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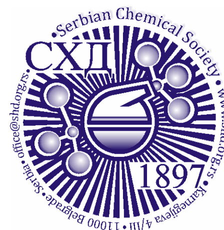

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Application of principal component and hierarchical cluster analysis to classify different edible *Cucurbita* based on *in vitro* antioxidant activity

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Abstract: This study investigated the variations in antioxidant profiles between nine edible *Cucurbita* species (pericarp and seed), using pattern recognition tools; classification was achieved based on the results of global antioxidant activity assays (DPPH, ABTS, FRAP, CUPRAC, Total reducing power, levels of total phenolics and flavonoids compounds). The pericarp samples showed significantly lower total phenol values than the seed samples. Spaghetti squash shows the highest ability to neutralize DPPH radicals among seed and pericarp extracts. This extract also shows the highest FRAP and CUPRAC values. The tested samples were grouped into pericarp extract groups and seed extract groups based on the principal component analysis of antioxidative profiles. Hierarchical cluster analysis confirmed the PCA analysis. These results can guide breeders in selecting *Cucurbita* varieties with enhanced antioxidant traits, contributing to the development of nutritionally superior crops.

Keywords: Cucurbita genus; total phenolic content; total flavonoid content; antioxidant activity; chemometric approach.

INTRODUCTION

Cucurbitaceae is a large family of plants, also known as cucurbits, with 130 genera and 800 species, mainly tropical or subtropical, with a few species extending into temperate climate.[1] The name of the *Cucurbitaceae* family came from Latin, where the word *corbis* means bottle or basket. *Cucurbitaceae* is the most diverse plant family and is cultivated worldwide in various environmental conditions. Humans utilize over 300 plant species, with 150 being extensively cultivated, and 30 of these are essential for global food production. The edible plants from this family that are frequently consumed for nutrition are mainly found

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in the genera *Cucurbita* (squash, pumpkin, zucchini), genus *Citrullus* (watermelon) and genus *Cucumis* (cucumber, various melons).[2] In the *Curcubitaceae* family, pumpkin seeds are the only ones not discarded as waste, while melon and watermelon seeds are mostly thrown away despite being abundant and edible. The *Cucurbita* genus comprises 14 species with six subspecies and two wild varieties.[3] The cultivated cucurbits are quite similar in their requirements for growth and development but their fruit morphology (sizes, shapes, colors, pulp structure) is highly variable. The original *Cucurbita* genus has been subject to intensive breeding that yielded the varieties, and their hundreds of hybrids, we know today.

The most popular vegetables from this family are cucumber and pumpkin, but squash and zucchini are increasingly used in the kitchen. Pumpkin is one of the first domesticated plants and has been grown worldwide for several hundred years, thus giving farmers enough time to obtain and introduce cultivars with unique characteristics. Courgette, classified as a summer squash, belongs to *Cucurbita pepo*.[4,5] Today, courgette is called summer squash, marrow, or zucchini, depending on the place of its cultivation.[6] Summer squash (*Cucurbita pepo* subsp. *pepo*) is frequently consumed worldwide. Its shape resembles a ridged cucumber, but zucchini is available in yellow and green colors. *Cucurbita* fruits can often be used when they are not yet fully ripe. Botanically, this vegetable is considered as fruit, but in gastronomic terms, this is a vegetable. Zucchini has a firm texture with ripened fruit and a characteristic flower. It contains several beneficial micronutrients such as minerals (potassium, magnesium, phosphorus, and calcium), carotenoids, vitamin C, phenolic compounds, etc. [7-10] This fruit contains approximately 93.5–95% of water, fiber (1.1 g/100 g f.w.), proteins (1–2.5 g/100 g f.w.), carbohydrates (2.3–4.2 g/100 g f.w.).[11,12] Fruits of the *Cucurbita* genus are also sources of functional compounds with nutraceutical and preventive potential against cardiovascular diseases and diseases derived from eating disorders. A characteristic feature of the *Cucurbita* genus is its low fat content and low glycemic index, attributed to its high dietary fiber content, particularly pectin[13] *Cucurbitaceae* plants are grown for their seeds, flowers, roots, leaves, and fruits, all of which are edible. [14]

Certain seeds from the *Cucurbitaceae* family have been traditionally utilized in folkloric medicine.[15,16] Due to bioactive nutrients, species from the *Cucurbita* genus are assumed to be used as a remedy for lowering blood cholesterol or depression.[17,18] The folk medicinal properties attributed to zucchini are diverse and include its use in the treatment of benign prostatic hyperplasia, and irritable bladder conditions.[19,20]

Due to its antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic, antiviral, and analgesic activities[21-24] summer squash has been used in traditional folk medicine to treat colds and to alleviate aches. The natural plant components

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found in pumpkin could recover the liver against alcohol-induced liver toxicity and oxidative stress in rats.[25] The presence of bioactive nutrients suggests it could be used to lower blood cholesterol or treat depression. Pumpkin flesh exhibits hypoglycemic, *in vitro* antioxidant, cholinesterase and tyrosinase inhibitory effects, along with hypolipidemic activity.[26-30] The cell growth inhibition of prostate, breast, and colon cancer cells by ethanolic seed extract authenticates the ethnomedical use of pumpkin seeds for the treating of benign prostate hyperplasia urinary dysfunction.[15] Pumpkin also exhibits various pharmacological actions, such as anticancer, antihypertension, antioxidant, anti-inflammation, antihyperlipidemic, antimicrobial, hypoglycemic and immunomodulation.[31-36]

Natural antioxidants have been demonstrated to be more effective and devoid of the adverse health effects associated with synthetic antioxidants.[37,38] Considerable attention has been given to the potential applications of natural antioxidants found in vegetables, fruits, and grains, such as carotenoids, tocopherols, flavonoids, phenolic acids, vitamin C, and minerals, in the prevention of numerous diseases.

The purpose of this study was to examine extracts from pericarp and seed of nine varieties of *Cucurbita* genus: (*Curcubita pepo Ghost rider*, *Curcubita maxima Roter zentner, Spaghetti squash, Marrow Long Green Bush, Sweet gourmet zucchini, Summer Green Tiger Zucchini, Round Zucchini green, Green zucchini*), concerning their content of total phenolics, flavonoids and antioxidant activity. As a result of the large amount of data that analytical techniques generate, the use of advanced multivariate chemometric tools is mandatory for extracting as much valuable information as possible.[39-41] Obtained data were processed using principal component analysis (PCA) and hierarchical cluster analysis (HCA). The findings of this study may allow the identification of *Cucurbita* species with desired phenolic and flavonoid levels, aiding in the selection of antioxidant-rich varieties.

EXPERIMENTAL

Sample collection

Nine varieties of *Cucurbita* genus were acquired from local supermarkets in the Nis region, Southern Serbia. Examined species were cultivated at multiple locations within the Nis region. The fruits of nine varieties of *Cucurbita* genus underwent a rapid cold-water wash, the pericarps (*Curcubita pepo Ghost rider* (P1), *Curcubita maxima Roter zentner* (P2), *Spaghetti squash* (P3), *Marrow Long Green Bush* (P4), *Sweet gourmet zucchini* (P5), *Summer Green Tiger Zucchini* (P6), *Round Zucchini green* (P7), *Round Zucchini yellow* (P8), *Zuchinni green* (P9)) and seeds (*Curcubita pepo Ghost rider* (S1), *Curcubita maxima Roter zentner* (S2), *Spaghetti squash* (S3), *Marrow Long Green Bush* (S4), *Sweet gourmet zucchini* (S5), *Summer Green Tiger Zucchini* (S6), *Round Zucchini green* (S7), *Round Zucchini yellow* (S8), *Zuchinni green* (S9)) of each sample were separated. Fresh samples were pulverized with an electric blender and immediately analyzed.

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Preparation of extracts

The protocol used for extracting polyphenolic compounds from pericarp and seed of different edible *Cucurbita* varieties was based on a previously employed procedure.[42] The pericarp and seed extracts, obtained from grinding 4 g of fresh samples, were extracted four times by stirring with 40 mL of 80% methanol (v/v) in an ultrasonic bath (power supply: 220 V/ 50 Hz, 10 A) at 25°C for 15 minutes. Samples were left in the solvent for 15 minutes, filtered, and diluted to a final concentration of 100 mg mL^{-1}.

Chemicals and instruments

Except 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), $FeCl_3x6H_2O$, $AlCl_3x6H_2O$, $CuCl_2$, Na_2CO_3, Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid (Trolox) and methanol were purchased from Sigma Co. St. Louis, Missouri, USA, all chemicals were purchased from Merck, Darmstadt, Germany. Spectrophotometric assays were performed on a double-beam UV–VIS spectrophotometer Perkin Elmer lambda 15 (Massachusetts, USA).

Total phenolic content (TPC)

The Folin–Ciocalteu redox method is commonly used to analyze total phenolic content, as phenolics are a major group of bioactive plant compounds that function as primary antioxidants or free radical scavengers. This assay is particularly important for evaluating total antioxidant capacity, as a high phenolic content is strongly associated with enhanced antioxidant activity. This assay is significant in measuring total antioxidant capacity since high phenolic content has been linked to high antioxidant capacity. Total phenolic content was determined using the Folin–Ciocalteu reagent as described by Mitic et al.[42] The reaction mixture consisted of 0.05 mL of extract, 2 mL of sodium carbonate solution, 5.0 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. The reaction was carried out in the dark for 30 minutes, and then the absorbance was measured at 750 nm. Results were expressed as mg gallic acid equivalents per 100 g of fresh weight (mg GAE 100 g^{-1} f.w.) since gallic acid was used to calculate the standard curve ($y = 0.0163x + 0.0121$, $R^2 = 0.9751$).

Total flavonoid content (TFC)

The total flavonoid content was determined using the aluminum chloride method, a widely used assay for quantifying flavonoids in plant extracts. In this method, aluminum chloride reacts with the carbonyl group at position C4 and the hydroxyl groups at positions C3 and C5 in flavonols and flavones, forming a stable complex. Additionally, it can form labile acid complexes with ortho-dihydroxyl groups (catechol groups) present in the flavonoid structure. Total flavonoid content of analyzed samples was determined by a method described by Dimitrijević et al.[43] Extract aliquot (50 µL) was mixed with 0.15 mL of a $NaNO_2$ solution. After 5 minutes, 0.75 mL of $AlCl_3$ solution was added, and the solution was kept 5 min at room temperature. Then, 1 mL of NaOH solution was added to the mixture, and water was added to a final volume of 5 mL. The absorbance was measured at 510 nm. Rutin solution was used for calibration curve construction ($y = 0.0356x + 0.0214$ $R^2 = 0.9907$) and results were expressed as mg rutin equivalents (RE) per 100 g of fresh weight (mg RE 100 g^{-1} f.w.).

DPPH free radical scavenging assay

Diphenyl-1- picrylhydrazyl (DPPH) is a popular, quick, easy, and affordable approach for the measurement of antioxidant properties. This test measures the scavenging capacity of antioxidants against the free radical DPPH. The advantage of this method lies in its ability to allow DPPH to interact with the entire sample, providing sufficient time for the reaction to

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occur, even with weak antioxidants that react more slowly. Quantitative tests of methanol extracts for DPPH radicals were performed according to the method of Mitic et al.[42] The reagent was prepared 2 h before use to ensure all the DPPH had dissolved. The flask containing DPPH solution was covered with aluminum foil to protect it from light. 1.5 mL of methanol solution of DPPH radical at the concentration of 100 mmol L^{-1}, 0.1 mL of extract concentration of 100 mg mL^{-1}, and methanol to a total volume of 4 mL were added to a test tube. The mixture was shaken vigorously and left in the dark at room temperature for 60 minutes. The reduction in the color of the solution caused by free radicals (DPPH) was measured at 515 nm. Trolox dissolved in methanol in appropriate dilution was used as a standard. A linear calibration curve was obtained in the range of 0.5 – 4 µg mL^{-1} Trolox, with regression equation $y = 0.0405x - 0.0495$ and linear regression coefficient $R^2 = 0.9963$. The antioxidant capacities of the samples were expressed as mg Trolox equivalents per 100 g of fresh weight g (mg TE 100 g^{-1} f.w.).

ABTS cation radical decolorization assay

ABTS cation radical "scavenging" activity assay is based on the ability of antioxidant molecules to quench ABTS radical, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analog. The $ABTS^{\bullet+}$ cation radical was prepared by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1). The flask containing this solution was covered with aluminum foil to protect it from light and it was stored at room temperature for 12-16 h before use as described by Mitic et al.[42] The prepared solution was then diluted with methanol to obtain an absorbance of 0.7±0.02 units at 734 nm. An aliquot of each extract, concentration 100 mg mL^{-1} was mixed with 1.8 mL of diluted ABTS solution and diluted with methanol to a total volume of 4 mL, absorbance was measured 6 min after initial mixing. An appropriate solvent blank was used in each assay. The same procedure was repeated for all standard Trolox solutions (0.1 – 2.5µg mL^{-1}) and a standard curve ($y = 0.0322x - 0.0141$, $R^2 = 0.9997$) was constructed. Results were expressed as mg Trolox equivalents per 100 g of fresh weight (mg TE 100 g^{-1} f.w.).

Ferric reducing antioxidant power (FRAP) assay

The FRAP analysis (ferric reducing antioxidant power) indicates the reducing ability of the extract based on the reduction of colorless Fe^{3+}-TPTZ to blue Fe^{2+}-TPTZ complex. This assay was performed according to the protocol described.[42] The FRAP reagent was prepared by mixing a freshly prepared acetate buffer (pH 3.6), the 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution, and ferric chloride (20 mM), (10 mM in 40 mM HCl) in a 10:1:1 ratio. An aliquot of the extract (10 µL) was mixed with 3 mL of the freshly prepared FRAP solution and diluted with water to a final volume of 4 mL. After incubation of the mixture at 37 °C (for 5 min) the absorbance at 595 nm was recorded. The value of FRAP was determined by plotting on a standard curve ($y = 0.0955x - 0.0185$, $R^2 = 0.9941$) prepared by adding ferrous sulfate (0.7 - 7 µg mL^{-1}) to the FRAP reagent and the results were expressed as mg Fe(II) per 100 g of fresh weight (mg Fe(II) 100 g^{-1} f.w.). The control solution was prepared as above but contained distilled water instead of extract samples.

Cupric reducing antioxidant capacity (CUPRAC) assay

Cupric reducing antioxidant capacity (CUPRAC) spectrophotometric method is based on the absorbance measurement of Cu(I)- neocuproine (Nc) chelate formed as a result of the redox reaction of chain-breaking antioxidants with the CUPRAC reagent, Cu(II)-Nc, where absorbance is recorded at the maximal light absorption wavelength of 450 nm; thus this is an electron-transfer (ET)-based method. In a test tube, the following were added in sequence: 0.05 mL of the extract, 1 mL of phosphate buffer (pH 7.0), neocuproine, copper(II) chloride. The

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mixture was then diluted with water to a total volume of 4.1 mL.[42] The reaction mixtures were incubated at room temperature for 30 minutes. Trolox dissolved in methanol in appropriate dilution (5 - 25 µg mL^{-1}) was used as the calibration curve's standard under the same parameters. The stability of the Trolox standards was monitored over a range of concentrations (in triplicate) by measuring their absorbance values and plotting their average against a range of time points. It was confirmed that the working Trolox standards were stable for the first three hours of the assay thereby removing any absorbance measurement inaccuracies due to the decomposition of standards in the assay system. Cupric reducing antioxidant capacity was expressed as mg Trolox equivalents per 100 g of fresh weight (mg TE 100 g^{-1} f.w.).

Total reducing power (TRP) assay

The reducing power of the extracts was determined according to the potassium ferricyanide-ferric trichloroacetic acid method based on the ability of antioxidants to reduce Fe(III) hexacyanate to Fe(II) hexacyanate, resulting in an increase in the absorbance in the absorbance of the reaction mixtures. The higher absorbance of the extracts indicated greater ferric reducing capacity. The reaction mixtures were prepared by mixing 1 mL of 1% solution $K_3[Fe(CN)_6]$, 1 mL of phosphate buffer (pH 6.6), 0.01 mL of the extract and diluted with water to 3.7 mL. The mixtures were incubated at 50 °C for 30 minutes and then 1 mL of 10% solution of trichloroacetic acid and 0.6 mL of $FeCl_3$ were added.[42] The absorbance was measured at 700 nm against the blank sample. A standard curve ($y = 0.1056x - 0.0452$, $R^2 = 0.9855$) was plotted using different concentrations of ascorbic acid (2–50 µg mL^{-1}), so that the results were expressed as mg ascorbic acid equivalents per 100 g of fresh weight (mg AAE 100 g^{-1} f.w.). Working ascorbic acid standards were stable for the first three hours of the assay, thereby avoiding absorbance measurement inaccuracies due to the decomposition of standards in the assay system.[44]

Statistical analysis

Statistical analyses were performed using the Statistica version 12.0 software package. All the experiments were conducted in triplicate. To eliminate uncertain data, the Q-Dixon test was performed. All data were checked for normality using the Shapiro–Wilk test ANOVA's Tukey multiple comparison test was used to compare the variance in the response variables: total phenolic concentration (TPC), total flavonoids content (TFC) and the antioxidant capacity: DPPH, ABTS, CUPRAC, TRP of nine varieties of *Cucurbita* genus. Differences were considered statistically significant at a level of 0.05 (that is, $p < 0.05$). Agglomerative hierarchical clustering was used as a multivariate analysis tool to cluster samples based on similar characteristics and then plotted as a dendrogram. The distance matrix was calculated using the Euclidean Pythagorean distance differences, and hierarchical cluster analysis was performed using Ward's method. Pattern recognition methods were applied to the data collection; these were principal component analysis (PCA). Principal Component Analysis facilitated the identification of similarities among samples and revealed the primary associations between variables contributing to the total variability in the analyzed data.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

Polyphenols include simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, condensed tannins, lignans, and lignins. According to the human physiological standpoint, phenolic compounds play a significant role in defense mechanisms, such as antioxidant, anti-

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inflammatory, anti-aging and anti-proliferative activities. Quantifying polyphenols is a standard practice for identifying foods and food products with high potential to combat free radicals. In this process, polyphenols in extracts react with a specific redox reagent, the Folin-Ciocalteu reagent, forming a blue complex that can be measured using visible-light spectrophotometry. For an approximate estimation of the total phenolic content (TPC) *Cucurbita* species extracts the Folin-Ciocalteu phenol reagent assay is used. The mean content values of the phenolics in the pericarp and seeds of the nine *Cucurbita* species are listed in Fig. 1a. TPC values for pericarp and seed samples were in the intervals 8.88 - 19.76 mg GAE 100 g^{-1} f.w. and 20.21–48.71 mg GAE 100 g^{-1} f.w., respectively.

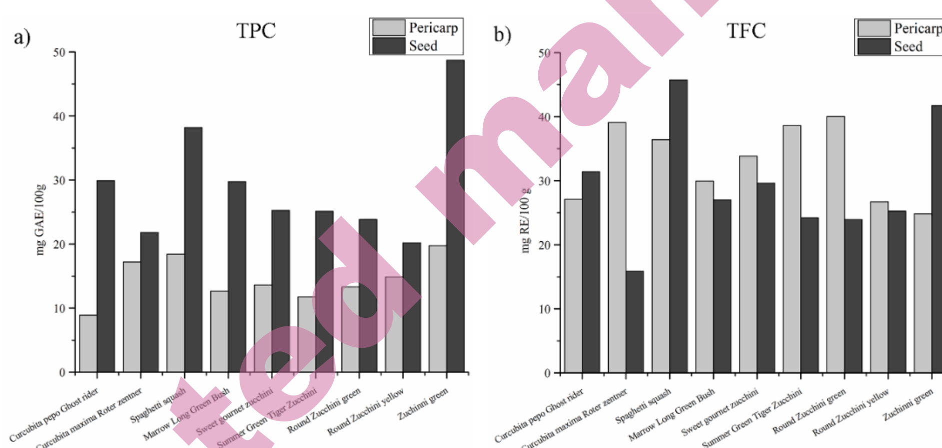

Figure 1. Results of a) total phenolic contents (TPC) and b) total flavonoid content (TFC) in the pericarp and seed of nine varieties of *Cucurbita* genus

The pericarp samples showed significantly lower TPC values than the seed samples. The TPC in four seed samples (*Zuchinni green, Spaghetti squash, Curcubita pepo Ghost rider, Marrow Long Green Bush*) were the highest (48.71, 38.20, 29.89 and 29.75 mg GAE 100 g^{-1} f.w., respectively). The pericarp samples *Curcubita pepo Ghost rider* and *Summer Green Tiger Zucchini* have the lowest values (8.88 and 11.78 mg GAE 100 g^{-1} f.w., respectively). Among the nine *Cucurbita* species examined, seeds and pericarp of *Zuchinni green* stands out with notable higher TPC when compared to the other tested varieties.

As Tejada et al. (2020) indicated, factors such as variety, harvest period, growth conditions, and ripening stage significantly influence the antioxidant profiles of courgette fruits. For example, frozen raw zucchini showed reduced phenolic content (0.12 µg GAE g^{-1} dry extract) compared to fresh samples (0.15 µg GAE g^{-1}). Stir-frying increased the phenolic content (0.14 µg GAE g^{-1}) compared to steaming (0.12 µg GAE g^{-1}) or raw samples. Other studies revealed varying TPC levels across *Cucurbita* species and parts, influenced by genetic,

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environmental, and extraction factors. In frozen raw zucchini (0.12 µg GAE g^{-1} dry extract) a decrease in the content of phenolic compounds is observed compared to fresh samples (0.15 µg GAE g^{-1} dry extract). On the other hand, stir-fried frozen zucchini had a higher phenolic content (0.14 µg GAE g^{-1} dry extract) compared to steamed frozen zucchini (0.12 µg GAE g^{-1} dry extract) and raw (0.12 µg GAE g^{-1} dry extract), probably as a result of a gain in phenolic content from the oil used to stir-fry the zucchini. [45] For *Curcubita pepo*, the highest levels of total phenolics were noted in the fruits of 'Kamo Kamo' (51.5 mg hlorogenic equivalents per 100 g of fresh weight) and 'Sweet Dumpling' (48.1 mg hlorogenic equivalents per 100 g of fresh weight) cultivars.[14] The total phenolic content of aqueous extracts from seed, peel, leaf, and flower powder of *C. maxima* was found to be as follows: seed - 0.79 mg GAE g^{-1}, peel - 2.46 mg GAE g^{-1}, leaf - 0.27 mg GAE g^{-1}, flower - 15.73 mg GAE g^{-1}. The isopropanol extracts from the same plant parts obtained the following TPC values: seed 41.05 mg GAE g^{-1}, peel 0.20 mg GAE g^{-1}, leaf 0.66 mg GAE g^{-1}, and flower 33.27 mg GAE g^{-1}.[46] In an India-based study, TPC of pumpkin flowers was found to be 49.6 mg GAE 100 g^{-1} dried powder.[47] In contrast, the Ethiopia-based research reported that the total phenolic content in pumpkin peel and seed ranged from 354 to 380 mg GAE 100 g^{-1} and 80 to 102 mg GAE 100 g^{-1} powder, respectively.[48]

Other studies revealed varying TPC levels across *Cucurbita* species and parts, influenced by genetic, environmental, and extraction factors. The total phenolic content of pumpkin peel and pulp extracts was comparable, measuring 5.21 mg GAE g^{-1} for the peel and 5.19 mg GAE g^{-1} for the pulp.[49] The content of total phenolic compounds in the analyzed *Cucurbita* spp. peel extracts varied from 17.599 ± 0.124 to 4.623 ± 0.082 mg GAE g^{-1} d.w. as published by Gaweł-Beben et al.[50] The average total phenolic compounds recorded in zucchini was 8.67 GAE g^{-1} f.w. reported by Hamissou et al.[51]

The variation in phenolic content is known to be due to genetic factors, degree of maturity, and environmental conditions. Secondly, the extractability of phenolic compounds is governed by the type of solvent (polarity) used, the degree of polymerization of phenolics, the interaction of phenolics with other constituents, as well as the extraction time and temperature. The complete extraction of all phenolic compounds from plant materials cannot be achieved using a single, universally effective method. In addition, different ways of expressing the content of polyphenolic components (concerning the equivalents of gallic acid, equivalents of pyrocatechol, equivalents of quercetin according to fresh sample, according to dry sample, according to extract) make it difficult to compare all the results.

Total flavonoids content (TFC)

Flavonoids are a diverse group of natural compounds with the ability to scavenge free radicals, chelate metal ions, and modulate enzymatic activities. This antioxidant activity is primarily attributed to their phenolic hydroxyl groups, which

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can donate hydrogen atoms or electrons to neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS). As a basis for the quantitative determination, flavonoid contents in selected plant extracts were determined using aluminium chloride in a colorimetric method. The basic principle involved in this method is that $AlCl_3$ forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. The TFC values of the samples assayed in this study differed according to pericarp and seed of the *Cucurbita* species (Fig. 1b). The TFC values for pericarp samples were in the intervals 24.82 (*Zuchinni green*) - 40.02 (*Round Zucchini green*) mg RE 100 g^{-1} f.w.), while the values 15.88 (*Curcubita maxima Roter zentner*) - 45.73 (*Spaghetti squash*) mg RE 100 g^{-1} f.w. were found in seed samples. Extract of the seed of the *Curcubita maxima Roter zentner* showed the lowest values in this test, also this sample has one of the lowest values of the content of total phenolic compounds (21.81 mg GAE 100 g^{-1} f.w.). At the same time, the content of flavonoids (39.09 mg RE 100 g^{-1} f.w.) in the pericarp of *Curcubita maxima Roter zentner* is one of the highest. Although less abundant in *Cucurbita* species, flavonoids are highly effective as antioxidants, even at low concentrations.[52] A survey of past literature reports found that Mokhtar et al. analyzed the flavonoids of *Curcubita moschata* at various stages of ripening (unripe, mature, ripened) and determined antioxidant activity. The content of the flavonoids compound in ripe pumpkin was 28.6 mg QE g^{-1}.[53] The total flavonoid content in pumpkin peel and seeds was found to range from 130 to 153 mg QE per 100 g^{-1} in the peel, and from 51 to 67 mg QE per 100 g^{-1} in the seed powder, as reported by Hagos et al. [48] Kar et al. reported that aqueous extracts from the seed and peel of *C. maxima* contain 71.06 mg QE g^{-1} and 63.80 mg QE g^{-1}, respectively.[46] The total flavonoid content in the analyzed *Cucurbita* spp. peel extracts, varied from 2.598 ± 0.127 (*C. moschata* 'Muscat') to 7.108 ± 0.120 (*C. maxima* 'Hokkaido') mg QE g^{-1} d.w.[46] Rutin was the flavonol found in all analyzed cultivars (the content of 51.92 ± 0.03 to 5.09 ± 0.01 mg 100 g^{-1}. Kaempferol, quercetin, isoquercetin, astragalin, and myricetin were detected only in some of the varieties. No significant differences in the content of the listed flavonols were found between the *C. pepo* and *C. moschata* cultivars, except for quercetin, which was most abundant in *C. pepo* (3.29 ± 3.43 vs. 1.07 ± 1.74 mg 100 g^{-1}) as publishes by Gaweł-Beben et al.[50] Saha et al. found that 1 g of dry extract *C. maxima* contained 26.50±1.40 mg equivalent of quercetin.[54] The results of the total flavonoid content obtained in previous studies by Astutik and Yanti were 0.146 mg QE 100 g^{-1} extract of unripe pumpkin, 0.221 mg QE 100 g^{-1} extract of mature pumpkin and 0.191 mg QE 100 g^{-1} extract of ripened pumpkin.[55] Previous research highlighted significant variability in flavonoid content across *Cucurbita* species, influenced by extraction methods, solvent types, and environmental factors. Such

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differences underscore the importance of standardizing extraction and measurement methods for reliable comparisons. Direct comparisons were not possible between the results of our study and most of the previously published assay values due to the vast differences in the published literature in terms of extracting solvents, assay incubation time, selection of different reference standards, presentation of results in various units, etc...

Antioxidant capacity of Cucurbita species

Due to their complex mechanisms, antioxidants exhibit diverse responses to different radicals or oxidants. Consequently, various assay systems are required to comprehensively and accurately evaluate antioxidant activity. *In vitro* methods for assessing antioxidant activity are notably diverse—some include a distinct oxidation step followed by measurement, while others do not delineate these procedural stages. [56]

In this study, five spectrophotometric methods were used to monitor variations in antioxidant capacity: DPPH free radical scavenging assay, ABTS cation radical decolorization assay**,** cupric reducing antioxidant capacity, (CUPRAC) ferric reducing antioxidant power assay (FRAP) and total reducing power (TRP). The findings are summarized in Fig 2 (a,b) and Fig 3 (a,b,c).

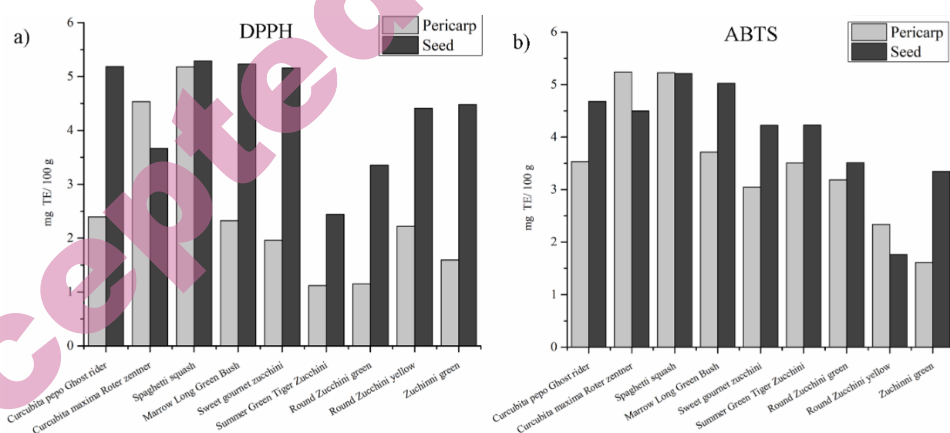

Figure 2. Results of a) DPPH free radical scavenging assay and b) ABTS cation radical decolorization assay in the pericarp and seed of nine varieties of *Cucurbita* genus

DPPH free radical scavenging assay

The radical scavenging activity (RSA) of extracts from nine *Cucurbita* species was assessed using the DPPH free radical scavenging assay, which primarily operates through electron transfer and hydrogen atom abstraction mechanisms. As shown in Fig. 2a, *Spaghetti Squash* demonstrated the highest ability to neutralize DPPH radicals among both seed extracts (5.29 mg TE 100 g^{-1} f.w.) and pericarp extracts (5.18 mg TE 100 g^{-1} f.w.). Other seed extracts, including those from

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Marrow Long Green Bush, *Curcubita pepo Ghost Rider*, and *Sweet Gourmet Zucchini*, also exhibited intense DPPH radical-scavenging activities, with values of 5.23, 5.19, and 5.16 mg TE 100 g^{-1} f.w., respectively. DPPH activity values ranged from 1.12 mg TE 100 g^{-1} f.w. for pericarp extracts. (*Summer Green Tiger Zucchini*) to 5.18 mg TE 100 g^{-1} f.w. (*Spaghetti Squash*). Notably, the pericarp of *Curcubita maxima Roter Zentner* showed vigorous scavenging activity (4.53 mg TE 100 g^{-1} f.w.). In comparison, *Round Zucchini Green* and *Zucchini Green* exhibited minimal activity (1.15 and 1.59 mg TE 100 g^{-1} f.w., respectively).

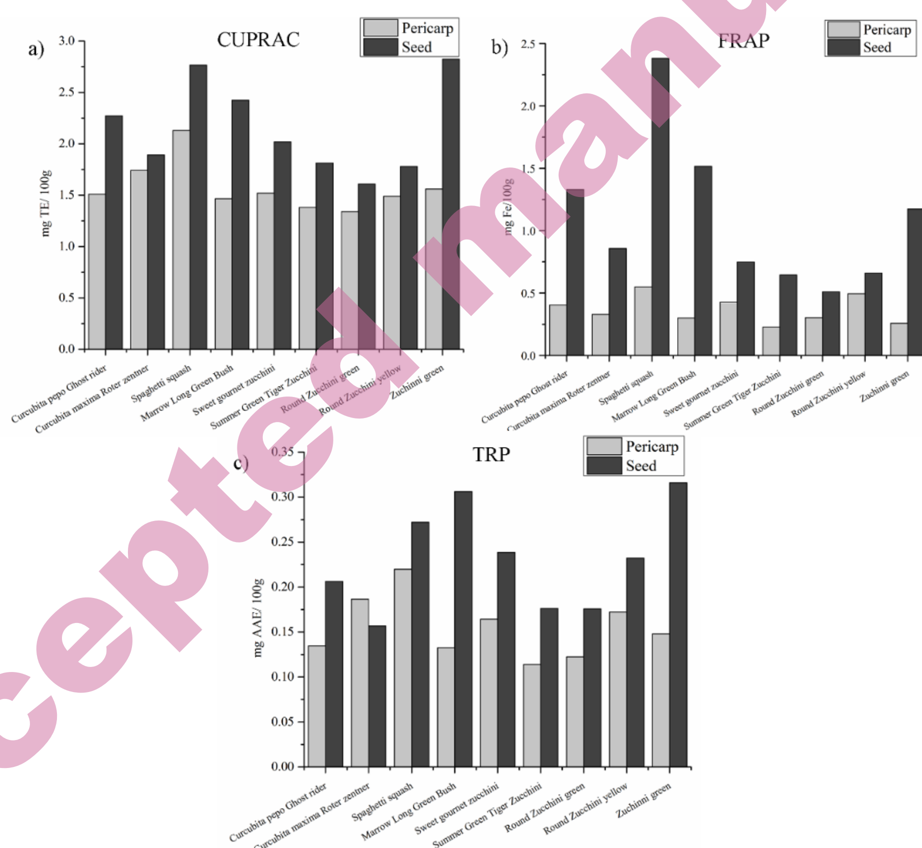

Figure 3. Results of a) ferric reducing antioxidant power (FRAP), b) total reducing power (TRP), and c) cupric reducing antioxidant capacity (CUPRAC) in the pericarp and seed of nine varieties of *Cucurbita* genus

Several studies have explored the antioxidant activity of *Cucurbita* species using the DPPH assay. Kostecka-Gugała et al. reported DPPH values ranging from 3.31–32.1 µmol TE 100 g^{-1} f.w. for *C. pepo*, 1.01–13.08 µmol TE 100 g^{-1} f.w. for *C. maxima*, and 2.45–16.08 µmol TE 100 g^{-1} f.w. for *C. moschata*.[14] In some

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research, DPPH results are expressed as the amount of antioxidants required to reduce the initial DPPH concentration by 50% (EC_{50}). For example, Mala and Kurian (2016) found that methanol extracts of pumpkin peel and pulp achieved 50% inhibition (IC_{50}) at a concentration of 18 mg mL^{-1}, while at 50 mg mL^{-1}, scavenging activity reached approximately 80%.[49] Kar et al. (2023) reported that aqueous extracts of dried *C. maxima* seeds, peels, leaves, and flowers showed DPPH scavenging activities of 48.41%, 55.71%, 83.91%, and 58.28%, respectively. Interestingly, isopropanol extracts exhibited higher scavenging activities of 67.99%, 90.35%, 49.80%, and 74.60%, respectively.[46] Various methods exist for expressing antioxidant activity results, which can be as diverse as the measurement techniques. For instance, while some results are expressed regarding EC_{50} or Trolox equivalents, others report percent scavenging activity, complicating direct comparisons between studies.

ABTS cation radical decolorization assay

The ABTS cation radical decolorization assay, also known as the Trolox Equivalent Antioxidant Capacity (TEAC) assay, evaluates the ability of antioxidants to donate electrons or hydrogen atoms to neutralize radical species. The reduction of the ABTS radical cation (ABTS•+) to ABTS serves as an indicator of antioxidant effectiveness. All extracts from *Cucurbita* species demonstrated their capacity to decolorize ABTS solution, underscoring their efficacy in scavenging organic radicals. The trends observed in the ABTS and DPPH assays were generally consistent. The ABTS scavenging activity of all examined seed extracts ranged from 1.76 to 5.21 mg TE 100 g^{-1} f.w.. The highest scavenging activity was observed in the pericarp of *Cucurbita maxima Roter Zentner* (5.24 mg TE 100 g^{-1} f.w.) and *Spaghetti squash* (5.23 mg TE 100 g^{-1} f.w.). In comparison, the lowest activities were recorded in *Zucchini Green* pericarp (1.61 mg TE 100 g^{-1} f.w.) and *Round Zucchini Yellow* seed (1.76 mg TE 100 g^{-1} f.w.). Seed extracts of *Curcubita pepo Ghost rider* and *Curcubita maxima Roter Zentner* also showed high ABTS scavenging activity 4.68 mg TE 100 g^{-1} f.w. and 4.49 mg TE 100 g^{-1} f.w., respectively. In this assay, pericarp extracts of *Curcubita maxima Roter Zentner*, *Spaghetti squash* and *Round zucchini yellow* displayed greater ABTS binding capacity (5.24, 5.23, 2.34 mg TE 100 g^{-1} f.w.) than their corresponding seed extracts (4.49, 5.21, 1.76 mg TE 100 g^{-1} f.w.). Additionally, a study by Mala and Kurian reported ABTS radical scavenging activity for pumpkin peel and pulp extracts, with IC_{50} values around 6 mg ml^{-1} and 10 mg ml^{-1}, respectively.[49]

Comparing antioxidant results across assays remains challenging due to differences in mechanisms, solvents, and reaction conditions, as well as the variability in how results are expressed (e.g., in µmol, mmol, or mg of standard equivalents per g, 100 g, or kg of fresh/dry weight).[44]

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CUPRAC - cupric-reducing antioxidant capacity

The CUPRAC method assesses antioxidant activity at near-physiological pH, contrasting with the basic (pH 10) or acidic conditions required for Folin or FRAP assays. Among the tested extracts, *Spaghetti squash* showed the highest antioxidant activity, with values of 2.77 mg TE 100 g^{-1} f.w. in seeds and 2.13 mg TE 100 g^{-1} f.w. in the pericarp. In contrast, *Round Zucchini Green* displayed the lowest values, measuring 1.61 mg TE 100 g^{-1} f.w. in seeds and 2.77 mg TE 100 g^{-1} f.w. in the pericarp. Notably, the seed extracts of *Zucchini Green* and *Marrow Long Green Bush* also demonstrated elevated CUPRAC values, recording 2.77 mg TE 100 g^{-1} f.w. and 2.42 mg TE 100 g^{-1} f.w., respectively. The cupric-reducing antioxidant capacity of extracts can be attributed to the content of phenolics, since the phenolic content in seed samples is higher than in pericarp samples. Comparative data on ferric-reducing antioxidant power can be found in the study by Kostecka-Gugała et al. (2020). The published FRAP values for different species are as follows: *Cucurbita pepo*: 11.7–57.8 µmol TE 100 g^{-1} f.w., *Cucurbita maxima*: 21.7–47.9 µmol TE 100 g^{-1} f.w., *Cucurbita moschata*: 21.2–139.9 µmol TE 100 g^{-1} f.w..[14]

Ferric-reducing antioxidant power (FRAP)

Determination of ferric-reducing antioxidant power (FRAP) is a typically simple, rapid, and cost-effective ET-based method performed under acidic pH conditions. The transformation ability of compounds from Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous form serves as a reliable indicator for antioxidant activity. The FRAP assay of antioxidants is convenient, reproducible, and linearly concentration-dependent.[57] The results (Fig 3b) indicated that *Spaghetti squash* showed the highest FRAP activity in seeds (2.38 mg Fe 100 g^{-1} f.w.) and pericarp extracts (0.55 mg Fe 100 g^{-1} f.w.), correlating with its high polyphenol content (seeds - 18.42 mg GAE 100 g^{-1} f.w., pericarp - 38.20 mg GAE 100 g^{-1} f.w.). The seed extract of *Marrow Long Green Bush* also shows high activity in this test 1.52 mg Fe 100 g^{-1} f.w..

The available data for ferric-reducing antioxidant power can be seen in the Kostecka-Gugała et al. paper [14] where published values in the interval 11.7 - 57.8 µmol TE 100 g^{-1} f.w. (*C. pepo*) 21.7 - 47.9 µmol TE 100 g^{-1} f.w. (*C. maxima*) 21.2 - 139.9 µmol TE 100 g^{-1} f.w. (*C. moschata*).

Our results cannot be directly compared with previously published findings due to differences in testing methodologies and the presentation of results.

The reducing power assay (TRP)

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reducing power of extracts is related to their electron transfer ability and may serve as a significant indicator of potential antioxidant activity. The results of the reducing power assay of the tested extracts

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are summarized in Fig. 3c. The seed extract of *Marrow Long Green Bush* exhibited the highest reducing power (0.31 mg AAE 100 g^{-1} f.w.), correlating with its phenolics (29.75 mg GAE 100 g^{-1} f.w.) and flavonoids (27.00 mg QE g^{-1} 100 g^{-1} f.w.) content. Among pericarp extracts, *Spaghetti squash* showed the highest reducing power (0.22 mg AAE 100 g^{-1} f.w.). In comparison, *Summer Green Tiger Zucchini* had the weakest activity (0.11 mg AAE 100 g^{-1} f.w.), aligning with its low DPPH activity. To the best of our knowledge, there are no available studies in the literature on the antioxidant activity of *Cucurbita* species extracts determined by this method.

The analytical findings of antioxidant profiles from *Cucurbita* species assist in identifying varieties with high antioxidant activity, providing a valuable benchmark for breeding programs. Detailed analysis of antioxidant profiles allows breeders to pinpoint which specific compounds (e.g., specific phenols or flavonoids) are most responsible for high antioxidant activity. This understanding enables targeted breeding for varieties enriched in those compounds rather than merely increasing overall antioxidant levels. Varieties with high antioxidant activity are attractive for their potential health benefits, including reducing oxidative stress and lowering the risk of chronic diseases.

Statistic analysis

The interpretation of chemical data from phenol and flavonoid content and antioxidant activity has recently gained importance through chemometrics. These methods facilitate the evaluation of similarities and differences between various extracts or project samples onto a two-dimensional factorial plane based on distinct characteristics using diverse mathematical and statistical approaches. Multivariate statistical techniques, particularly chemometrics, are increasingly applied in science and technology due to their ability to extract comprehensive information from chemical data, including chemical composition and antioxidant activity. This study employed pattern recognition techniques such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). PCA, an unsupervised classification approach, and HCA, an unsupervised learning method, effectively identified patterns and grouped species with high antioxidant content. These analyses identify patterns and groupings of species with high antioxidant content, enabling targeted selection of varieties for specific purposes, and can guide breeders in selecting *Cucurbita* varieties with enhanced antioxidant traits, contributing to developing nutritionally superior crops, so PCA and HCA streamline the selection process, saving time and resources in breeding programs.

Before the chemometrics application, all variables were autoscaled (transformation into z-scores) to standardize the statistical importance of all responses. After this mathematical operation, each parameter contributes equally to the data set variance and carries equal weight in the principal component calculation. Then, a matrix of samples (n = 18) and response variables (n = 7) was

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built, in which samples were adopted as lines and variables as rows, totaling 126 data points. The correlation matrix was constructed to analyze the relation between the values of antioxidant capacities for all the analyzed cultivars, and the content of phenolics and flavonoids. (Table 1). A correlation matrix provides quantitative insights into the relationships between multiple variables in a dataset: values of Pearson's correlation coefficients indicate the strength and direction of the relationship between each pair of variables. Also, It highlights how one variable changes in response to another, which can help identify trends, dependencies, or redundancies within the data.

Table 1. The Pearson's correlation coefficients for selected antioxidant parameters of nine varieties of *Cucurbita* genus

Correlations							
	ABTS	DPPH	TRP	CUPRAC	FRAP	TPC	TFC
ABTS	1.000000	0.65**	0.33	0.51*	0.48*	0.28	0.28
DPPH		1.00	0.82**	0.81**	0.68**	0.64**	0.13
TRP			1.00	0.91**	0.75**	0.84**	0.25
CUPRAC				1.00	0.87**	0.91**	0.33
FRAP					1.00	0.77**	0.27
TPC						1.00	0.25
TFC							1.00

Abbreviations: ABTS-2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay, DPPH-diphenyl picrylhydrazyl free radical scavenging assay, TRP-total reducing power assay CUPRAC-cupric reducing antioxidant capacity assay, FRAP-ferric reducing antioxidant power assay TPC-total phenolic content, TFC-total flavonoids content, * values of Pearson's correlation coefficients significant at $p < 0.05$, ** values of Pearson's correlation coefficients significant at $p < 0.001$.

The correlation analysis revealed numerous statistically significant connections between the contents of bioactive compounds with antioxidative properties. A significant positive correlation was observed between the total phenolic compound content and antioxidant capacity, as measured by the CUPRAC (r = 0.91**) and TRP (r = 0.84**) methods. The content of phenolics was also positively correlated with FRAP (r = 0.77**) and DPPH (r = 0.64**) assays. These results emphasized the importance of phenolic compounds in the antioxidant behavior of examined extracts and indicated that the phenolic compounds contributed significantly to the total antioxidant activity. The high correlation coefficient for antioxidant capacities measured by FRAP and CUPRAC (r = 0.87**) confirms the similarity of the two methods in terms of their mechanisms of action. Antioxidant capacity as measured by CUPRAC and by DPPH was positively correlated (r = 0.81**). There were also positive correlations

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between the content of total phenolic compounds and FRAP (r = 0.77**), the content of total phenolic compounds and DPPH (r = 0.64**), and between the FRAP and TRP (r = 0.75**). No negative correlation was observed between the investigated variables. Principal components (PCs) were determined from the eigenvalues of the correlation matrix of observations. The eigenvalues were calculated as 4.5926 for PC1 and 0.9723 for PC2. The contribution of the variables to the PCs is shown in Figure 4, and as can be seen, the first two PCs explain 79.50 % of the total variance (65.61% (PC1) and 13.89% (PC2) of the total variance, respectively.)

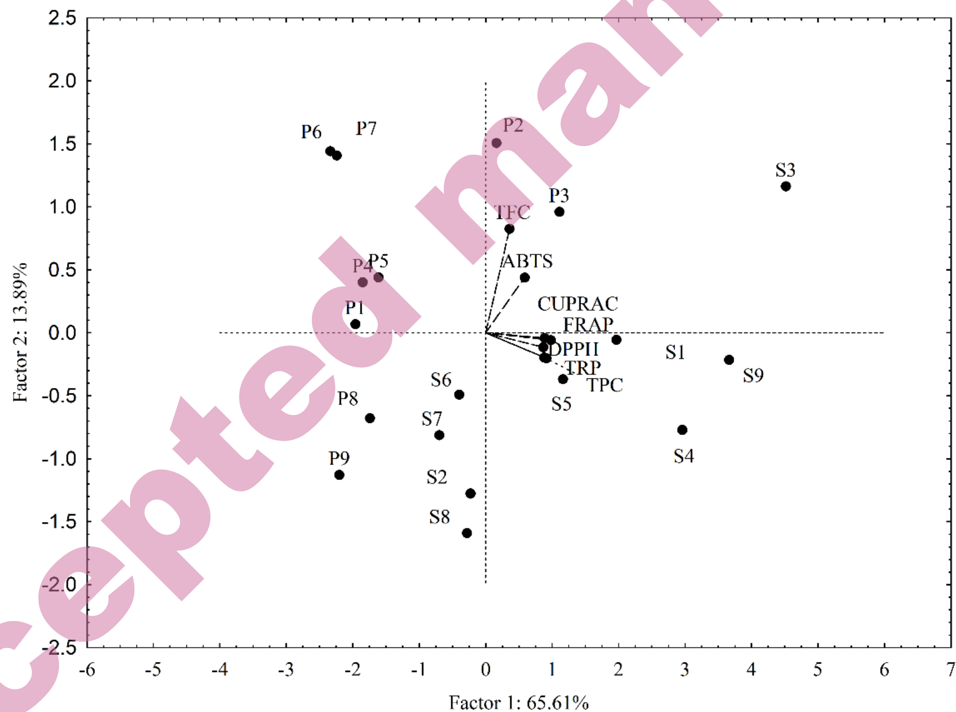

Figure 4. Principal component analysis (PCA) of total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging assay, ABTS cation radical decolorization assay, ferric reducing antioxidant power (FRAP), total reducing power (TRP), and cupric reducing antioxidant capacity (CUPRAC) in the pericarp and seed of nine varieties of *Cucurbita* genus. The first principal component (PC1 - 65.61%) and the second principal component (PC2- 13.89%) represent the total variance of the data (79.50%).

PC1 is generally better correlated with the variables than PC2. This is expected, as principal components (PCs) are extracted sequentially, with each one accounting for the maximum possible remaining variance. The CUPRAC and TRP values strongly correlate with PC1 in the negative direction (0.9819, 0.9152), while

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the ABTS values show a positive correlation with PC2 (0.4384). Based on the principal component analysis, all tested samples were clustered into two groups: one comprising seed extracts and the other comprising pericarp extracts. All applied methods for determining the antioxidant activity as well as the content of phenolics and flavonoids are clustered together on the right-hand side of the loading plot. These parameters are significantly correlated as evidenced by their Pearson correlation coefficients. TPC and ABTS are found in opposition to FRAP, CUPRAC, DPPH, TRP and TFC. Among tested extracts, *Spaghetti squash* seed, has the highest loading (4.5177) on PC1. High loadings on PC1 are also shown by the samples *Marrow Long Green Bush* seed (2.9591) Summer *Green Tiger Zucchini* pericarp (-2.3387), *Round Zucchini green* seed (-2.2413). Pericarp extract of *Curcubita maxima Roter zentner Sweet gourmet zucchini*, *Round Zucchini green* and *Summer Green Tiger Zucchini* have the highest loading on PC2 (1.5061, 1.4409, 0.4402 1.4080, respectively). By using the plots in Fig 4., it is possible to suggest reasons for the location of the varieties based on their antioxidant activity. The location of *Spaghetti squash pericarp* and *Curcubita maxima Roter zentnerin* pericarp in the first quadrant of Fig 4. may be explained by their high values of TPC and ABTS which are co-located in this region of the PC space.

The same data matrix used in the principal component analysis was applied for cluster analysis, utilizing Euclidean distance and Ward's method. The results obtained following HCA are shown as a dendrogram (Figure 5) in which three well-defined clusters are visible. Samples will be grouped in clusters in terms of their nearness or similarity. Seed extracts of *Zuchinni green* and *Spaghetti squash* have a strong relationship with each other, forming a distinct cluster separate from all other groups. These species are associated in the first cluster with high antioxidant activity measured by ABTS, DPPH, CUPRAC, FRAP and TRP. The third cluster contains pericarp samples from all tested extracts except *Zuchinni green.* The second cluster includes samples of seven seed extracts and the pericarp extract of *Zuchinni green.* The high flavonoid concentration in the pericarp of *Zucchini Green* plays a key role in this clustering.

The high phenolic and flavonoids content in *Green zucchini* seed suggests a notable antioxidant potential, as phenolic compounds are commonly linked to such activity. However, not all phenolic compounds possess equivalent antioxidant efficacy. While the seed of *Zuchinni green* has a high total phenol concentration and exhibits significant antioxidant activity, as determined by the CUPRAC and TRP methods, the specific phenolic compounds it contains may be less effective at scavenging free radicals. Antioxidant efficiency often relies on the synergistic interaction between phenolics and other bioactive compounds, such as carotenoids, vitamins, tannins. Furthermore, the bioavailability and solubility of certain phenols in the seed of *Green zucchini* may limit their functionality in biochemical assays, resulting in an underutilization of their antioxidant potential. Consequently,

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despite its elevated phenol levels, the seed of *Green zucchini* demonstrates suboptimal performance in DPPH, ABTS and FRAP antioxidant activity. In the second cluster (seed cluster), the greatest similarity was observed between *Summer Green Tiger Zucchini* and *Round Zucchini green*- Euclidean distance 1.8 and between *Curcubita pepo Ghost rider* and *Marrow Long Green Bush* Euclidean distance of 4.4. In the third cluster, the pericarp cluster, varieties *Summer Green Tiger Zucchini* and *Round Zucchini green* also exhibit the highest similarity, forming a subcluster with the smallest Euclidean distance of 2.1. Additionally, a high similarity (Euclidean distance of 3) is observed between the samples *Curcubita maxima Roter Zentner* and *Spaghetti squash*.

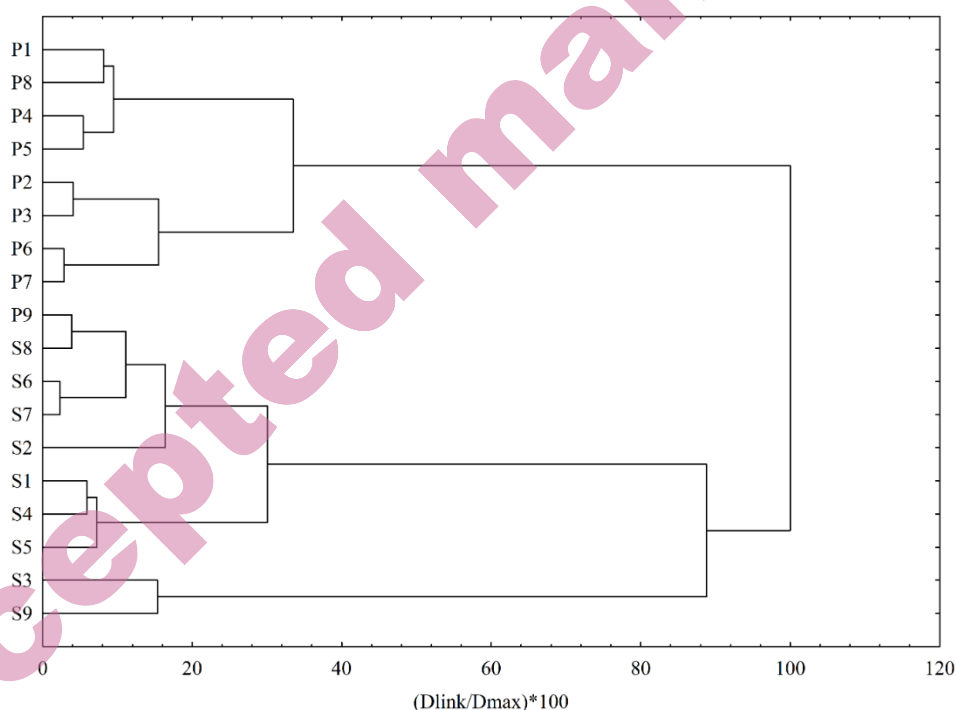

Figure 5. Hierarchical Cluster Analysis (HCA) of total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging assay, ABTS cation radical decolorization assay, ferric reducing antioxidant power (FRAP), total reducing power (TRP), and cupric reducing antioxidant capacity (CUPRAC) in the pericarp and seed of nine varieties of *Cucurbita* genus. Dlink/Dmax represents the quotient between the linkage distances for a particular case divided by the maximal linkage distance. The dendrogram was generated using Ward's method, with Euclidean distance applied to assess similarity.

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CONCLUSION

In the present study analysis of free radical scavenging activity and total phenolics and flavonoid content showed that methanol extract from pericarp and seed of nine varieties of *Cucurbita* genus can be a potent source of natural antioxidants. Considerable variations were observed between different examined varieties in terms of total antioxidant activity and different antioxidant polyphenolic compounds. *Spaghetti squash* is the species whose pericarp extract showed the best antioxidant characteristics in all methods (ABTS, DPPH, TRP, CUPRAC, FRAP). Also, *Curcubita maxima Roter zentner* pericarp extract shows significant antioxidant characteristics in the method. *Spaghetti squash* seed extract also has the highest results in four (ABTS, DPPH, CUPRAC, FRAP) of the five applied methods.

Principal component analysis obtained from antioxidative profiles grouped the tested samples into two groups, a group of pericarp extracts and a group of seed extracts. Hierarchical cluster analysis confirmed the PCA analysis, with one small difference. In addition to the two clusters containing only seed samples (Cluster 1) and only pericarp samples (Cluster 3), the hierarchical analysis identified a mixed cluster, which includes one pericarp sample (*Green zucchini*) alongside seven seed samples. The combined use of PCA and HCA demonstrated clear differentiation among *Cucurbita* extracts based on their antioxidant profiles. These insights are instrumental for breeding programs to enhance specific antioxidant traits in *Cucurbita* varieties, enabling the development of nutritionally superior crops.

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И З В О Д

ПРИМЕНА АНАЛИЗЕ ГЛАВНИХ КОМПОНЕНАТА И ХИЈЕРАРХИЈСКЕ КЛАСТЕР АНАЛИЗЕ ЗА КЛАСИФИКАЦИЈУ РАЗЛИЧИТИХ ЈЕСТИВИХ *CUCURBITA* НА ОСНОВУ *ИН ВИТРО* АНТИОКСИДАТИВНЕ АКТИВНОСТИ

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Ова студија је истраживала варијације у антиоксидативним профилима девет јестивих *Cucurbita* врста (перикарп и семе), користећи алате за препознавање образаца; класификација је постигнута на основу резултата тестова антиоксидативне активности (DPPH, ABTS, FRAP, CUPRAC, укупна редукујућа моћ, нивои укупних фенолних једињења и флавоноида). Узорци перикарпа су имали значајно ниже вредности укупних фенола од узорака семена. Екстракти семена и перикарпа врсте *Spaghetti squash* показују највећу способност неутралисања DPPH радикала. Ови екстракти такође показују и највеће вредности у FRAP и CUPRAC тестовима. Анализа главних компоненти вредности антиоксидативних профила испитиваних екстраката груписала је испитиване узорке у две

групе: екстракти перикарпа и екстракти семена. Хијерархијска кластер анализа потврдила је резултате анализе главних компоненти. Добијени резултати могу да усмере узгајиваче у одабиру *Cucurbita* врста са побољшаним антиоксидативним особинама, доприносећи развоју нутритивно супериорних усева.

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