxidant, anti-inflammatory, and antithrombotic properties (Andualem, 2023; Tahir et al., 2023; Zhang et al., 2023). Regular consumption of a variety of fruits has been associated with a reduced risk of

several diseases, particularly cancer and cardiovascular disorders, due to their bioactive potential (Karasawa & Mohan, 2018).

The Cucurbitaceae family is the second-largest group of fruit and vegetable plants, ranking just behind Solanaceae in global importance (Schaefer and Renner, 2011; Guo et al., 2020). It comprises approximately 115 genera and 960 species, predominantly herbaceous annual vines or perennial lianas, often equipped with tendrils (Schaefer, Heibl, and Renner, 2009).

The Cucurbitaceae family includes several economically significant species, such as cucumber, melon, watermelon, calabash, squash, and pumpkin. In 2023, the global production of melon and watermelon reached approximately 105 million tons, being cultivated across 4.2 million hectares of land. (<http://faostat.fao.org>). China, India, and Turkey are the leading producers of melon and watermelon.

In 2023, Romania produced a total of 179,100 metric tons of watermelon and yellow melon, cultivated over an area of 10,200 hectares. The average yield was 17,482 kg/ha (INSSE, 2023). The watermelon and yellow production was divided across macro-regions, development

regions, and counties as follows: macro-region 1 (North-West, Center), macro-region 2 (North-East, South-East), macro-region 3 (Bucharest-Ilfov, South Muntenia), and macro-region 4 (South-West Oltenia, West). Accordingly, the production of watermelon and yellow melon was 4,980 tons in macro-region 1, 66,196 tons in macro-region 2, 41,279 tons in macro-region 3, and 66,652 tons in macro-region 4 (INSSE, 2023) (Figure 1).

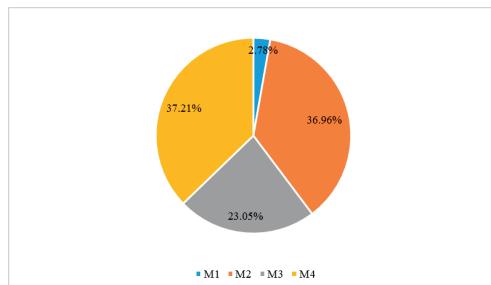


Figure 1. Production of green and yellow watermelons in 2023, by macroregions

Based on the development region and county, the counties with the highest annual production in 2023 are Bihor, Alba, Bacău, Brăila, Argeș, Dolj, and Arad.

Cucurbitaceae fruits are rich in carotenoids and phenolic compounds, known for their potent antioxidant properties (Hafeez, 2024). As a result, they represent a significant source of bioactive phytochemicals. Furthermore, research has shown that different components of fruits within this family display varying degrees of antioxidant activity (Kubola and Siriamornpun, 2011).

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (+)-rutin, gallic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Sigma Chemical Co. (Switzerland). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany). All chemicals used were of analytical grade. Standard solutions were prepared with distilled deionized water.

Plant material

The selected Romanian fruit samples (watermelon, yellow watermelon, and melon) were

obtained from a local market in Bucharest, Romania. The fruits were purchased one day before the experiment and stored at 4 °C in a refrigerator until analysis. Prior to processing, the samples were washed with tap water, and any damaged parts were removed. The edible portions (20-30 g of unpeeled fruit) were then homogenized using a laboratory mixer.

Methods

Extraction procedure

A 3.0 g portion of fruit was weighed and mixed with 30 mL of 50% aqueous methanol. The mixtures were subjected to vortex mixing at 2,000 rpm for one hour using a Heidolph Instruments Multi Reax vortex. Following this, the extracts were centrifuged at 10,000 rpm for 10 minutes at 4 °C (Mulțescu and Susman, 2024). The obtained supernatant was then stored at -20 °C until further analysis

Determination of total phenolic content (TPC)

The determination of TPC was performed using the Folin-Ciocalteu assay with minor modification (Mulțescu and Susman, 2024). Briefly, 1 mL of the extract was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of a 20% sodium carbonate solution. The mixture was incubated in the dark for 20 minutes, after which the absorbance was measured at 752 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Bremen, Germany). A standard curve was created using different concentrations (10 to 50 µg/mL) of Gallic acid, prepared under the same conditions as the samples ($R^2=0.9990$). The total phenolic content was calculated as mg of Gallic acid equivalent per gram of fresh weight (mg GAE/g f.w.).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the aluminum chloride ($AlCl_3$) method (Mulțescu and Susman, 2024). In short, 0.1 mL of extract was combined with 0.1 mL of 10% sodium acetate and 0.12 mL of 2.5% $AlCl_3$, with the final volume adjusted to 1 mL using 70% ethanol. The mixture was vortexed and then incubated in the dark for 45 minutes. Absorbance was recorded at 510 nm. A standard curve was generated using rutin at concentrations ranging from 10 to 60 µg/mL

($R^2=0.9996$). The total flavonoid content was expressed as mg of rutin equivalent per 100 g (mg RE/100 g f.w.).

Determination of Total Anthocyanin Content (TAC)

The total anthocyanin content (TAC) was assessed using the pH differential method, which takes advantage of the changes in spectral absorbance of anthocyanin-containing samples under different pH conditions, following the AOAC (2005) protocol with minor modifications. In brief, 1 mL of extract was mixed with 4 mL of a pH 1.0 buffer solution (0.025M potassium chloride), while another 1 mL was combined with 4 mL of a pH 4.5 buffer solution (0.4M sodium acetate). The absorbance of these solutions was recorded at 520 nm and 700 nm using a Specord 210 UV-VIS spectrophotometer (Analytik Jena, Germany). TAC was determined using the formula outlined in the AOAC (2005) method, with results expressed as mg cyanidin-3-glucoside per 100 g of fresh weight (mg C3G/100 g f.w.).

$$\frac{AxMWxDFx10^3}{ex1}$$

Where: A = (A₅₂₀ nm-A₇₀₀ nm) pH 1.0 – (A₅₂₀ nm-A₇₀₀ nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (C3G); DF = dilution factor; 1 = pathlength in cm; ϵ = 26 900 molar extinction coefficient, in L x mol⁻¹ x cm⁻¹, for C3G; and 10³ = conversion factor (g to mg).

Determination of Antioxidant Activity through DPPH

The DPPH radical scavenging activity was evaluated by measuring the reduction of the DPPH radical, following the method of Culetu et al. (2016) with slight modifications. The reaction mixture included 1 mL of methanolic extract and 6 mL of DPPH radical solution, which was incubated in the dark for 20 minutes. Absorbance was recorded at 517 nm using a Specord 210 UV-VIS spectrophotometer (Analytik Jena, Bremen, Germany). Antioxidant activity was quantified using a calibration curve (0.0156-0.0625 μ g/mL) prepared with Trolox as a reference standard ($R^2=0.9998$). The results

were expressed as μ mol of Trolox equivalent per 100 g (μ mol TE/100 g f.w.).

Photochemiluminescence Assay – hydrophilic system (PCL-ACW)

The reactions were carried out using specialized kits for assessing the antioxidant capacity of water-soluble compounds (Analytik Jena, Jena, Germany). The reaction mixture contained 1500 μ L of water (reagent 1), 1000 μ L of buffer solution (reagent 2), 25 μ L of luminol (reagent 3), and 10 μ L of extract. Measurements were taken using a Photochem device equipped with PCL Soft software (Analytik Jena). A calibration curve was generated using ascorbic acid as a standard, and the results were expressed as μ mol of ascorbic acid per 100 g (μ mol AA/100 g f.w.).

Photochemiluminescence Assay - lipophilic system (PCL-ACL)

The reactions were carried out using specialized kits for assessing the antioxidant capacity of water-soluble compounds (Analytik Jena, Jena, Germany). The reaction mixture contained 2300 μ L of methanol (reagent 1), 200 μ L of buffer solution (reagent 2), 25 μ L of luminol (reagent 3), and 10 μ L of extract. Measurements were taken using a Photochem device equipped with PCL Soft software (Analytik Jena). A calibration curve was generated using Trolox as a standard, and the results were expressed as μ mol of Trolox per 100 g (μ mol Trolox/100 g f.w.).

Statistical analysis

All methods were applied for samples characterisation in at least two repetitions. Results are expressed as mean values \pm standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's test to evaluate differences between means (Minitab software, Minitab Inc., Coventry, UK). A significance level of $p < 0.05$ was considered statistically significant. The principal component analysis (PCA) was also carried out using Minitab considering as variables TPC, TFC, TAC and DPPH; the type of matrix was correlation.

RESULTS AND DISCUSSIONS

Bioactive compounds content

In Table 1. the TPC, TFC and TAC of the selected Cucurbit fruits are presented. The TPC ranged from 7.40 to 15.43 mg GAE/100 g f.w. Yellow melon had the highest phenolic content of 15.43 mg GAE/100 g f.w., whereas yellow watermelon showed the lowest TPC value of 7.40 mg GAE/100 g f.w. The concentration of polyphenols was significantly different ($p < 0.05$) among the fruit samples. Choudhary et al. (2014) determined the phenolic content among the selected genotypes of watermelon from India. The results indicated that total phenol content varied significantly among the tested genotypes, ranging from 16.77 to 21.41 mg/g DW. A substantial variation in total phenolic content has also been reported in watermelon fruits, ranging from 13.05 to 18.08 mg gallic acid equivalent per 100 g fresh weight (Nagal et al., 2012). Singh et al. (2016) analysed TPC in watermelon pulp using various solvent systems: methanol, ethanol and acetone at three different concentrations in distilled water (50, 70, and 100%) and 100% distilled water. The results ranged between 30.45 mg GAE/100 g in 100% distilled water and 48.63 mg GAE/100 g in acetone: water 50/50%. For methanol:water 70/30% the phenolic content was 35.54 mg GAE/100g. In a study, Tili et al. (2011) reported that the TPC in six watermelon cultivars ranged between 89.0 mg GAE/kg fw and 147.3 mg GAE/kg fw. Furthermore, Brat et al. (2006) reported a moderate phenolic content of 116 mg GAE/kg f.w. in watermelon obtained from French national markets. Significantly higher values, ranging from 870 to 910 mg GAE/kg f.w., were observed in red-fleshed watermelon (Perkins-Veazie, 2002). The phenolic content in cantaloupe flesh was 1.68 mg GAE/g (Ismail et al., 2010). The TPC of six melon varieties was in the range of 41.36-315.96 mg GAE/100 g d.w (Wang et al., 2023).

Variations in phenolic values observed in this study compared to previous research may be influenced by differences in cultivar origin, environmental conditions, and fruit maturity levels (Hegedus et al., 2010). Likewise, previous studies have shown that factors such as sunlight exposure, soil composition, seasonal changes, agronomic practices (Joshi et al., 1991), and the

choice of analytical method can lead to discrepancies in phenolic compound levels (Hegedus et al., 2010; Leccese et al., 2011).

Table 1. Total phenolic, flavonoid and anthocyanins content in selected fruits

Sample	TPC	TFC	TAC
Watermelon	8.49±0.25 ^b	1.22±0.07 ^b	0.89±0.02 ^a
Yellow watermelon	7.40±0.03 ^c	1.35±0.02 ^{a,b}	0.76±0.01 ^b
Yellow melon	15.43±0.16 ^a	1.43±0.02 ^a	0.16±0.01 ^c

TPC: mg GAE/100 g FW

TFC: mg RE/100 g FW

TAC: mg C3G/100 g FW

The values are expressed as means ± standard deviations (n = 2). Values followed by different letters in the same column are significantly different ($p < 0.05$)

The flavonoid content in *Cucurbitaceae* fruits ranged from 1.22 mg RE/100 g f.w. to 1.43 mg RE/100 g f.w. Data in Table 1. showed that yellow melon had the highest content of TFC, 1.43 mg RE/100 g f.w., followed by yellow watermelon, 1.35 mg RE/100 g f.w. The concentration of flavonoids was significantly different ($p < 0.05$) among the studied fruits, except yellow watermelon and yellow melon, and yellow watermelon and watermelon ($p > 0.05$).

The TFC in six varieties of watermelon were from 111.30 mg GAE/kg to 176.10 mg GAE/kg (Tili et al., 2011). The flavonoid content in red fleshed watermelon genotypes varied from 55.60 to 100.93 mg/100 g f.w. (Choudhary et al., 2015).

In a study conducted by Ismail et al. (2010), cantaloupe flesh showed a TFC of 2.03 µg RE/g. The results revealed considerable variability in total flavonoid content, influenced by both the specific cultivars and the distinct anatomical parts of the fruits.

The TACs in the analyzed Romanian fruits were between 0.16-0.89 mg C3G/100 g f.w. High levels of anthocyanins were measured in watermelon. In contrast, melon exhibited the lowest anthocyanin content, with a recorded value of 0.16 mg C3G/100 g f.w. The anthocyanin levels showed a statistically significant difference ($p < 0.05$).

Antioxidant activity of *Cucurbitaceae* fruits

As shown in Figure 2, the antioxidant activity of the selected fruits ranged from 55.98 to 273.76 µmol TE/100 g f.w. Yellow watermelon extract exhibited the highest activity at 273.76 µmol

TE/100 g f.w., whereas watermelon extract had the lowest DPPH scavenging activity. The DPPH values were significantly different ($p < 0.05$).

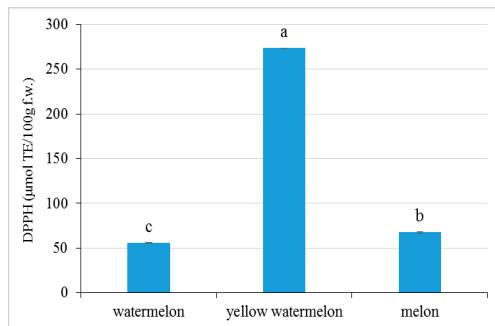


Figure 2. Antioxidant activity of 50% methanolic extracts from analyzed *Cucurbitaceae* fruits

A correlation was made between TPC, TFC, and TAC with DPPH method.,

The correlation coefficients (r) for these relationships were 0.8596, 0.1731, and 0.3086 respectively (Table 2).

Table 2. The correlation coefficients between TPC, TFC and TAC with antioxidant activity, DPPH

	TPC	TFC	TAC
DPPH	0.8596	0.1731	0.3086

The average antioxidant activity of different watermelon genotypes were 40.13 to 84.05 $\mu\text{mol TE/100 g f.w.}$ as determined by the CUPRAC assay (Choudhary et al., 2015).

Antioxidant Capacity of Water Soluble Compounds (ACW) and Lipid Soluble Compounds (ACL)

The results of ACW and ACL antioxidant capacity, determined by the PCL method, are presented in Table 3.

Table 3. Values of antioxidant capacity of the water soluble (ACW) and lipid soluble compounds (ACL)

Sample	ACW-PCL	ACL-PCL
Watermelon	48.72 \pm 0.04 ^b	38.78 \pm 0.06 ^b
Yellow watermelon	10.92 \pm 0.47 ^c	25.66 \pm 0.78 ^c
Melon	99.73 \pm 0.05 ^a	51.02 \pm 0.15 ^a

ACW: $\mu\text{mol TE/100 g f.w.}$

ACL: $\mu\text{mol AA/100 g f.w.}$

The values are expressed as means \pm standard deviations ($n = 2$). Values followed by different letters in the same column are significantly different ($p < 0.05$).

The ACW values ranged from 10.92 to 99.73 $\mu\text{mol AA per 100 g f.w.}$ As shown in Table 3, the melon sample exhibited the highest ACW value, reaching 99.73 $\mu\text{mol AA/100 g f.w.}$ Obtained data for the yellow watermelon extract was the lowest, 10.92 $\mu\text{mol AA/100 g f.w.}$ The lipid-soluble antioxidant capacity of *Cucurbitaceae* fruits ranged from 25.66 to 51.02 $\mu\text{mol TE/100 g f.w.}$ (Table 2). Among the analyzed fruits, melon exhibited the highest value at 51.02 $\mu\text{mol TE/100 g f.w.}$, whereas yellow watermelon had the lowest ACL value, measuring 25.66 $\mu\text{mol TE/100 g f.w.}$ Strong positive correlations were found between DPPH, ACW, and ACL (Table 4).

Table 4. The correlation coefficients between DPPH, ACW, and ACL

Method	DPPH	ACW-PCL	ACL-PCL
DPPH	1	0.9752	0.9931
ACW-PCL	-	1	0.9944
ACL-PCL	-	-	1

Principal Component Analysis

The aim of the principal component analysis (PCA) is to reduce a big number of variables to a few variables, referred to as principal components (PCs) (Granato et al., 2018). PCA was employed to explore similarities among the fruit samples in relation with the analyzed parameters (TPC, TFC, TAC, DPPH, ACL, and ACW); these parameters are called variables within the statistical software. PCA graph projected onto the first principal component (PC1)/second principal component (PC2) plane is presented in Figure 3.

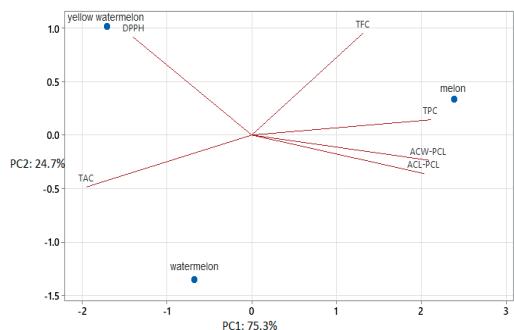


Figure 3. Principal component analysis

The PC1 and PC2 described 75.3% and 24.7% of variance, respectively, and the total

contribution rate of PC1 and PC2 was 100.0%. The plot indicates differences between all the analyzed samples (located in different sides of the graph). The analyzed parameters (TPC, ACW-PCL, and ACL-PCL) are positively correlated, being in close proximity and on the same side of the graph.

In a study investigating the properties of common Indian fruits and vegetables, PCA analysis revealed positive correlations between TPC, TFC, DPPH, and ABTS antioxidant activities. This emphasizes that phenolic compounds were the main contributors to the antioxidant properties of these fruits and vegetables (Singh et al., 2016). Likewise, in various Citrus species, PCA analysis revealed that the distinct flavonoids present in each species played a significant role in determining their antioxidant capacity (Chen et al., 2020).

CONCLUSIONS

In this study, we investigated the phenolic, flavonoid, and anthocyanin content, along with the antioxidant activity and antioxidant capacity in both water- and lipid-soluble systems, of three *Cucurbitaceae* fruits. Our analysis revealed that these fruits contain phenolics in moderate quantities while still exhibiting notable biological activity.

Antioxidant activity was assessed using the DPPH method, while the PCL method was employed to evaluate water-soluble and lipid-soluble compounds. The results demonstrated significant variations in antioxidant capacities among the studied *Cucurbitaceae* fruits. Notably, watermelon and melon exhibited the highest antioxidant capacity.

Moreover, differences in polyphenol content and antioxidant capacity were influenced by the species of the fruits. In conclusion, our findings highlight the *Cucurbitaceae* family's potential as a source of phenolic compounds with antioxidant properties.

Further research aims to evaluate the total antioxidant capacity of fruits commonly consumed in Romania and to establish a comprehensive database with the obtained results.

ACKNOWLEDGEMENTS

The authors acknowledge that this work was conducted in the context of the Core program (PN 19 02), supported by the Ministry of Research, Innovation, and Digitalization (project number 19 02 02 04).

The authors gratefully acknowledge the financial support of the METROFOOD-EVOLVE project (SMIS 309287).

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