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THE PERICARP OF CITRUSES: WASTE PRODUCT OR POLYPHENOL RESOURCE

BY

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Abstract. Citruses, members of the *Rutaceae* family, are globally renowned for their nutritional and health benefits. While the edible parts are widely consumed, the peels are often discarded despite their rich polyphenol content, particularly flavonoids like hesperidin, naringin, and quercetin. These polyphenols offer significant antioxidant, anti-inflammatory, and antimicrobial properties. The aim of our study was to evaluate the secondary metabolites profile and antioxidant potential of peels from four citrus species: sweet oranges, mandarins, grapefruits, and lemons. Fresh peels were cut and prepared for a microscopic identification, then methanolic maceration extracts were obtained in a ratio of 1:20 (m/v). The major group of active compounds were first screened by chemical reactions with sodium hydroxide, aluminium chloride, Folin-Ciocâlteu Reagent and by thin-layer chromatography (TLC). Total flavonoids, total polyphenols and carotenoids were quantified with spectrophotometric tests. Moreover, the antioxidant capacity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay. The obtained results indicated that although the chemical composition varies depending on species, there is a similar chemical profile for all samples. Nevertheless, lemon peel extracts exhibited the highest antioxidant activity, followed by mandarins and oranges, with grapefruit showing the lowest. The findings underscore the potential of citrus peels as a valuable resource for bioactive compounds, advocating for their

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utilization in nutritional and medicinal applications to promote sustainability and reduce waste.

Keywords: citrus peels, polyphenols, flavonoids, antioxidant activity, sustainable utilization.

1. Introduction

Citruses are members of the *Rutaceae* family and constitute a major group of fruit crops grown globally. They are highly valued not only for their refreshing taste and nutritional benefits but also for their health-enhancing properties. This diverse genus includes oranges, lemons, limes, grapefruits, and tangerines, all of which are abundant in bioactive compounds (Richa *et al*., 2023; Cernătescu *et al*., 2023a).

Among these, polyphenols are particularly notable for their strong antioxidant, anti-inflammatory, and antimicrobial effects. While the flesh of citrus fruits is commonly consumed and celebrated for its high vitamin C content and other essential nutrients, the peels are often discarded as waste, although contain a high concentration of these beneficial polyphenols (Harikrishnan *et al*., 2020).

Polyphenols, a varied group of naturally occurring compounds, are primarily categorized into flavonoids, phenolic acids, tannins, and stilbenes. In the peels of citrus fruits, flavonoids such as hesperidin, naringin, and quercetin are prevalent. These compounds not only contribute to the distinctive flavour and colour of citrus fruits but also offer numerous health benefits. Extensive research has demonstrated their role in the prevention and management of chronic diseases, including cardiovascular diseases, cancers, and neurodegenerative disorders (Chen *et al*., 2018, Lungu *et al*., 2020).

Despite their significant potential, the polyphenols found in citrus peels remain largely underutilized. This is mainly due to a lack of awareness and the challenges associated with efficiently extracting these compounds. However, recent advancements in extraction technologies and a growing interest in sustainable practices have sparked renewed interest in citrus peel polyphenols.

By valorising this by-product, it is possible to reduce waste while harnessing a rich source of bioactive compounds for both nutritional and medicinal applications. This shift not only promotes environmental sustainability, sugar preservation, oil and vinegar maceration, as showed in a previous study (but also opens new avenues for the development of health-promoting products (Satari and Karimi, 2023).

2. Materials and methods

For this study we have acquired 4 different species of citruses, bought from the local market, taking into account the country of origin and the bio/NON-GMO label.

The fruit samples were as follows: sweet oranges: P (*Citrus sinensis*) – Greece; mandarins: M (*Citrus reticulata*) – Turkey; grapefruits: G (*Citrus* x *paradisi*) – No data; lemons: L (*Citrus limon*) – Italy. Only lemons were stated as organic, all the other samples were indicated as being pulverised with fungicides prior to transportation.

For the microscopical assessment, the samples were used under powder form, and then clarified using heat and an 80% chloralhydrate solution directly on the microscope slide. The clarification was repeated several times (5) to allow the observation of all cell layers.

Alcohol extracts were prepared by macerating 2.5 g of each sample's peel in 50 mL of analytical grade methanol at room temperature for 10 days. This was done for the qualitative analysis that followed.

For the thin layer chromatography (TLC), the research team has used the following:

- solvent mixture: ethyl acetate-anhydrous formic acid-water in a 75:15:10 ratio;
- chromatography plate: Alufoil F 254 (Merck);
- samples: methanolic extracts from the peels of citruses (P, M, G, L);
- application: each sample was dotted as a thin spot 5 times 10 μL of the extract on the TLC plate;
- visualising: the plate was sprayed with diphenylboric acid 2-aminoethyl-ester (NSR, also called Natural Substances Reagent) (Sigma-Aldrich Switzerland) and examined in the UV range at $\lambda = 365$ nm.

Firstly, the chromatographic plate was saturated with a mixture of solvents for a minimum of 30 minutes. All of the steps up to the analysis and the determination itself were executed at room temperature. The samples were applied on the chromatography plate as small spots using capillary tubes. When the samples were dried, the plate underwent development with the specific solvent mixture, after which it was further dried in an oven (105ºC, 10 min.).

The qualitative screening used various reagents to confirm the identification of different compounds. The determination of total flavonoid levels was done using a mixture of aluminium chloride ($AlCl_3$, 2.5%) and sodium acetate (CH_3COONa, 10%), and left to incubate at room temperature. This reaction would be confirmed by the formation of an intern complex between flavonoids and aluminium which is yellow-green coloured and fluorescent at 430 nm. For the quantitative determination the comparison was made with a rutin standard curve evaluated with a spectrophotometer at the same wavelength (Gorduza *et al*., 2000).

The assay for identifying phenolates was done using a solution of sodium hydroxide (NaOH, 10%), the hydroxide reacts with phenolic groups to produce a red compound (phenolates) that gives the solution an orange-red colour.

For the analysis of the total polyphenol profile of the extracts, the Folin-Ciocâlteu reagent was employed in a sodium carbonate environment (Na_2CO_3). The Folin-Ciocâlteu reagent is a mixture of phosphomolybdic and phosphotungstic acid, which relies on redox principles to oxidize hydroxyl groups to ketones. The reduction of Mo^{6+} to Mo^{5+} and W^{6+} to W^{5+} results in the formation of brightly coloured blue complexes that can be further quantified with a spectrophotometer at 660 nm, and the results can be expressed as gallic acid equivalents.

The DPPH• method is used for the assessment of the antioxidant properties of certain compounds and extracts, those being acids, phenols and polyphenols. This method is based on the reduction of the free stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), which has a deep purple colour, to 2,2-diphenyl-1-picrylhydrazine (DPPH-H) which is yellow. This reaction is confirmed by a shift in colour from deep purple to pink or orange-yellow (Shahidi *et al*., 2020).

The quantitative analysis of the total carotenoids was evaluated based on the intense colour of these compounds at three different wavelengths, 440.5, 662 and 664 nm. First, 0.1 mL of the sample was placed in a 25 mL volumetric flask and acetone was added up to the mark. Total carotenoids are then calculated as a difference between the absorbance obtained at the 440.5 nm and the other two; 662 nm is the wavelength at which chlorophyll a absorbs most intensively, and 664 nm corresponds for chlorophyll b.

3. Results and discussions

While orange peel is predominantly used in the culinary world, the European Pharmacopeia has a specific monography for orange essential oil. This product is utilized as an aromatic compound in different pharmaceutical formulations from topical ointments, like creams for its soothing properties and pleasant scent, to capsules and tables for its potential digestive benefits and as a flavoring agent. *Aurantii pericarpium* is the plant product harvested from oranges (*Citrus aurantium* ssp. *dulcis*) and is obtained from the peel of fruits. Citrus peels have a specific structure, being composed of key components: **flavedo** (the colored outer layer which can range from yellow to orange and pink and contains carotenoids, limonene and linalool, the peel's oil glands), and **albedo (**the inner white layer which has a spongy texture and no pharmaceutical significance.

The macroscopic observation of the peel of these fruits indicated that all of the samples have the specific aspect of citruses, with a dimpled coloured peel and a spongy deeper layer. The pericarp presented no abnormal aspects and has the characteristic colour, aroma and smell of species from the *Citrus* genus. In Fig. 1 are presented the dissected peels and down below the red arrow indicates the oil glands that give citruses their specific smell.

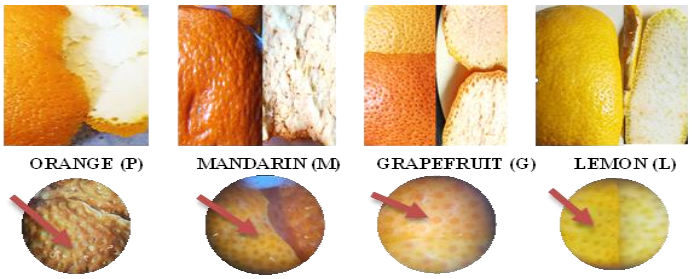


Fig. 1 – Macroscopic assessment of the samples.

The microscopic examination revealed common characteristics for all of the samples. The reported findings are presented bellow in Figs. 2-6.

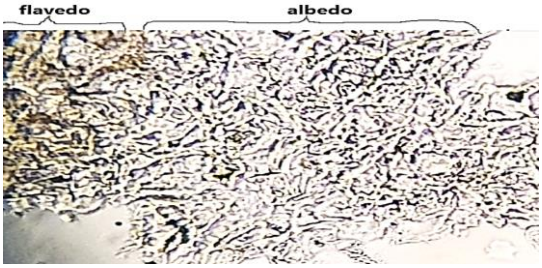


Fig. 2 – Lateral view of the orange pericarp and hypodermis at 10x.

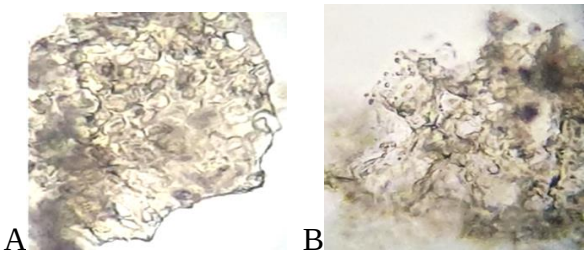


Fig. 3 – Front facing view of the grapefruit epicarp (cells with thickened walls) – A; Hypodermis (albedo) containing calcium oxalate crystals – B (10x).

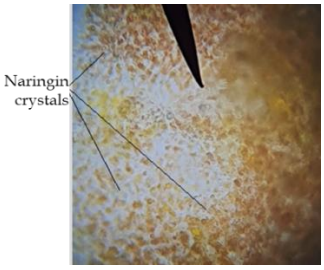


Fig. 4 – Mandarin epicarp with small orange naringenin crystals present.

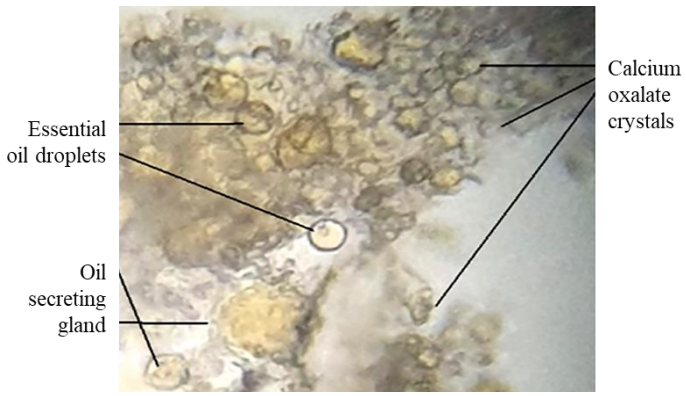


Fig. 5 – Oil secreting glands in the citrus epicarp and free oil droplets.

Qualitative analysis

All qualitative reactions were performed using the methanolic extracts described in subsection 2. The preliminary evaluation aimed to identify using specific reactions, the most important natural compound species (flavones and polyphenolic acids) from the sampled citruses.

TLC analysis indicates a very similar chemical profile for all of the samples, with slight differences. The most important compounds identified using this technique were naringenin that presents a yellow-green fluorescence and caffeic acid which fluoresces slightly blue. Besides these, the research team has also identified chlorogenic acid which fluoresces blue and has a retention factor of 0.7. The TLC plates are presented down below in Fig. 6.

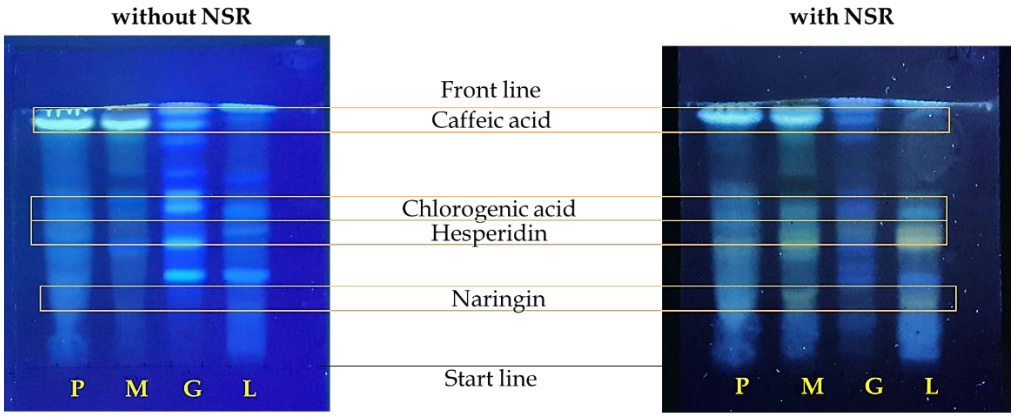


Fig. 6 – TLC plates of the samples under UV light.

The chemical assessment of the most important secondary metabolite groups was done using the specific chemical reagents as described in subsection 2. The obtained results indicated that all samples gave positive results for all reactions, however the intensity of the observed interaction varied significantly, depending on the origin of the sample. For the better understanding, the results are presented comparatively in Table 1.

Table 1
Comparative study of the analysed extracts

Nr.	Secondary metabolites	Sample			
		M	**P**	**G**	**L**
1	**Flavonosides** ➢ $AlCl_3$(benzopyran structure)	+++	++	+	++++
2	**Flavonoidic aglycons** ➢ NaOH(hydroxyl groups with phenol character)	+++	++	++	++++
3	**Total polyphenols** ➢ Folin-Ciocâlteu	++++	++++	++++	++++
4	**Antioxidants** ➢ 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenger activity	++++	+++	+	++++

The orange extract (P) presented a medium reactivity, fact that confirmed the presence of polyphenols and flavonoids. Reactions 1 and 2 indicated the presence of flavones and the 3rd reaction further endorsed a rich polyphenol profile. The reactivity with Folin-Ciocâlteu reagent demonstrated a moderate antioxidant potential and by correlation with the results from the DPPH radical assay, we can state that the orange extract contains antioxidants.

Similar results were obtained for the mandarin extract (M), but the reactions have proceeded with high intensity, indicating a high quantity of flavones and polyphenols. The first two reactions have confirmed the presence of flavones, from these we could also deduce that the extract was very rich in flavones by the intensely coloured solutions. Reactions 3 and 4 indicated a high antioxidant profile.

For the grapefruit extract (G) all reactions gave a positive result, nevertheless, the slight colour change confirmed the presence of flavones but in a small quantity. Similar results were observed for all assays, the reduced amount of total phenols was correlated with the low capacity to inhibit the formation of DPPH radicals.

The lemon peel extract (L) had considerable results with all reagents which confirms the presence of polyphenols and flavonoids. By reacting with R1

and R2 it was found that it had a rich content of flavones. In the Folin-Ciocâlteu reaction the extract exhibited a high polyphenol content and by correlating it with the results from DPPH assay, this extract had the most intense antioxidant potential of the tested samples. A comparative view of these tests is given in Fig. 7.

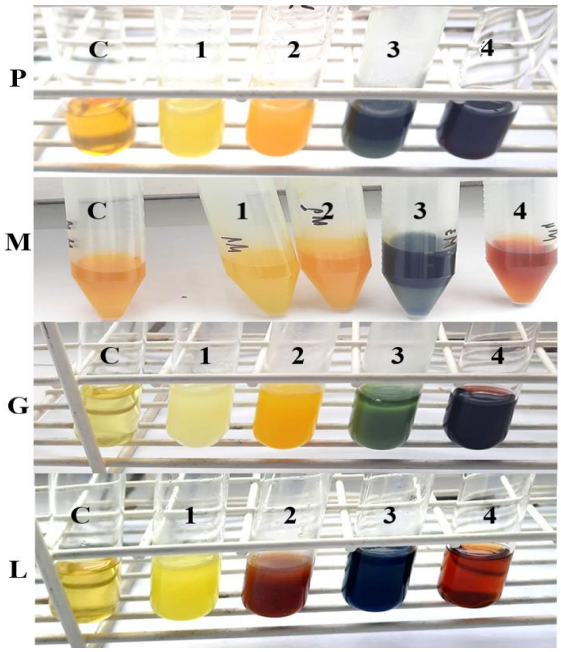


Fig. 7 – Comparative results of the qualitative screening, where:
C- control color of the initial extract, 1- reaction with aluminium chloride (positive – yellow/green fluorescence), 2- reaction with natrium hydroxide (positive – orange/red), 3- reaction with Folin-Ciocâlteu (positive – intense blue color), 4- DPPH assay (intense activity - discoloration).

Quantitative analysis

Flavonoids are natural components with known antioxidant properties which lead to a widespread use of these compounds for the prevention and treatment of multiple disorders (Gorduza *et al*., 2000; Lungu *et al*., 2020).

Total flavonoids were quantified as indicated by European Pharmacopoeia, using a spectrophotometric method. In general, orange peels are known for the rich content in such compounds, however they vary depending on the variety and the country of origin. Most studies indicated that polyethoxylated flavonoids (naringin, hesperidin etc.) have good antioxidant, anti-inflammatory, antiviral and antitumorigenic potential (Cernătescu *et al*., 2023b).

Our samples contained different amounts of total flavonoids (Fig. 8), similar results being given in the initial screening tests.

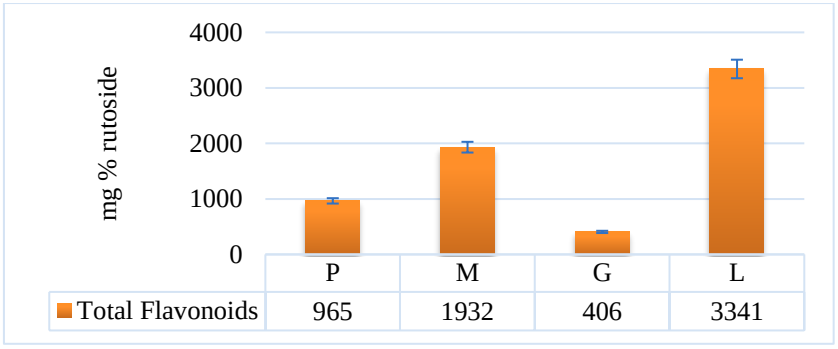


Fig. 8 – The total amount of flavonoid compounds quantified in the citruses peel extracts.

We noted that the lemon peels were the richest in such compounds, we need to take into account that this was the only ecological (bio) sample we were able to buy on the market at the time we began our study.

As indicated before, the Folin-Ciocâlteu assay was used to evaluate the quantity of total phenolic compounds from the investigated extracts. This reagent also indicates that the species that interact with it have also a reducing activity. This is why the results from this determination is also directly correlated with the antioxidant capacity of the sample. Such correlations have been observed for our samples (Fig. 9).

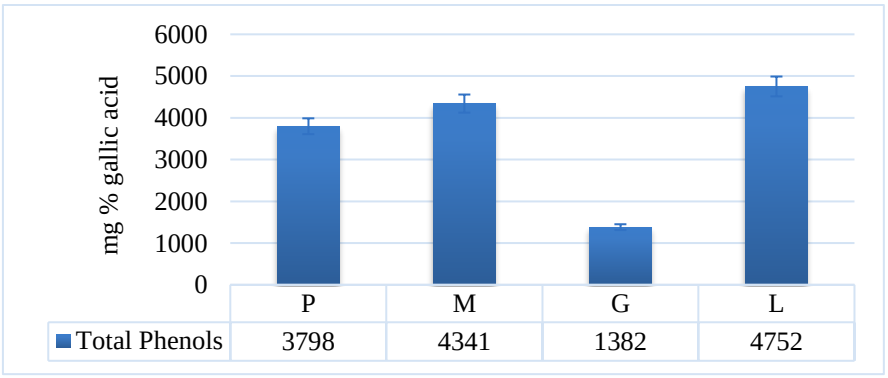


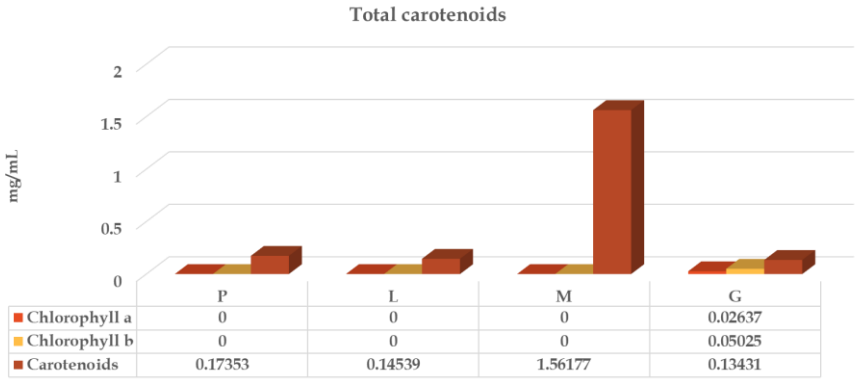
Fig. 9 – The total phenolic compounds quantified in the citruses peel extracts.

In this test, the variations are less obvious between the orange, mandarin and lemon extracts, but the grapefruit sample is still the last in terms of the content of phenolic compounds. These results are directly correlated with all other tests.

Carotenoids are coloured compounds found in various plants that have an important role in the protection against UV radiations. Chlorophyll derivatives

may interfere in the quantification of the carotenoids; therefore, the methodology ensure the elimination of positive results.

For our samples the obtained results are indicated in Fig. 10.



	P	L	M	G
Chlorophyll a	0	0	0	0.02637
Chlorophyll b	0	0	0	0.05025
Carotenoids	0.17353	0.14539	1.56177	0.13431

Fig. 10 – The quantification of carotenoid pigments in the citrus samples.

It is observed that the mandarin pericarp is the richest, followed by oranges and lemons at a long distance. Grapefruit pericarp contains the lowest amounts. Moreover, traces of chlorophyll were dosed in the pericarp of grapefruit, which may be most likely due to the fact that these fruits were harvested when they were still green.

4. Conclusions

Our research aimed to evaluate the main components found in the pericarp of different species from the Citrus genus, trying to understand their specific morphologies and chemical profiles.

Morphological assessment of the samples has uncovered different structures like calcium oxalate and small orange naringenin crystals, essential oil secreting glands and free oil droplets.

The chemical profile of the samples presented a lot of similarities, with slight differences. For example, TLC analysis revealed that the grapefruit extract had the lowest content of flavonoids, while the extracts made from orange, mandarin and lemon peels presented naringenin and hesperidin in higher quantities.

All of the extracts gave positive results to the analytical reactions for flavones and polyphenols; the grapefruit extract had the lowest reactivity while the lemon one presented the highest, the mandarin and orange extract presented medium reactivity. The Folin-Ciocâlteu and DPPH reactions presented promising results for the antioxidant potential of the extracts.

Further research is needed to fully understand the chemical profile and quantification of the compounds contained in these extracts.

Future research may try to fully understand the stability of these extracts and how their antioxidant potential can be harnessed for the use in pharmaceutical formulations and food supplements.

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PERICARPUL CITRICELOR: DEŞEU SAU SURSĂ DE POLIFENOLI

(Rezumat)

Citricele, membre ale familiei *Rutaceae,* sunt renumite la nivel mondial pentru beneficiile lor nutriționale și pentru sănătate. În timp ce părțile comestibile sunt consumate pe scară largă, cojile sunt adesea aruncate deși sunt bogate în polifenoli, în special flavonoide precum hesperidina, naringina și cvercetolul. Acești polifenoli oferă proprietăți antioxidante, antiinflamatorii și antimicrobiene semnificative. Scopul studiului nostru a fost de a evalua profilul metaboliților secundari și potențialul antioxidant al cojilor de la patru specii de citrice: portocale dulci, mandarine, grepfrut și lămâi. Cojile proaspete au fost tăiate și pregătite pentru o identificare microscopică, apoi s-au obținut extracte metanolice de macerare în raport de 1:20 (m/v). Identificarea grupului major de compuși activi a fost verificată mai întâi prin reacții chimice cu hidroxid de sodiu, clorură de aluminiu, reactiv Folin-Ciocâlteu și prin cromatografie în strat subțire (TLC). Flavonoidele totale, polifenolii totali și carotenoizii au fost cuantificați prin teste spectrofotometrice. Mai mult, capacitatea antioxidantă a fost evaluată utilizând testul 2,2-difenil-1-picrilhidrazil (DPPH•).

Rezultatele obținute au indicat că, deși compoziția chimică variază în funcție de specie, există un profil chimic similar pentru toate probele. Cu toate acestea, extractele de coajă de lămâie au prezentat cea mai mare activitate antioxidantă, urmate de mandarine și portocale, extractul din grepfrut având efectul cel mai slab. Datele noastre subliniază potențialul cojilor de citrice ca o resursă valoroasă pentru compușii bioactivi, susținând utilizarea lor în aplicații nutriționale și medicinale, dar și pentru a promova sustenabilitatea și a reduce deșeurile.