STUDIA UBB CHEMIA, LXVIII, 4, 2023 (p. 93-110)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2023.4.07

COMPARISON OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC, FLAVONOID, PROANTHOCYANIDIN, SAPONIN CONTENTS OF EGGPLANT'S (*SOLANUM MELONGENA L.*) PULP AND PEEL – A CHEMOMETRIC APPROACH

Violeta D. MITIĆ[a], Jelena S. NIKOLIĆ[a], Marija V. DIMITRIJEVIĆ[a], Jelena M. MRMOŠANIN[a,*], Snežana B. TOŠIĆ[a], Aleksandra N. PAVLOVIĆ[a], Vesna P. STANKOV JOVANOVIĆ[a]

ABSTRACT. The eggplant is a vegetable that has been used more in recent years thanks to the low calories versus high content of phenolic compounds. This study aimed to determine total phenolic, flavonoid, proanthocyanidin, and saponin content using spectrophotometric assays and antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2'-azinobis(3-ethylbenzothiazol-6sulpho-nate (ABTS), total reducing power (TRP), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) assassin peel and pulp of eggplant cultivars Robi, Tudela, Vernal, Aragon, and Rosa Bianca. The average total saponin content (TSC) in peel was 116.34 µg DSGE mg^{-1} DW (diosgenin equivalents per mg of extract's dry weight), whereas in pulp was slightly lower (107.86 µg DSGE mg^{-1} DW). A similar trend was observed for total phenolic content (TPC) (24.54 µg GAE mg^{-1} DW (gallic acid equivalents per mg of extract's dry weight) in peel and 15.88 µg GAE mg^{-1} DW in pulp); total proanthocyanidin (TP) (2.74 µg CE mg^{-1} DW (catechin equivalents per mg of extract's dry weight) in peel and 1.24 µg CE mg^{-1} DW in pulp) and total flavonoid content (TFC) (0,05 µg RE mg^{-1} DW (rutin equivalents per mg of extract's dry weight) in peel and 0.02 µg RE mg^{-1} DW in pulp). Principal component analysis (PCA) and cluster analysis (CA) methods were applied on the antioxidant activity (ABTS, DPPH, TRP, FRAP, and CUPRAC) and total bioactive compounds

[a] *University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Višegradska 33, P.O. Box 224, 18000 Niš, Serbia*
* *Corresponding author: jelena.mrmosanin@pmf.edu.rs*

content (TSC, TPC, TP and TFC) parameters in order to reveal the relationships between analyzed samples. As a result of these approaches, analyzed samples were grouped into two groups.

Keywords: *Eggplant, Solanum Melongena, Antioxidants, Chemometric analysis*

INTRODUCTION

Eggplant (*Solanum melongena L.*), also known as aubergine, comes in wide varieties with various shapes (oval, ovoid shape, long club-shaped) and colours (white, yellow, green, and purple) [1, 2]. It is grown worldwide and is used in a diet because of the high concentration of bio-friendly metals (K, Mg, Ca, and Fe), low-calorie content, and affordable price [3].

Due to its high phenolic, alkaloid, and vitamin contents, the eggplant-rich diet positively affected hypercholesterolemia, asthma, diabetes, bronchitis, and digestive difficulty [4]. It has anti-inflammatory, hepatoprotective, hypolipidemic, antiallergic, and anticancer activity [5]. Many studies have shown that phenol compounds are responsible for the antioxidant activity of eggplant [6]. Kaushik et al. [7] found that eggplant has the most outstanding total phenolic acid content among 21 different vegetables, and the major soluble phenolic acid in eggplant is chlorogenic acid and its isomers. Caffeic acid and *p*-coumaric acid were also present in eggplant samples. The polyphenol oxidase catalyzes the enzymatic oxidation of phenol compounds to the quinones, which polymerize into water-soluble brown melanin [8]. The main anthocyanin present in eggplant peel was nasunin [9].

Previous studies have dealt with the identification and quantification of polyphenolic compounds using high-performance liquid chromatography (HPLC) [9,10], total phenolic content (TPC), and total flavonoid content (TFC) [5, 9-12]. Antioxidant activity was also estimated using ABTS [10, 12], DPPH [5], and FRAP assays [13]. Depending on the thermodynamic conditions and reactivity of the phenols' hydroxyl groups, it has been found that different oxidants transform the substrate into different products. Therefore, using multiple antioxidant assays is recommended to get a comprehensive picture of one's antioxidant characteristics.

The present study aimed to determine and compare the antioxidant activity of five eggplant cultivars peel and pulp, dominantly present in the Serbian markets, using five spectrophotometric assays (DPPH, ABTS, FRAP, TRP, and CUPRAC) and total flavonoid content (TFC), total phenolic (TPC) content, total proanthocyanidin (TP), and total saponin (TS) content. To the

best author's knowledge, total proanthocyanidin and saponin content in eggplant samples is evaluated for the first time. The obtained data were subjected to chemometric analysis to estimate the relations between peel and pulp of different eggplant varieties and used assays.

RESULTS AND DISCUSSION

Total phenolic, flavonoid, proanthocyanidin, saponin contents, and antioxidant activities of eggplant extracts

Phenolic compounds are secondary metabolites often combined with mono and polysaccharides via phenolic groups [14]. The main dietary phenolics are phenolic acids, flavonoids, and polyphenols [15]. Many studies have shown [16, 17] a positive and high correlation between phenolic compounds and the antioxidant potential of vegetables and fruits. The hydroxyl groups' hydrogen atoms in the o-position in rings A, B, and C, double bonds in aromatic rings, and C=O bonds are responsible for their high antioxidant activity [18].

DPPH, ABTS, CUPRAC, FRAP, and TRP are widespread since they apply to various samples, are reproducible, low-cost, and, above all, reliable. The antioxidant potentials of eggplant peel and pulp extracts were determined using five different spectrophotometric assays, and the results are presented in Table 1.

Table 1. Antioxidant activity of eggplant samples using five different assays (DPPH, ABTS, TRP, FRAP, and CUPRAC)

Name	Part of the eggplant	DPPH[1] (μg TE mg^{-1} DW) [2]	ABTS (μg TE mg^{-1} DW) [2]	TRP (μg AAE mg^{-1} DW) [2]	FRAP (μg FE mg^{-1} DW) [2]	CUPRAC (μg TE mg^{-1} DW) [2]
Robi	Pulp	1.15 ± 0.04	59.0 ± 0.4	18.30 ± 0.07	16.02 ± 0.03	84.72 ± 0.07
	Peel	11.0 ± 0.1	3.16 ± 0.06	24.7 ± 0.2	52.52 ± 0.04	80.48 ± 0.06
Tudela	Pulp	3.2 ± 0.1	8.6 ± 0.3	26.13 ± 0.04	21.72 ± 0.02	62.31 ± 0.08
	Peel	9.5 ± 0.3	3.18 ± 0.08	29.15 ± 0.07	47.32 ± 0.03	88.67 ± 0.09
Vernal	Pulp	4.6 ± 0.1	6.14 ± 0.009	22.7 ± 0.1	22.63 ± 0.03	63.68 ± 0.7
	Peel	11.1 ± 0.1	2.60 ± 0.03	29.8 ± 0.2	47.82 ± 0.07	90.3 ± 0.1
Aragon	Pulp	1.79 ± 0.02	11.44 ± 0.03	17.03 ± 0.04	14.22 ± 0.03	52.74 ± 0.05
	Peel	8.4 ± 0.3	7.2 ± 0.1	22.1 ± 0.1	43.32 ± 0.02	68.32 ± 0.03
Rosa Bianca	Pulp	2.19 ± 0.09	9.52 ± 0.07	17.80 ± 0.07	20.52 ± 0.05	73.24 ± 0.09
	Peel	5.07 ± 0.08	9.16 ± 0.01	16.61 ± 0.01	19.82 ± 0.04	56.70 ± 0.04

[1] mean ± SD,
[2] TE - Trolox equivalent, AAE - ascorbic acid equivalent, FE - Fe(II) equivalent, DW – dry weight.

Pulp extracts showed lower antioxidant activity, ranging from 52.74 to 84.72 μg TE mg^{-1} DW, than peel extracts (56.7 to 88.64μg TE mg^{-1} DW) according to the CUPRAC method. Antioxidant activity for eggplant in the study of Pasli et al. [19] was 72.59 μg TE mg^{-1} DW, which agrees with the results we obtained. Antioxidant activity determined using ABTS and DPPH was lower than those obtained by the CUPRAC assay. These results are most likely a consequence of the reaction mechanism on which the assays are based. In DPPH and ABTS assays, antioxidants react with organic radicals, so only smaller molecules can react due to the steric effect [20]. ABTS radical cation is formed in a reaction between ABTS and potassium persulfate. ABTS radical cation solution is then mixed with antioxidants from a sample and reduced to ABTS again. Its reduction depends on antioxidant concentration and reaction time. For peel samples, ABTS assay results ranged from 2.6 μg TE mg^{-1} DW (Vernal) to 9.16 μg TE mg^{-1} DW (Rosa Bianca). Results using the same assay for pulp extracts gave higher results, ranging from 6.14 μg TE mg^{-1} DW (Vernal) to 59 μg TE mg^{-1} DW (Robi). DPPH assay results differ from results obtained using ABTS assay, with slightly higher results for peel extract ranging from 5.07 μg TE mg^{-1} DW (Rosa Bianca) to 11.1 μg TE mg^{-1} DW (Vernal); than pulp samples ranging from 1.15μg TE mg^{-1} DW (Robi) to 4.6 μg TE mg^{-1} DW (Vernal). Pasli et al. [19] also analyzed eggplant extracts and recorded 27.87 μg TE mg^{-1} for ABTS and 23.43 μg TE mg^{-1} for DPPH assay. Colak et al. [20] have determined DPPH activity; the activity ranges 0.66 μg TE mg^{-1} FW (fresh weight) for white eggplant peel, 0.84 μg TE mg^{-1} FW for purple eggplant peel and 2.04 μg TE mg^{-1} FW for black eggplant peel; 0.166 μg TE mg^{-1} FW for white eggplant pulp, 0.364μg TE mg^{-1} FW for purple eggplant pulp and 0.584 μg TE mg^{-1} FW for black eggplant peel. Colak et al. [20] also obtained higher DPPH activities in peel samples than in pulp samples, which is consistent with our results.

FRAP and TRP assays both include the reduction of Fe^{3+} to Fe^{2+} with antioxidants from the sample. Results obtained using TRP and FRAP methods were similar: TRP assay's lowest values were 16.61 μg AAE (ascorbic acid equivalent) mg^{-1} DW for Rosa Bianca peel extract and 29.8 μg AAE mg^{-1} DW for Vernal peel extract. Unlike peel samples, pulp samples have slightly higher values using TRP assay than FRAP assays, except for the pulp sample Rosa Bianca which is lower. TRP activity ranges from 17.03 μg AAE mg^{-1} DW for Aragon pulp extracts to 26.13 μg AAE mg^{-1} DW for Tundela pulp extracts. FRAP values were in the range of 14.22 μg FE mg^{-1} DW for Vernal pulp extracts to 21.72 μg FE mg^{-1} DW for Tundela pulp extracts, and 19,82 μg FE mg^{-1} DW for Rosa bianca peel extracts to 52,52 μg FE mg^{-1} DW to Robi peel extracts. Also, the FRAP assay gave higher results for the peel than the pulp samples, except for the Rosa Bianca sample. Boulekbache-Makhlouf et al. [21] analyzed the byproduct of eggplants and recorded a reducing power of 39 mg QE 100 g^{-1} DW for methanolic extract.

Saponins are a class of compounds characterized by a skeleton derived from the 30-carbon precursor oxide-squalene to which glycosyl residues are attached [22]. Saponins found in eggplant act as pancreatic lipase inhibitors, which can be used in obesity treatment [23]. Total saponin content ranged from 102.3 μg DSGE mg^{-1} DW in Rosa Bianca to 140.4 μg DSGE mg^{-1} DW in Tudela peel (Table 2). Similar total saponin content was found in the pulp samples, from 100.5 μg DSGE mg^{-1} DW in Tudela to 121.8 μg DSGE mg^{-1} DW in Robi. In the review of published papers, only Hoang et al. [24] determined the TSC (total saponin content) by spectrophotometric method. According to these authors, the content is from 18.05 μg mg^{-1} DW for aqueous extracts to 38.34 μg mg^{-1} DW for methanolic extracts.

The bitterness of eggplant can come from a high concentration of saponins [25]. It was reported that some processing techniques (milling, boiling, and steaming) reduced bitterness and saponin content [26]. Also, *Solanum melongena L.* can be used to treat several diseases like bronchitis, arthritis, asthma, and diabetes. The main phenolic compounds are in the pulp and peel, so unpeeled eggplant should be consumed.

Table 2. Total phenolic, saponin, flavonoid, and proanthocyanidin content of eggplant samples

Name	Part of the eggplant	TPC[1] (μg GAE mg^{-1} DW)[2]	TSC (μg DSGE mg^{-1} DW)[2]	TFC (μg RE mg^{-1} DW)[2]	TP (μg CE mg^{-1} DW)[2]
Robi	**Pulp**	12.58 ± 0.04	121.8 ± 0.7	0.012 ± 0.001	1.04 ± 0.06
	Peel	26.4 ± 0.1	108.3 ± 0.6	0.064 ± 0.005	1.61 ± 0.06
Tudela	**Pulp**	21.52 ± 0.08	100.5 ± 0.9	0.014 ± 0.001	1.30 ± 0.03
	Peel	29.49 ± 0.08	140.4 ± 0.4	0.043 ± 0.003	4.7 ± 0.1
Vernal	**Pulp**	16.49 ± 0.06	104.5 ± 0.2	0.033 ± 0.001	2.05 ± 0.02
	Peel	29.4 ± 0.1	126 ± 1	0.075 ± 0.003	3.71 ± 0.09
Aragon	**Pulp**	13.09 ± 0.08	106.5 ± 0.2	0.0113 ± 0.0006	0.77 ± 0.01
	Peel	22.74 ± 0.04	104.7 ± 0.6	0.053 ± 0.002	2.5 ± 0.1
Rosa Bianca	**Pulp**	15.70 ± 0.06	106 ± 1	0.011 ± 0.001	1.02 ± 0.05
	Peel	14.65 ± 0.07	102.3 ± 0.3	0.022 ± 0.002	1.18 ± 0.06

[1] mean ± SD,
[2] GAE - gallic equivalent, DSGE - diosgenin equivalent, RE - rutin equivalent, CE – catechin equivalent, DW – dry weight.

Phenolic compounds are listed as the primary antioxidants in plant tissue, so determining their content can serve as quick antioxidant activity estimation. Cao et al. [27] characterized eggplant as among the top ten vegetables with high antioxidant activity. The total phenolic content ranged from 14.65 μg GAE mg^{-1} DW for Rosa bianca to 29.49 μg GAE mg^{-1} DW for Tudela peel extracts. Pulp samples had lower TPC, ranging from 12.58 μg GAE mg^{-1} DW for Robi to 21.52 μg GAE mg^{-1} DW for Tudela. According to Djounadi et al. [28], the total phenolic content was 41.3 to 82.31 μg GAE mg^{-1} for peel samples and 15.29 to 23.78 μg GAE mg^{-1} for pulp samples for two types of eggplant. Salerno et al. [5] also investigated total phenolic content in eggplant samples, and their results were lower, ranging from 4.49 to 4.80 μg GAE mg^{-1} DW for pulp samples and 6.12 to 6.78 μg GAE mg^{-1} DW for peel samples. According to Arkoub-Djermoune et al. [29], the total phenolic content in eggplant samples using 70% methanol as solvent was 6.69 μg GAE mg^{-1}. Ferarsa et al. [30] reported that TPC ranged from 0.02 μg GAE mg^{-1} DW to 1.99 μg GAE mg^{-1} DW. Somewhat different values obtained in this study may be due to differences in antioxidant activity assay conditions and/or different antioxidant activities of analyzed eggplants species. The present study results follow the trend established in previous studies - the higher total phenolic content in eggplant peel versus its pulp.

The total flavonoid content ranged from 0.022 μg RE mg^{-1} DW for Rosa Bianca peel extracts to 0.075 μg RE mg^{-1} DW for Vernal peel samples. TFC ranged from 0.011 μg RE mg^{-1} DW for Rosa Bianca pulp extracts to 0.033 μg RE mg^{-1} DW for Vernal pulp samples. Total flavonoid content was higher in all peel extracts than in pulp extracts of the same type of eggplants. According to Nayanathara et al. [31], total flavonoid content ranged from 22.62 μg CE mg^{-1} DW for the Long green eggplant sample to 102.01 μg CE mg^{-1} DW for the Violet uphold eggplant sample. Boulekbache-Makhlouf et al. [21] reported total flavonoid content in methanolic extract eggplant was 0.1626 μg QE mg^{-1} DW. Comparing the results presented in this study with the previous studies is problematic since, in the previous study, experiments were done with different standards (catechin and quercetin), and the results were recalculated to fresh weight while our results refer to dry weight. The big issue for comparison still stands - water percentage, which can vary significantly depending on species, climate and storage sample conditions. Nevertheless, the trend is the same-higher flavonoid content in the peel versus pulp.

Among phenolics in fruits and vegetables, there is a large group of compounds called proanthocyanidins, also known as condensed tannins [32]. The total proanthocyanidin contents in tested samples were higher in the peel than in pulp extracts. Peel extracts' total proanthocyanidin content varied from 1.18 to 4.7 μg CE mg^{-1}, while pulp extracts ranged from 0.77 to 2.05 μg CE mg^{-1}.

Considering the present results, we can conclude that the total proanthocyanidin's content were higher in the peel than in the pulp samples.

Antioxidant Composite Index (ACI)

Because the antioxidant activity of extracts was obtained by five different assays: two assays based on reactions with free radicals (ABTS and DPPH), two assays based on the reducing power of iron (TRP and FRAP), and one assay based on the reducing power of cupric ion (CUPRAC), results are challenging to compare. Therefore, the antioxidant activity index (ABTS, DPPH, TRP, FRAP, and CUPRAC index) and antioxidant composite index - ACI index were calculated and presented in Table 3. The results of the antioxidant index and ACI index were given as relative percentages. The highest ACI index, based on the results of five antioxidant activity assays, was recorded for the Vernal peel sample (78.1), followed by Robi peel (75.3) and Tudela peel (74.4). All peel samples had a higher ACI index than pulp samples of the same type. The sample Vernal peel had the highest $\mathrm{DPPH_{index}}$, $\mathrm{TRP_{index}}$ and $\mathrm{CUPRAC_{index}}$. The lowest ACI index was noticed for sample Aragon pulp.

Table 3. The antioxidant composite index for eggplant samples calculated from five different antioxidant activity assays

Name	Sample	$\mathrm{DPPH_{index}}$	$\mathrm{ABTSi_{index}}$	$\mathrm{TRP_{index}}$	$\mathrm{FRAP_{index}}$	$\mathrm{CUPRAC_{index}}$	$\mathrm{ACI_{index}}$
Robi	**Pulp**	10.4	100.0	61.4	28.8	93.8	58.9
	Peel	99.1	5.4	82.9	100.0	89.1	75.3
Tudela	**Pulp**	28.8	14.6	87.7	39.1	69.0	47.7
	Peel	85.6	5.4	97.8	85.2	98.2	74.4
Vernal	**Pulp**	41.4	10.4	76.2	40.8	70.5	47.9
	Peel	100.0	4.4	100.0	86.1	100.0	78.1
Aragon	**Pulp**	16.1	19.4	57.1	25.6	58.4	35.3
	Peel	75.7	12.2	74.2	78.0	75.7	63.2
Rosa Bianca	**Pulp**	19.7	16.1	59.7	37.0	81.1	42.7
	Peel	45.7	15.5	55.7	35.7	62.8	43.1

Statistical analysis

Statistical analysis was performed to understand further the relations between analyzed samples and methods used. The correlation matrix is given in Table 4. The correlation between the results of spectrophotometric assays - ABTS, DPPH, TRP, FRAP, CUPRAC, TPC, TFC, TSC, and TP was calculated.

Table 4. Correlation coefficient between results obtained using spectrophotometric methods

	DPPH	ABTS	TPR	FRAP	CUPRAC	TPC	TSC	TFC	TP
DPPH	1.00								
ABTS	-0.56	1.00							
TPR	**0.74**	-0.43	1.00						
FRAP	**0.97**	-0.48	**0.75**	1.00					
CUPRAC	0.54	0.17	0.61	**0.64**	1.00				
TPC	**0.90**	-0.55	**0.92**	**0.93**	0.63	1.00			
TSC	0.41	0.14	0.55	0.46	**0.81**	0.53	1.00		
TFC	**0.96**	-0.47	**0.70**	**0.92**	0.56	**0.83**	0.35	1.00	
TP	**0.74**	-0.39	**0.81**	**0.74**	**0.64**	**0.83**	0.77	**0.67**	1.00

The strongest correlation was noticed between DPPH and FRAP assays (0.97, $p<0.05$), followed by TRP and FRAP (0.75, $p<0.05$), DPPH, and TRP (0.74, $p<0.05$). A strong correlation between DPPH and FRAP was reported in the literature [33, 34], which is expected because both assays share a similar mechanism, which includes electron transfer from an antioxidant to reduce an oxidant. Since the TRP assay includes a reduction of Fe^{3+} to Fe^{2+} in the FRAP assay, the correlation between them is also expected. ABTS assay showed a poor and negative correlation with other spectrophotometric assays. Also, the negative and low correlation was between ABTS assay and TPC, TSC, TFC, and TP. ABTS reacts with small molecules and can give real pictures of the antioxidant activity of plant extract [35].

A high positive correlation was recorded between TRP and TPC (0.92, $p<0.05$), DPPH, and TPC assays (0.90, $p<0.05$). Also, a high positive correlation was noticed between DPPH/TFC (0.96, $p<0.05$), TRP/TFC (0.7, $p<0.05$), and FRAP/TFC (0.7, $p<0.05$). This positive correlation confirms that

phenolic and flavonoid compounds in eggplant samples contribute to their antioxidant activity. Proanthocyanidin also showed good correlations with TRP, FRAP, DPPH, and CUPRAC, so they also affected the antioxidant activity of eggplant samples.

Of the many chemometric techniques, the most common are used multivariate techniques that allow systematic data extraction, usually in large sets. As this method reduces the dimensionality of the initial data without losing essential information and retaining variability, the components with the most significant variance were used [36]. PCA analysis gave two principal components (PC) with eigenvalues>1, which is acceptable according to the Kaiser criterion [37]. For PC analysis, we used eggplant samples as a case, antioxidant assays (ABTS, DPPH, FRAP, and TRP), and assays for determining total phenolic, flavonoid, proanthocyanidin, and saponin content as a variable. These two principal components explain 87.26% of the total variance. The first principal component (PC1) had the highest eigenvalue (6.22), explaining 69.11% of the total variance. The second component had 1.64 eigenvalues, explaining 18.15% of the total variance. Based on the first principal component, all tested samples were grouped into two groups. The first group is on the positive sides of PC1 and PC2 and consists of eggplant pulp samples Tudela, Vernal, and Aragon and one eggplant peel samples Rosa Bianca. Aragon and Rosa Bianca pulp samples have the lowest $DPPH_{index}$ values (16.1 and 19.7, respectively). Robi pulp sample is also in this group, on the negative side of PC2. This sample stands out as a sample with the highest loading (-3.08) and the highest contribution from ABTS ($ABTS_{index}$ =100), which can also be seen in Figure 1, and Tables 1 and 3. Rosa Bianca peel and Aragon pulp samples are in the upper right quadrant (Figure 2), and CUPRAC is in the lower-left quadrant, indicating they have the lowest value of CUPRAC (CUPRAC index value is 62.8 and 58.4, respectively). The second group is on the negative side of PC1. This group consists of Robi, Tudela, Vernal, and Aragon eggplant peel samples. According to the component PC2, Robi and Aragon peel samples stand out on the positive side of PC2; these are the samples with the highest values of TPC, TFC, and the high values of DPPH (Figure 1 and Tables 1, 2 and 3). The Tudela and Vernal peel samples are on the negative side of PC2, as samples with the highest value of TSC (140.4 and 126 μg DSGE mg^{-1} DW, respectively), TP (4.7 and 3.7 μg CE mg^{-1} DW, respectively) and CUPRAC (88.67 and 90.3 μg TE mg^{-1} DW, respectively). Figure 1 shows that grouping spectrophotometric assays DPPH and FRAP with TPC and TFC; CUPRAC with TSC and TP are consistent with the Pearson correlation (Table 4).

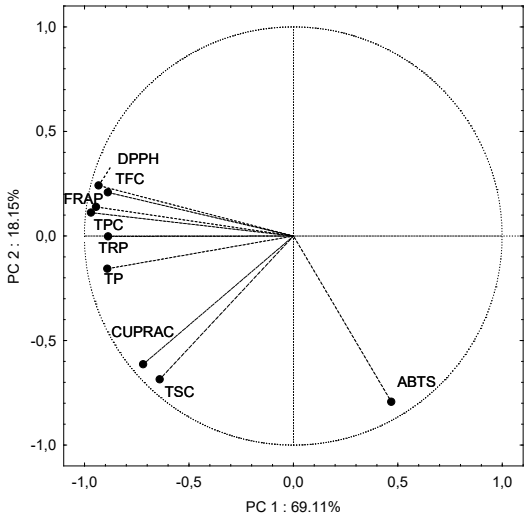

Figure 1. Grouping spectrophotometric assays based on the antioxidant activity in tasted eggplant samples using PCA

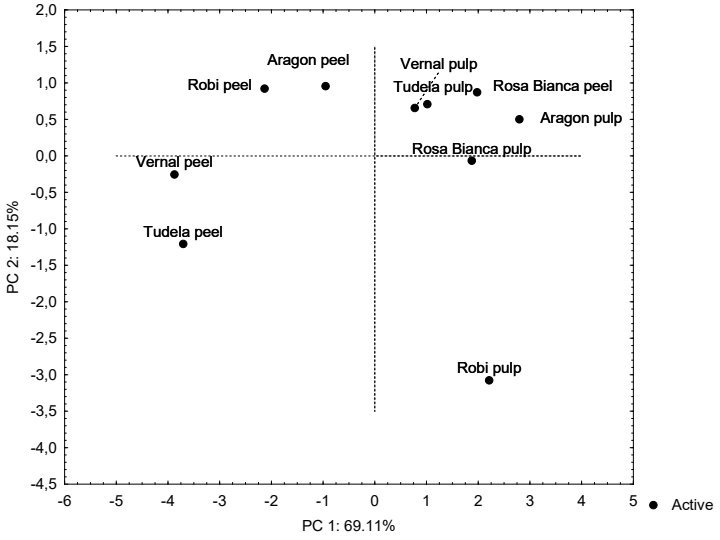

Figure 2. Grouping peel and pulp eggplant samples using PCA

From the results of the factor analysis, we can infer which assays or samples contribute to the differentiation between the peel and pulp. The original correlation matrix of the principal components underwent orthogonal varimax rotation, and Table 5 present the factor loadings of the principal components PC1 and PC2.

Table 5. The Varimax rotated matrix of principal components, along with factor loading values and communalities

	Component		Communalities			Component		Communalities	
	PC1	PC2	Initial	Extraction		PC1	PC2	Initial	Extraction
DPPH	**0.916**	0.308	1.00	0.933	**Robi peel**	**0.878**	0.465	1.00	0.987
TPC	**0.872**	0.433	1.00	0.947	**Vernal peel**	**0.856**	0.514	1.00	0.997
FRAP	**0.871**	0.397	1.00	0.916	**Aragon peel**	**0.843**	0.532	1.00	0.994
ABTS	**-0.826**	0.413	1.00	0.853	**Tudela peel**	**0.840**	0.543	1.00	0.999
TFC	**0.815**	0.313	1.00	0.762	**Vernal pulp**	**0.771**	**0.633**	1.00	0.995
TRP	**0.735**	0.486	1.00	0.777	**Tudela pulp**	**0.765**	**0.637**	1.00	0.991
Total proanthocyanidins	**0.665**	**0.612**	1.00	0.817	**Rosa Bianca pulp**	**0.741**	**0.667**	1.00	0.994
Total saponins	0.167	**0.922**	1.00	0.878	**Rosa Bianca peel**	**0.739**	**0.668**	1.00	0.993
CUPRAC	0.272	**0.906**	1.00	0.895	**Robi pulp**	0.454	**0.881**	1.00	0.983
Eigen-values	6.158	**1.620**	9.00	7.778	**Aragon pulp**	0.693	**0.709**	1.00	0.983
Variance (%)	53.202	**33.218**	100	86.42	**Eigen-values**	9.656	0.26	10.00	9.913
					Variance (%)	58.818	40.341	100	99.159

For instance, DPPH, TPC, FRAP, ABTS assay show high loadings on PC1, whereas total saponins and CUPRAC exhibit hight loading on PC2. This indicates that these assays have a greater influence on the formation of the components.

High communalities for individual samples (e.g. Robi peel, Vernal peel and Aragon peel) suggest that these samples share similar characteristics or variations that are well-explained by the extracted components (PC1 and PC2). Additionally, the communalities for pulp samples (e.g. Vernal pulp, Tudela pulp) also show similar patterns.

The same data matrix as in principal component analysis was used for cluster analysis. This chemometric technique was used as a supplement to PCA to obtain the most accurate classification of tested samples. The results are shown in Figures 3 and 4. For clustering, Euclidean distance and Ward's method were used.

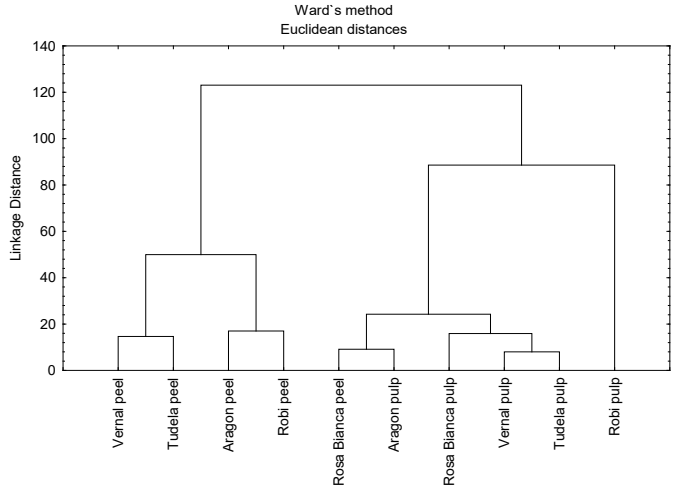

Figure 3. Grouping tested peel and pulp samples based on the antioxidant activity using CA

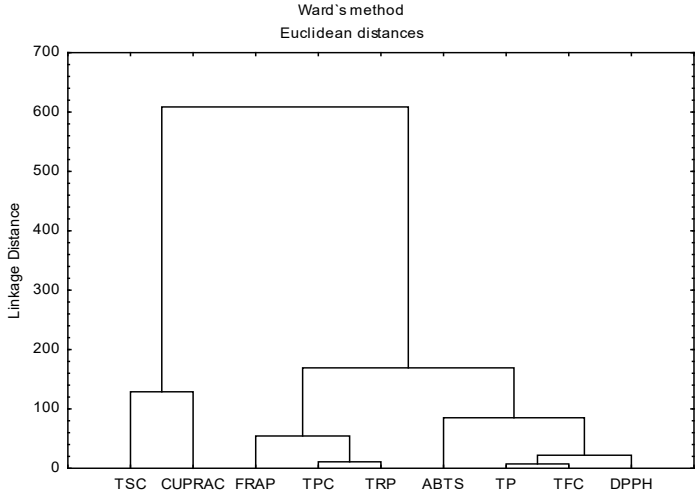

Figure 4. Grouping spectrophotometric assays based on the antioxidant activity in tested eggplant samples using CA

The first cluster consists of three subclusters: the first subcluster includes Tudela, Vernal, and Aragon pulp samples; the second cluster includes Rosa Bianca peel and Aragon pulp samples; and the third includes Robi pulp samples. The second cluster comprises two subclusters: the first subcluster includes Robi and Aragon peel samples, and the second subcluster includes Tudela and Vernal peel samples. The explanation for this grouping of the samples is the same as for PCA.

CONCLUSIONS

Antioxidant activity and bioactive compounds (phenolics, flavonoids, proanthocyanidins and saponins) in five eggplant peel and pulp samples commonly consumed in Serbia were analyzed using spectrophotometric assays. According to the results, analyzed eggplant samples have a high content of phenolic compounds and saponins. Higher total phenolic content was recorded for peel than pulp samples, which is also the case with total proanthocyanidin content. Total saponin and phenolic content strongly correlated with all assays used for antioxidant activity determination, indicating those compounds contribute the most to overall antioxidant activity. Chemometric statistical techniques (PCA and cluster analysis) formed two groups of tested samples according to antioxidant activity and total content of phenol, flavonoid, proanthocyanidin, and saponin. Using the PCA analysis the parameters differentiating the Rosa Bianca peel were revealed. They are low values of TRP, TPC and CUPRAC assay.

EXPERIMENTAL SECTION

Methods and Materials

Sample preparation and extraction procedure

Five eggplant varieties, Robi, Tudela, Vernal, Aragon, and Rosa Bianca, were purchased at the local market in Niš, Serbia. The samples were prepared according to Dranca and Oroian [38], with some modifications. The peel was washed and separated from the pulp; samples were cut into pieces and homogenized. Eggplant peel and pulp were lyophilized and kept in polyethene bags at -20°C until analysis. Lyophilized samples (1 g) were extracted twice for 15 min at 25 °C in an ultrasonic bath, using methanol as a solvent. Samples were left in the solvent overnight, filtered, and evaporated to dryness, using a vacuum rotary evaporator. Dimethyl sulphoxide (DMSO) dissolved extracts to a final concentration of 100 mg mL^{-1}.

Chemicals and instrumentation

2,2-Diphenyl-1-picrilhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent, iron(III) chloride hexahydrate, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, ascorbic acid, (+)-catechin hydrate, vanillin, diosgenin, rutin, and methanol were purchased from Sigma (St. Louis, Missouri, USA). Copper(II) chloride dihydrate, 2,9-dimethyl-1, 10-phenanthroline (neocuproine), sodium carbonate, hydrochloric acid, 2, 4, 6-Tris(2-pyridyl)-s-triazine (TPTZ), potassium hexacyanoferrate(III), phosphate buffer (NaH_2PO_4/Na_2HPO_4), ammonium acetate buffer, trichloroacetic acid, potassium peroxodisulfate, $AlCl_3$ x $6H_2O$, DMSO, H_2SO_4, hydrochloric acid, sodium hydroxide, sodium nitrite, and iron(II) sulfate heptahydrate were purchased from Merck (Darmstadt, Germany), all listed chemicals are of analytical grade.

For spectrophotometric analysis, UV/VIS spectrophotometer was used (Perkin Elmer Lambda 15, Massachusetts, USA).

A lyophilizer (Christ, Germany) was used to dry the samples.

DPPH radical scavenging activity

The DPPH radical assay was conducted according to the method described by Brand-Wiliams et al. [39]. The extract aliquot (10 μL) was mixed with DPPH solution and diluted with methanol to a final volume of 4 mL. After the mixture was incubated for 60 minutes in the dark at room temperature, absorbance was recorded at 515 nm. The results were expressed as μg Trolox equivalent (TE) per mg of extract's dry weight (DW).

ABTS radical scavenging activity

The ABTS radical activity assay was conducted according to the method described by Re et al. [40]. Previously prepared ABTS solution was mixed with extract aliquot (10 μL), and the mixture was diluted with methanol to a final volume of 4 mL. After the mixture was incubated (6 min; room temperature), absorbance was recorded at 734 nm. The results were expressed as μg Trolox equivalent (TE) per mg of extract's dry weight (DW).

Total Reducing Power Assay (TRP)

The total reducing power assay was determined as per the method described by Oyaizu [41], based on the ability of antioxidants in samples can reduce Fe(III) to Fe(II). Briefly, 10 μL extracts were mixed with 1 mL of 1% $K_3[Fe(CN)_6]$ and the same volume of phosphate buffer pH 6.6 solution and water. This mixture was incubated for 30 minutes at 50 °C. After incubation, 1 mL of CCl_3COOH and 0.6 mL $FeCl_3$ were added. The absorbance was recorded at 700 nm, and results were expressed as μg ascorbic acid equivalent (AAE) per mg of extract's dry weight (DW).

Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay [42] was used method that uses antioxidants as reductants in colorimetric reaction where Fe^{3+}-TPTZ is reduced to blue Fe^{2+}-TPTZ complex. Extract aliquot (10 µL) was mixed with 3 mL of freshly prepared FRAP solution and diluted with water to a final volume of 4 mL. Then the mixture was incubated for 5 min at 37 °C, absorbance was recorded at 595 nm, and results were expressed as µg Fe(II) per mg of extract's dry weight (DW).

Cupric Ion Reducing Antioxidant Capacity Assay (CUPRAC)

CUPRAC assay of antioxidant measurement was used. The Cu(II)-neucuproine complex oxidize to orange-yellow Cu(I)-neocuproine complex [43]. The stable complex of Cu(I) with neocuproine was obtained by mixing 10 µL of extract solution, 1 mL of neocuproine, 1 mL of ammonium acetate solution, 1 mL of $CuCl_2$, and 1.9 mL of ethanol. The absorbance of this complex was measured at 450 nm after incubation for 30 minutes at room temperature. The results were expressed as µg Trolox (TE) per mg of extract's dry weight (DW).

Total Phenolic Content (TPC)

Total phenolic content was determined using modified Folin-Ciocalteu method [44]. The extract's solution volume of 0.1 mL was mixed with Folin-Ciocalteu reagent, 20% sodium carbonate, and deionized water. After incubation for 30 minutes in the dark, absorbance was measured at 750 nm. Results were expressed as µg gallic acid equivalents (GAE) per mg of extract's dry weight (DW).

Total Saponin Content (TSC)

Total saponin content was determined using spectrophotometric method as per the method described by Hiai et al. [45]. The method is based on the reaction of triterpene, oxidized by sulfuric acid with vanillin, which produces a coloured product with maximum absorbance at 544 nm. The extract's solution aliquot was mixed with vanillin and H_2SO_4, and then the mixture was incubated in a water bath for 10 minutes at 60 °C. The results were expressed as µg diosgenin equivalents (DSGE) per mg of extract's dry weight (DW).

Total Flavonoid Content (TFC)

A mixture for total flavonoid content determination was prepared by mixing 0.1 mL of the extract, deionized water, and $NaNO_2$. The obtained mixture was incubated for 5 minutes at room temperature, then $AlCl_3$ solution was added, followed by incubation for 5 minutes, and NaOH was added. The absorbance was recorded at 510 nm, according to Jia et al. [46]. The results were expressed as µg of rutin equivalents (RE) per mg of extract's dry weight (DW).

Total Proanthocyanidin Assay (TP)

This assay is a quick method for quantitatively determining proanthocyanidin (condensed tannin) in many plant materials [47]. Total proanthocyanidin was estimated using method previously described by Price et al. [48]. The extract's solution was mixed with 4% vanillin and conc. HCl. After 15 minutes of incubation, absorbance was recorded at 500 nm. The results were given as μg of catechin equivalents (CE) per mg of extract's dry weight (DW).

Antioxidant Composite Index

The antioxidant composite index – ACI and the antioxidant index were calculated using the following equations [49]:

$$ACI_{index} = \frac{(ABTS_{index}+DPPH_{index}+FRAP_{index}+TRP_{index}+CUPRAC_{index})}{5} \quad (1)$$

Where: $ABTS_{index}$, $DPPH_{index}$, $ABTS_{index}$, $FRAP_{index}$, TRP_{index} and $CUPRAC_{index}$ are calculated by taking the sample score, dividing it by the best score, and then multiplying the result by 100.

Statistical analysis

The results were presented as mean value ± standard deviation (SD) of triple measurements. Two statistical techniques were used for statistical data processing - the principal component analysis (PCA) and cluster analysis (CA) using Statistica 8.1 (StatSoft, Tusla) software. The principal component analysis is a method used to reduce the number of variables, with new variables independent of one another [50]. Cluster analysis is a chemometric technique examining variables' relationships [51]. At the same time, the dendrogram, which is obtained, gives an overview of the sample grouping based on the results of spectrophotometric assays.

ACKNOWLEDGMENTS

The authors thank the financial support from the Ministry of Education, Science and Technology Development of the Republic of Serbia (Agreement No. 451-03-47/2023-01/200124).

REFERENCES

1. M. H. Aminifard; H. Aroiee; H. Fatami; A. Ameri; S. Karimpour; *J. Cent. Eur. Agric.*, **2010**, *11*, 453–458
2. T. K. Koley; S. K. Tiwart; A. Sarkar; J. Nishad; A. Goswami; B. Singh; *Agric. Res.,* **2018**, *8*, 9–20
3. M. Zenia; B. Halina; *J. Elem.* **2008**, *13*, 269–274

4. G. E. Vural; E. Ari; *Sci. Hortic.* **2020**, *272*, 109472
5. L. Salerno; M. N. Modica; V. Pittalá; G. Romeo; M. A. Siracusa; C. Di Giacomo; V. Sorrenti; R. Acquaviva; *Sci. World J.,* **2014**, 1–6
6. M. Dimitrijević; V. Stankov-Jovanović; J. Cvetković; T. Mihajlov-Krstev; G. Stojanović; V. Mitić; *Anal. Methods,* **2015**, *7,* 4181–4191
7. P. Kaushik; L. Andujar; S. Vilanova; M. Plazas; P. Gramazio; F. J. Herraiz; N. S. Brar; J. Prohens; *Molecules*, **2015**, *20 (10),* 18464–18481
8. D. P. Uscanga-Sosa; M. B. Pérez-Gago; F. C. Gómez-Merino; J. A. Herrera-Corredor; A. S. Hernández-Cázares; A. Contreras-Oliva; *Not. Bot. Horti Agrobot. Cluj-Napoca,* **2020**, *48 (1)*, 79–89
9. G. Niño-Medina; V. Urías-Orona; M. D. Muy-Rangel; J. B. Heredia; *S. Afr. J. Bot.,* **2017**, *111*, 161–169
10. P. Šilarová; L. Boulekbache-Makhlouf; F. Pellati; L. Česlová; *Antioxidants,* **2019**, *8 (7),* 234–244
11. A. P. Singh; D. Luthria; T. Wilson; N. Vorsa; V. Singh; G. S. Banuuelos; S. Pasakdee; *Food Chem.,* **2009**, *114,* 955–961
12. B. Okmen; H. O. Sigva; S. Mutlu; S. Doganlar; A. Yemenicioglu; A. Frary; *Int. J. Food Prop.,* **2009**, *12,* 616–624
13. M. Kazemi; F. Khodaiyan; S. S. Hosseini; Z. Najari; *Waste Manag.,* **2019**, *100*, 101-111
14. J. B. Harborne; H. Baxter; G. P. Moss; *Phytochemical Dictionary: Handbook of Bioactive Compounds from Plants,* 2nd ed.; Taylor & Francis, London, **1999**, pp. 976
15. A. King; G. Young; *J. Am. Diet. Assoc.*, **1999**, *99*, 213–218
16. C. V. K. Reddy; D. Sreeramulu; M. Raghunath; *Food Res. Int.*, **2010**, *43*, 285–288
17. C. Sarawong; R. Schoenlechner; K. Sekiguchi; E. Berghofer; P. K. W. Ng; *Food Chem.*, **2014**, *143*, 33–39
18. C. A. Rice-Evans; N. J: Miller; *Free Rad. Biol. Med.*, **1996**, *20*, 933–956
19. A. A. Pasli; M. Yavuz-Düzgün; U. Altuntas; G. Altin; B. Özçelik; E. Firatligil; *Int. Food Res. J.*, **2019**, *26*, 793–800
20. N. Colak; A. Kurt-Celebi; J. Gruz; M. Strnad; S. Hayirlioglu-Ayaz; M. G. Choung; T. Esatbeyoglu; F. A. Ayaz; *Molecules*, **2022**, *27*, 2410
21. L. Boulekbache-Makhlouf; L. Medouni; S. Medouni-Adrar; L. Arkoub; K. Madani; *Ind.I Crops Bioprod.*, **2013**, *49*, 668–674
22. J. P. Vincken; L. Heng; A. de Groot; H. Gruppen; *Phytochem.*, **2007**, *68*, 275–297
23. S. Subandi; L. Zakiyaturrodliyah; T. Brotosudarmo; Saponin from purple eggplant (*Solanum Melongena* L.) and their activity as pancreatic lipase inhibitor. *IOP Conference Series: Materials Science and Engineering 509*, May 3, **2019**
24. T. N. N. Hoang; T. T. Phan; T. K. L. Phan; N. H. V. Nguyen; T. A. D. Dong; T. H. A. Le; *J. Food Process. Preserv.*, **2023**, article ID 2581641, 1–16
25. L. Heng; J. P. Vincken; G. van Koningsveld; A. Legger; *J. Sci. Food Agric.*, **2006**, *86*, 1225–1231
26. K. Sharma; R. Kaur; S. Kumar; R. Kumar Saini; S. Sharma; S. V. Pawde; V. Kumar; *Food Chem. Advanc.*, **2023**, 2, 100191
27. G. Cao; E. Sofic; R. L. Prior; *J. Agric. Food Chem.*, **1996**, *44*, 3426–3431
28. A. Djouadi; T. Lanez; C. Boubekri; *J. Fundam. Appl. Sci.,* **2016**, *8*, 223–231

29. L. Arkoub-Djermoune; F. Benmeziane; K. Madani; L. Boulekbache-Makhlouf; *Adv. Hortic. Sci.*, **2019**, *33*, 567–580
30. S. Ferarsa; W. Zhang; N. Moulai-Mostefa; L. Ding; M. Y. Jaffrin; N. Grimib; *Food Bioprod. Process.*, **2018**, *109*, 19–28
31. A. R. Nayanathara; A. Mathews; K. P. Aalolam; J. K. Reshma; *Emerg. Life Sci. Res.*, **2016**, *2*, 63–65
32. X. Han; T. Shen; H. Lou; *Int. J. Mol. Sci.*, **2007**, *8*, 950–988
33. H. X. Xu; J. W. Chen; *J. Sci. Food Agric.*, **2011**, *91*, 1057–2063
34. A. Szydłowska-Czerniak; I. Bartkowiak-Broda; I. Karlović; G. Karlovits; E. Szłyk; *Food Chem.*, **2011**, *127*, 556–563
35. R. L. Prior; X. Wu; K. Schaich; *J. Agric. Food Chem.*, **2005**, *53*, 4290–4302
36. D. Casoni; C. Sârbu; *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, **2014**, *118*, 343–348
37. H. F. Kaiser; *Educ. Psychol. Meas.*, **1960**, *0*, 141–151
38. F. Dranca; M. Oroian; LWT *J. Food Process Eng.* **2017**, *40*, e12312
39. W. Brand-Wiliams; M. E. Cuvelier, C. Berset; *Lebensmittel-Wissenschaft und-Technologie,* **1995**, *28*, 25–30
40. R. Re; N. Pellegrini; A. Proteggente; A. Pannala; M. Yang; C. Rice-Evans; *Free Radic. Biol. Med.,* **1999**, *26*, 1231–1237
41. M. Oyaizu; *Jpn. J. Nutr. Diet.,* **1986**, *44,* 307–315
42. I. F. F. Benzie; J. J. Strain; *Meth. Enzymol.,* **1999**, *299*, 15–27
43. R. Apak; K. Güçlü; M. Özyürek; S. E. Çelik; *Microchim. Acta*, **2008**, *160*, 413–419
44. R. L. Prior; X. Wu; K. Schaich; *J. Agric. Food Chem.*, **2005**, *53*, 4290–4302
45. S. Hiai; H. Oura; H. Hamanaka; Y. Odaka; *Planta Med.*, **1975**, *28*, 131–138
46. Z. Jia; M. Tang; J. Wu; *Food Chem.*, **1999**, *64*, 555–559
47. L. G. Butler; M. L. Price; J. E. Brotherton; *J. Agric. Food Chem.*, **1982**, *30*, 1087–1089
48. M. L. Price; S. V. Scoyoc; L. G. Butler; *J. Agric. Food Chem.*, **1978**, *26*, 1214–1218
49. N. P. Seeram; M. Aviram; Y. Zhang; S. M. Henning; L. Feng; M. Dreher; D. Heber; *J. Agric. Food Chem.*, **2008**, *56*, 1415–1422
50. A. C. Rencher; *Methods of Multivariate Analysis,* 2nd ed.; John Willey, New York, **1995**
51. F. Zolfaghari; H. Khosravi; A. Shahriyari; M. Jabbari; A. Abolhasani; *PLOS ONE*, **2019**, *14*, e0226355