ECLÉTICA QUÍMICA

Eclet. Quim. 51 | e-1563, 2026

Original Articles in Chemistry and Related Areas

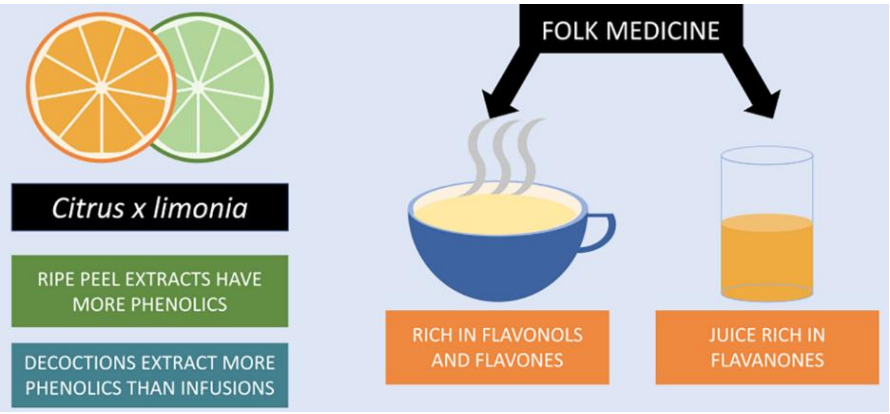
https://doi.org/10.26850/1678-4618.eq.v51.2026.e1563

Phenolics and antioxidant activity of Rangpur lime (*Citrus × limonia*) extracts from peels, leaves, and juice

Maycon Hudson **Contador**[1], Jacqueline Aparecida **Marques**[1], Romaiana Picada **Pereira**[1+]

Abstract

The Rangpur lime (*Citrus x limonia*) is a common fruit in Brazil. Although it is popular in medicine and cuisine, few investigations were conducted into the compositions and antioxidant capacities of infusions, decoctions and from the juice, leaves, and peels. The Folin-Ciocalteu reagent was used to evaluate phenolic content; aluminum chloride to measure flavonols; and sodium borohydride was used to determine flavanones. Antioxidant capacity was assessed through $ABTS^{\bullet+}$, DPPH• radical scavenging, and ferric ions reducing power. The evaluation of different extracts confirmed that ripe fruit peels contain more phenolics and flavonoids than unripe fruit peels. Furthermore, leaves decoction presents more flavonols and flavones and higher antioxidant activity than infusion and peels, both decoction and infusion. Flavonols were identified as the primary flavonoids in peel and leaf extracts, but no flavanones were found. Conversely, juices displayed a high amount of flavanones, a significant phenolic level, and the best antioxidant capabilities, the most beneficial form for widespread consumption. Further studies must identify the primary compounds in both juices and infusions.



Article History

Received May 11, 2024
Accepted August 07, 2025
Published June 30, 2026

Keywords

1. natural products;
2. flavones;
3. flavanones;
4. flavonols;
5. free radicals.

Section Editors

María Natalia Besil **Arismendi**

Highlights

- 1. Rangpur lime juices and infusions/decoctions have distinct phenolic compositions.
- Flavones and flavonols are the main phenolic compounds in infusions/decoctions.
- Juices contain a high number of flavanones.

[1]State University of Ponta Grossa, Ponta Grossa, Brazil. **+Corresponding author:** Romaiana Picada Pereira, **Phone:** +55422102-8122, **Email address:** romaiana@uepg.br

1. Introduction

Citrus fruits, including oranges, tangerines, lemons, and grapefruits, are the second most widely produced class of fruits, with an estimated production of over 160 million tons in 2021 (FAOSTAT, 2020). This fruit class encompasses all fruits that are part of the subtribe Citrinae, tribe Citreae, subfamily Aurantioideae, and Rutaceae family. The economically essential citrus fruits are categorized into three main genera in this subtribe: *Fortunella*, *Citrus*, and *Poncirus* (Penjor *et al.*, 2013).

Brazil cultivates citrus trees on different rootstocks, but the pink lemon tree (*Citrus x limonia*) is widely grown due to its resilience and high yield (Schäfer *et al.*, 2001). This hybrid citrus fruit is commonly consumed in Brazil due to its accessibility. It is versatile, as it can be consumed fresh, used as a seasoning, and utilized for making juices and infusions (Freitas *et al.*, 2015).

The essential oils in citrus fruits are abundant in terpenes (Fagodia *et al.*, 2017; Fancello *et al.*, 2016; González-Mas *et al.*, 2019; Othman *et al.*, 2016), while the leaves and peels contain flavonoids and para-coumaric acid derivatives, for example: Eriocitrin and Hesperidin - flavanones, dihydroferulic acid and 4-hydroxycinnamic acid - phenolic acid (González-Mas *et al.*, 2019; Rafiq *et al.*, 2016; Sanches *et al.,* 2022; Zhang *et al.*, 2014; Zou *et al.*, 2015). These compounds have been found to possess diverse biological activities, including antioxidant effects that play a role in reducing inflammation (Geronikaki and Gavalas, 2006), fighting tumors (Zhang *et al.*, 2015), preventing premature aging (Chatzianagnostou *et al.*, 2015), and benefiting individuals with diabetes, cardiovascular issues (Zhang *et al.*, 2015), and neurodegenerative disorders (Panahi *et al.*, 2018).

Despite its various uses in folk medicine, such as treating colds, sore throats, nausea, and anaemia, there is limited knowledge about the chemical composition and potential biological effects of Rangpur lime infusions and juices. The literature has previously emphasized the anti-inflammatory (Amorim *et al.*, 2016), schistosomicidal (Martins *et al.*, 2016), leishmanicidal (Estevam *et al.*, 2016), antibacterial (Millezi *et al.*, 2014), and larvicidal (*Aedes aegypti*) effects of essential oils derived from barks and leaves (Cavalcanti *et al.*, 2004). Thus, considering also that the most literature data is about another species of *Citrus* gennus (Couto and Canniatti-Brazaca, 2010; Siddhartha *et al.*, 2025), the current research evaluates the levels of phenolic compounds and antioxidant capacity of different extracts and juice from the *Citrus x limonia* hybrid, according to its use in folk medicine.

2. Experimental

2.1. Plant material

Citrus x limonia fruits (both ripe and unripe) and leaves were collected in Ponta Grossa (coordinates -25.093810 ° S, -50.101766 ° W, altitude 896 m), Paraná State, Brazil, during the winter season. A voucher specimen was identified and deposited at the Herbarium of Universidade Estadual de Ponta Grossa (HUPG-22142).

2.2. Preparation of extracts

Peels from ripe (R) and unripe (U) fruits were dried at 50 °C in an oven and ground using a blender. The hydroalcoholic crude extract was prepared by macerating 500 g of dried peels at room temperature in an ethanol (EtOH): distilled water (9:1) mixture for seven days; the extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The extracts were partitioned using hexane (H) (fractions HR and HU), the aqueous residues were partitioned again using ethyl acetate (A) (fractions AR and AU), solvents were removed, and the aqueous (W) residue was freeze-dried (fractions WR and WU).

One gram per liter (distilled water) of either fresh leaves or ripe fruit peels was used to make decoctions and infusions. A decoction was made by boiling the vegetal material for 10 min, and an infusion was made by pouring boiling water over the vegetal material. Both extracts remained undisturbed until they reached room temperature.

To obtain the juices, the fresh ripe fruits were pressed to extract the pulp, which was then filtered to remove fibers, while retaining the citrus cloud. All samples (infusions, decoctions, and juices) were prepared just before use.

2.3. Determination of flavonols and flavones content

The quantification of these flavonoid classes was determined by their ability to form complexes with aluminum (III) ion, as first described by Pękal and Pyrzynska (2014), with some modifications. By combining 40 µL of the sample, 40 µL of 2% (w/v) $AlCl_3$ aqueous solution, and 200 µL of water, a final volume of 280 µL was obtained. After incubating for 5 min at room temperature, the absorbance was measured at λ = 415 nm. The results were expressed in $\mu mol \cdot L^{-1}$ (for infusions, decoctions, and juice) and $\mu mol \cdot g^{-1}$ (for extracts), using the calibration curve of the quercetin standard.

2.4. Determination of flavanones content

The determination of flavanones was carried out using the acid-oxidized borohydride reduction method, with modifications from Rowell and Winter's (1959) protocol. The procedure involved adding 100 µL of the sample and 100 µL of 0.75% (w/v) $NaBH_4$ in DMSO to the wells, followed by the addition of 50 µL of AcOH and 50 µL of HCl after 5 min. The absorbance at λ 583 nm was measured right after adding HCl. The results were then expressed as hesperidin equivalents in $\mu mol \cdot L^{-1}$, using the calibration curve of the hesperidin standard.

2.5. Determination of total phenolic compound content (TPC)

The phenolic content was measured using the Folin-Ciocalteu reagent described by Pękal and Pyrzynska (2014). A mixture of 100 µL of the 10% (v/v) Folin-Ciocalteu reagent and 100 µL of the sample was prepared in a 96-well microplate. After 5 min, 100 µL of 7% (w/v) Na_2CO_3 aqueous solution was introduced, and the absorbance was measured at λ 765 nm after 30 min. The results from the calibration curve of gallic acid were converted to gallic acid equivalents (GAEq) in $\mu mol \cdot L^{-1}$.

2.6. Determination of the ferric ion reducing power

The ferric ion-reducing power was determined by measuring the extract's capacity to reduce solutions of tris(1,10-phenanthroline)iron(III) complex ($[Fe(phen)_3]Cl_3$), as described by Berker *et al.* (2007), with modifications. A stock solution of the complex was prepared by mixing 0.1600 g of $NH_4Fe(SO_4)_2.12H_2O$ with 2 mL of 37% HCl. Then, the minimum volume required (approximately 3.6 mL) of ethanol was used to dissolve 0.1802 g of 1,10-phenanthroline, this solution was mixed with the ammonium ferric sulphate solution, and the volume was adjusted to 100 mL with water, resulting in a $[Fe(phen)_3]Cl_3$ solution concentration of 3.3 $mmol \cdot L^{-1}$. A mixture of 50 µL of $[Fe(phen)_3]Cl_3$ reagent solution, 100 µL of water, and 150 µL of

the sample was prepared in a 96-well plate. The absorbance at λ = 510 nm was measured after incubating for 30 min at 50 °C. Water was used as the control, while ascorbic acid was the standard. The results were reported as ascorbic acid equivalent antioxidant capacity (VCEAC).

2.7. Determination of DPPH• radical scavenging ability

The DPPH• radical scavenging capacity of the extracts was measured using the procedure described by Brand-Williams *et al.* (1995), with modifications. In a 96-well plate, 100 µL of DPPH• solution in EtOH (120 µmol·L^{-1}), 80 µL of EtOH, and 20 µL of samples (concentration range: 10 to 1000 µg·mL^{-1}) were combined. The absence of light was maintained during a 30-min incubation at room temperature, and the absorbance was then measured at λ = 518 nm. Ethanol served as the control while ascorbic acid was used as the standard. From the inhibition percentage of each extract concentration, a curve of inhibition percentage versus extract concentration was plotted. From the generated equation, it was possible to calculate the IC_{50} value, which is the extract concentration required to inhibit 50% of the DPPH radical. All the results were expressed as IC_{50} values.

2.8. Determination of ABTS•+ radical cation scavenging ability

The ability of the extracts to scavenge the ABTS•+ radical cation was assessed using the method described by Re *et al.* (1999). To prepare the radical cation stock solution, 100 mL of water, 0.3602 g of ABTS, and 0.0662 g of potassium persulfate were mixed and left in the dark for 12 h to form ABTS•+. A mixture of 100 µL of this solution, 20 µL of the sample (concentration range: 10 to 100 µg·mL^{-1}), and 100 µL of ethanol was prepared in a 96-well plate. The absorbance was measured at λ = 734 nm following a 30-min incubation in the dark at room temperature. Ethanol was used as the control, while ascorbic acid was used as the standard. All results were expressed as IC50 values, calculated as described previously for the DPPH radical assay.

2.9. Mathematical analyses and statistics

The results were presented as mean ± standard error of means (sem). All analyses were performed in triplicate. The software Origin 9.0 was used for data analysis. IC_{50} values were obtained through logistic regression, and all data were evaluated using analysis of variance (ONE-WAY ANOVA) to compare the means of different groups using the Tukey's test. Correlation analyses were made using the Pearson and Spearman models. The level of significance for the analyses was set to $p < 0.05$.

2.10. Material

All chemicals and solvents used were of analytical grade and they were purchased from Sigma-Aldrich Chemical Company (Saint Louis, Missouri, USA). Aluminum chloride ($AlCl_3.6H_2O$), ammonium ferric sulphate [$NH_4Fe(SO_4)_2.12H_2O$], sodium borohydride ($NaBH_4$), sodium carbonate (Na_2CO_3), 37% hydrochloric acid (HCl), potassium persulfate ($K_2S_2O_8$), Folin-Ciocalteu reagent, quercetin, hesperidin, gallic acid, 1,10-phenanthroline monohydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ascorbic acid (AA), 96% Ethanol (EtOH), hexane, ethyl acetate, dimethyl sulfoxide (DMSO) and glacial acetic acid (AcOH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All aqueous solutions were prepared with distilled water.

For the spectrophotometric readings, a Varian Cary 100 double-beam spectrophotometer with a deuterium lamp was used for readings in the range of λ from 190–350 nm, and a tungsten lamp for readings above λ = 350 nm. Also, 96-well plate analyses were performed on a Biotek Epoch plate reader equipped with a 10W xenon flash lamp for readings across the ultraviolet and visible ranges.

3. Results and discussion

3.1. Determination of total phenolic content (TPC) in the fractions of the ripe (R) and unripe (U) *Citrus* x *limonia* peels

In the ripe fruit peels, the fractions obtained from hexane (HR) and ethyl acetate (AR) exhibited higher levels of phenolic compounds (**Fig. 1** and **Table 1**) compared to other fractions analyzed. There was no significant difference ($p < 0.05$) among the fractions from the unripe fruit peels.

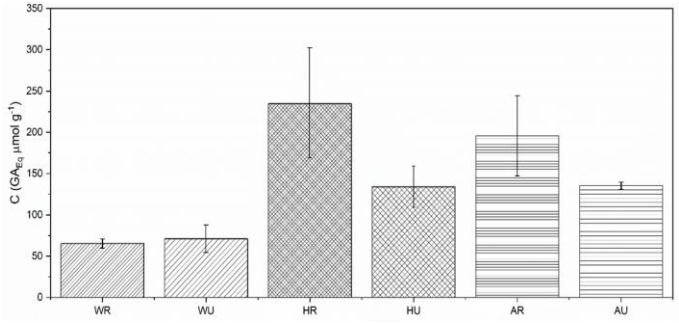


Figure 1. Total phenolic content (TPC), as gallic acid equivalents (GAEq), for: water fractions from ripe fruit peels (WR) and unripe fruit peels (WU); hexane fractions from ripe fruit peels (HR) and unripe fruit peels (HU); and ethyl acetate fractions from ripe fruit peels (AR) and unripe peels (AU) of *Citrus* x *limonia*. According to Tukey's test, values with identical indices show no significant difference at 0.05.
Source: Elaborated by the authors.

Phenolic degradation commonly occurs during fruit ripening due to polyphenol oxidase activity. The behavior of the fractions is unexpectedly distinct from *Citrus limon* (Dong *et al.*, 2019; Mcharek and Hanchi, 2017), but comparable to *Citrus reticulata* (Wang *et al.,* 2016; Yoo *et al.,* 2004). The genetic similarity of the hybrid to *Citrus reticulata* may explain this result (Curk *et al.*, 2016). Nonetheless, the dissimilarities in the collection, planting, and extraction techniques have a notable impact on the observed composition and should also be recognized as sources of this variation (Cardeñosa *et al.,* 2015; Loizzo *et al.,* 2012). Importantly, our results align with the literature data on organic extracts from the citrus genus, specifically those evaluating ripening peels, which demonstrate superior phenolic content in ripe fruits (Mehmood *et al.*, 2020).

https://doi.org/10.26850/1678-4618.eq.v51.2026.e1563

Table 1. TPC, flavonols and flavones content, and the ratio of quercetin (QEq) and gallic acid (GAeq) equivalents for: water fractions from ripe fruit peels (WR) and unripe fruit peels (WU); hexane fractions from ripe fruit peels (HR) and unripe fruit peels (HU); and ethyl acetate fractions from ripe fruit peels (AR) and unripe fruit peels (AU) of *Citrus* x *limonia*.

Fraction	TPC (GAeq μmol·g^{-1})	Flavonols and flavones (QEq μmol·g^{-1})	Ratio QEq/GA$_{eq}$
WR	65 ± 6^{b}	3.3 ± 0.2^{E}	0.052 ± 0.009^{a}
WU	71 ± 17^{b}	8 ± 2^{D}	0.12 ± 0.03^{a}
HR	235 ± 68^{a}	34 ± 2^{A}	0.16 ± 0.06^{a}
HU	134 ± 26^{b}	14.6 ± 0.9^{C}	0.11 ± 0.03^{a}
AR	196 ± 49^{a}	24 ± 4^{B}	0.13 ± 0.04^{a}
AU	135 ± 4^{b}	21 ± 1^{B}	0.152 ± 0.004^{a}

Note: Lowercase and uppercase letters: Values with the same letter do not differ significantly at a level of 0.05 according to Tukey's test. **WR:** Water fractions from ripe fruit peels; **WU:** Water fractions from unripe fruit peels; **HR:** Hexane fractions from ripe fruit peels; **HU:** Hexane fractions from unripe fruit peels; **AR:** Ethyl acetate fractions from ripe fruit peels; **AU:** Ethyl acetate fractions from unripe fruit peels
Source: Elaborated by the authors.

3.2. Determination of the flavonols and flavones content in the fractions of the ripe (R) and unripe (U) *Citrus* x *limonia* peels

Literature supports that citrus peels are rich in flavonoids (Dong *et al.*, 2019; Mcharek and Hanchi, 2017; Yoo *et al.*, 2004). The data analysis (**Table 1**, **Fig. 1** and **2**) supports a significant correlation (r = 0.970) between flavonoid and phenolic compounds. The study of the flavonoid/phenolic ratio reveals no significant difference in the distribution of these compounds between unripe and ripe peel samples ($p < 0.05$).

According to literature, the flavonoid content in different Citrus fruit species decreases as they mature (Dong *et al.*, 2019; Moriguchi *et al.*, 2001; Wang *et al.*, 2016). This behavior was only observed for the aqueous fraction in this study. The TPC of the hexanic ripe fruit fraction was noticeably larger than that of the unripe fraction. However, no differences were observed in the composition of the ethyl acetate fractions (**Table 1**and **Fig. 2**).

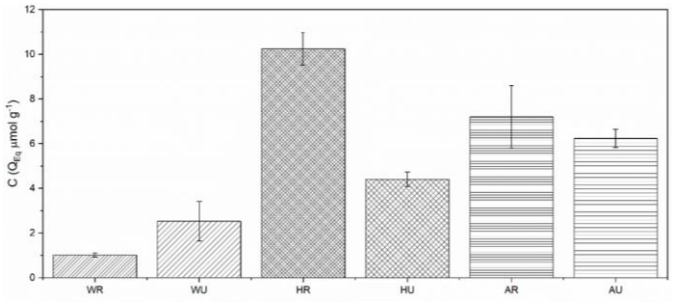


Figure 2. Comparison between the flavonoid contents in the different fractions of *Citrus* x *limonia* peel extracts. According to Tukey's test, values with identical indices show no significant difference at the 0.05 level.
Source: Elaborated by the authors.

3.3. Determination of ferric ion reducing capacity of the fractions from ripe (R) and unripe (U) *Citrus* x *limonia* peels

The phenolic compounds and flavonoids contents of citrus fruits are correlated with their antioxidant capacity (Martins *et al.*, 2016; Millezi *et al.*, 2014). The present study showed no significant correlation between the reducing power of ferric ions and flavonoids ($p = 0.64$) or phenolics ($p = 0.66$). The reduced potential may be due to undetected compounds, such as flavanones, which are known to reduce metal ions (Apak *et al.*, 2007).

Moreover, the ethyl acetate fractions extracted from unripe fruit peel exhibited a stronger reducing power compared to those from ripe fruit peel (**Fig. 3**). This result can be attributed to the increased level of hesperidin in unripe fruits, which decreases as the fruit matures (Moriguchi *et al.*, 2001).

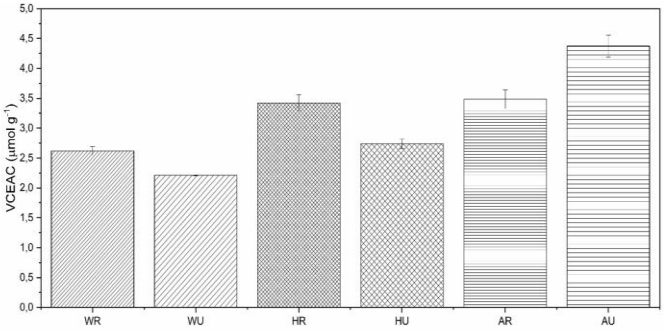


Figure 3. Antioxidant capacity measured by the ferric ion reducing power expressed as VCEAC in μmol g^{-1} for the fractions of *Citrus x limonia* peel extracts. According to Tukey's test, values with identical indices show no significant difference at 0.05.
Source: Elaborated by the authors.

3.4. Determination of total phenolic content (TPC) in *Citrus* x *limonia* decoctions (D) and infusions (I) from leaves (L) and peels (P)

The leaf and peel decoctions exhibited similar levels of phenolic content. In the infusions, the leaf extracts had the highest content of these compounds (**Fig. 4**). The content of phenolic compounds in the peel and leaves can vary among different species of the *Citrus* genus (Muthiah *et al.*, 2012) and within the same species for different cultivars and collection regions (Lagha-Benamrouche and Madani, 2013; Loizzo *et al.*, 2012).

Additionally, the results indicate that the decoction method was superior in extracting phenolic compounds (**Fig. 4**). It is generally noted that the extended contact of plant material with boiling solvent can assist in extraction or result in the breakdown of extracted compounds (Fotakis *et al.*, 2016; Sentkowska *et al.*, 2014; 2016), potentially yielding low molecular weight phenolic compounds (Jeong *et al.*, 2004).

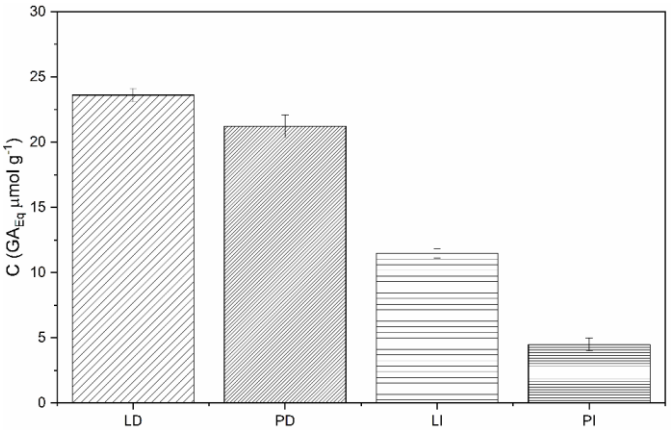


Figure 4. TPC in the decoctions and infusions obtained from leaves and peels of *Citrus* x *limonia*. According to Tukey's test, values with identical indices show no significant difference at the 0.05 level.
Source: Elaborated by the authors.

https://doi.org/10.26850/1678-4618.eq.v51.2026.e1563

3.5. Determination of flavonoid content in *Citrus* x *limonia* decoctions (D) and infusions (I) from leaves (L) and peels (P)

Flavonols, flavones, and flavanones are the predominant flavonoid classes in citrus fruits (Nogata *et al.*, 2006). Flavonols and flavones were present in the studied extracts, while no flavanones were detected. Additionally, **Fig. 5** highlights that the leaf decoction had the highest flavonoid content. HPLC analysis of methanolic extracts from *Citrus aurantium* leaves and peels yielded similar results (Loizzo *et al.*, 2012).

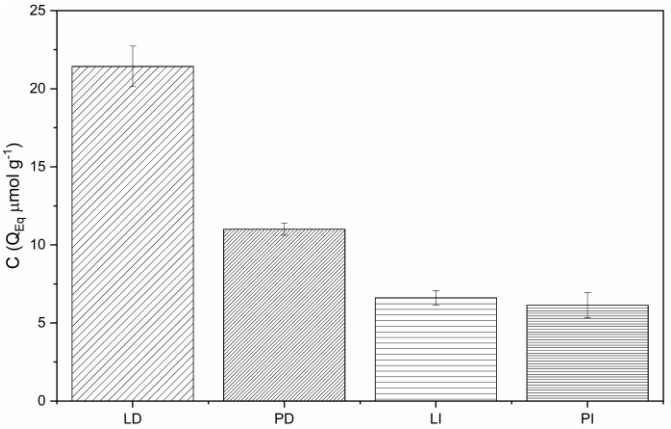


Figure 5. Flavonols and flavones contents in the decoctions and infusions obtained from *Citrus* x *limonia* leaves and peels. According to Tukey's test, values with identical indices show no significant difference at the 0.05 level.
Source: Elaborated by the authors.

3.6. Determination of the antioxidant capacity of *Citrus* x *limonia* decoctions (D) and infusions (I) from leaves (L) and peels (P)

Measurements of DPPH• radical and ABTS•+ radical cation scavenging abilities, as well as the ferric ion-reducing power assay, were conducted to evaluate the antioxidant capacity, considering the presence of phenolic compounds (**Fig. 4**) and flavonoids (**Fig. 5**).

In **Fig. 6a**, the extracts displayed effective inhibition of the $ABTS^{\bullet+}$ radical cation, while in **Fig. 6b**, they couldn't reach the IC_{50} in the $DPPH^{\bullet}$ radical assay. Literature indicates that the primary mechanism for inhibiting both radicals is electron transfer (SET) rather than hydrogen transfer (HAT), which explains why the differences between these tests are unrelated to the mechanism involved. The slower kinetics of the $DPPH^{\bullet}$ assay can be attributed to the steric hindrance caused by flavonoids and phenolic acids, indicating that ascorbic acid and phenolic acids do not play a significant role in the antioxidant capacity of these extracts (Chen *et al.*, 2015; Floegel *et al.*, 2011; Huang *et al.*, 2005; Schaich *et al.*, 2015).

The order of efficiency for $ABTS^{\bullet+}$ radical cation scavenging ability (**Fig. 7a**) can be verified as LD > LI > PD > PI. The correlation analysis confirms that there is no significant correlation between phenolics ($p = 0.30$) or flavonoids ($p = 0.31$), indicating that other secondary metabolites not included in these tests may contribute to the measured antioxidant capacity by inhibiting $ABTS^{\bullet+}$ radical cation.

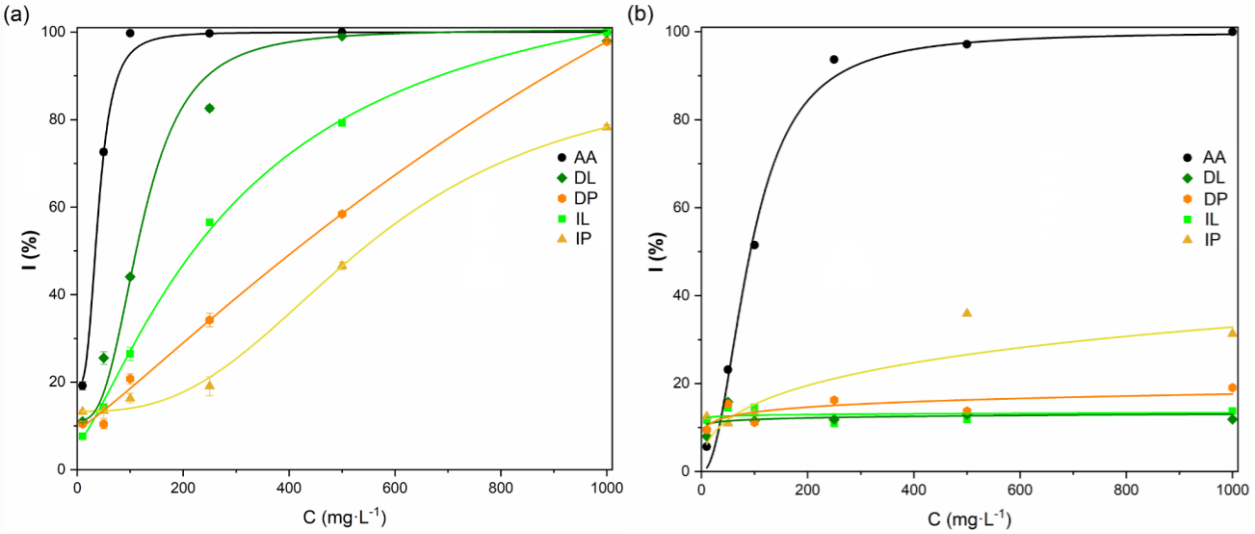


Figure 6. (a) Inhibition curves of $ABTS^{\bullet+}$ radical cation for the extracts obtained by decoction (D) and infusion (I), with ascorbic acid (AA) as standard; and **(b)** $DPPH^{\bullet}$ radical inhibition curves for the same extracts.
Source: Elaborated by the authors.

The ferric ion reducing capacity assay (**Fig. 7b**) exhibits a similar pattern to the flavone and flavonol assay (**Fig. 5**), which is confirmed by the strong correlation with VCEAC ($r = 0.990$). **Table 2** compares the antioxidant values, ferric ion-reducing power, and contents of phenolics, flavonols, and flavones.

https://doi.org/10.26850/1678-4618.eq.v51.2026.e1563

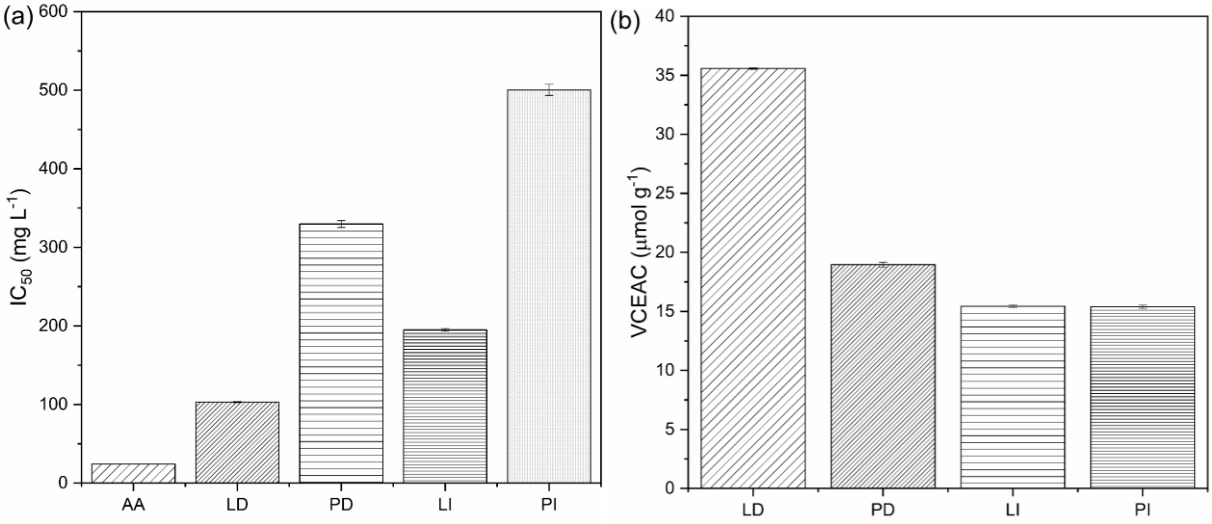


Figure 7. (a) IC_{50} values against $ABTS^{\bullet+}$ radical cation of extracts; and **(b)** VCEAC values of the same extracts. According to Tukey's test, values with identical indices show no significant difference at the 0.05 level.
Source: Elaborated by the authors.

Table 2. TPC (µmol eq gallic acid L^{-1}), flavonols and flavones (µmol eq quercetin L^{-1}), VCEAC (µmol eq. AA L^{-1}) and IC_{50} (mg·L^{-1}) for $DPPH^{\bullet}$ radical and $ABTS^{\bullet+}$ radical cation for the extracts obtained from leaves (L) and peels (P) of *Citrus* x *limonia*.

Extract	Phenolics	Flavonols and flavones	IC_{50} ($DPPH^{\bullet}$)	IC_{50} ($ABTS^{\bullet+}$)	VCEAC
LD	23.6 ± 0.5[a]	21 ± 1[a]	>1000	103.1 ± 0.8[a]	35.58 ±0.07[a]
PD	21.2 ± 0.9[a]	11.0 ± 0.4[b]	>1000	330 ± 4[c]	19.0 ± 0.2[b]
LI	11.5 ± 0.4[b]	6.6 ± 0.5[b]	>1000	195 ± 2[b]	15.4 ± 0.1[b]
PI	4.5 ± 0.5[c]	6.1 ± 0.8[b]	>1000	501 ± 7[d]	15.4 ± 0.1[b]

Note: Lowercase letters: Values with the same letter do not differ significantly at a level of 0.05 according to Tukey's test. **LD:** Decoctions from leaves; **PD:** Decoctions from peels; **LI:** Infusions from leaves; **PI:** Infusions from peels.
Source: Elaborated by the authors.

3.7. Determination of total phenolic content (TPC) and flavonoids in *Citrus* x *limonia* juice

The phenolic compound content of *Citrus* x *limonia* juice was significantly higher than that of infusions and decoctions, with a phenolic content forty times greater than the extracts mentioned in **Table 2**. This value is like the phenolic content observed in *Citrus reticulata* juices (Synowiec-Wojtarowicz *et al.*, 2018).

Despite this, the literature on *Citrus reticulata* juices reports no significant presence of flavonols and flavones (Gattuso *et al.*, 2007). Flavanones (**Table 3**) are abundantly found in *Citrus* x *limonia* juices, making them the primary phenolic compounds. The same behavior is observed in other citrus fruit juices (Dugo *et al.*, 2005; Gattuso *et al.*, 2007; Kimball, 1991; Letaief *et al.*, 2016).

Table 3. TPC (µmol eq gallic acid·L^{-1}), flavonols and flavones (µmol eq quercetin·L^{-1}) and flavanones (µmol eq hesperidin·L^{-1}) contents found in *Citrus* x *limonia* juice.

Compounds	Content
Phenolics	1028 ± 6
Flavonols e flavones	ND*
Flavanones	583 ± 3

Note: *ND = Not detected.
Source: Elaborated by the authors.

3.8. Determination of the antioxidant capacity of *Citrus* x *limonia* juice

Comparatively, the juice has a more intense antioxidant capacity (**Table 4**) than the infusions and decoctions (**Table 2**). The presence of phenolics in the juice did not result in $DPPH^{\bullet}$ radical inhibition, possibly due to steric factors. However, it did show notable inhibition of the ABTS•+ radical cation (**Fig. 8**).

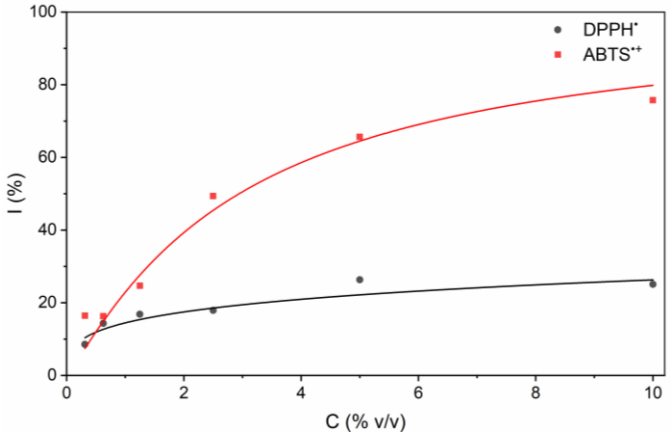


Figure 8. Comparison of the inhibitions of $DPPH^{\bullet}$ radical and $ABTS^{\bullet+}$ radical cation by *Citrus* x *limonia* juice.
Source: Elaborated by the authors.

The presence of flavanones in these juices, with their phenolic and flavanone contents, suggests that their antioxidant activity is primarily attributed to flavanones. This is supported by ascorbic acid, which effectively scavenges $DPPH^{\bullet}$ radicals, whereas the juices did not exhibit $DPPH^{\bullet}$ radical inhibition. These data are essential, since most studies found in literature investigate other species of the citrus genus and do not compare juices with extracts (Huynh *et al.*, 2023; Rahmiati *et al.*, 2024; Sanches *et al.*, 2022).

Table 4. IC_{50} values (% v/v) of the DPPH$^{\bullet}$ radical and ABTS$^{\bullet+}$ radical cation and the values of VCEAC (μmol eq. ascorbic acid·L^{-1}) for *Citrus* x *limonia* juice.

Assay	Antioxidant Capacity
DPPH$^{\bullet}$	>100%
ABTS$^{\bullet+}$	2.9 ± 0.2%
VCEAC	986 ± 14

Source: Elaborated by the authors.

4. Conclusions

The antioxidant compounds in the ripe fruits were higher than in the unripe fruits, showing an increased reduction in the power of ferric ions and containing more phenolics and flavonoids.

The ethyl acetate fraction of the unripe fruit peel exhibits the highest antioxidant capacity in this study and should be further examined for its composition. This data is essential since the sample could be explored as a possible source for a future nutraceutical product.

The extracts revealed that the decoctions contained higher levels of phenolics, flavonols, and flavones and demonstrated superior antioxidant capabilities compared to infusions. When comparing leaves and peels, it was found that preparations with leaves had higher phenolic and flavonoid levels and a greater antioxidant capacity.

While the leaf decoction (the best extract) had a lower phenolic content, the juice surpassed it by over 40 times. Surprisingly, the juice had no detectable levels of flavones and flavonols but had a significant presence of flavanones, which can be attributed to the juice cloud. It can be concluded that consuming Citrus x limonia (Rangpur lime) juice in popular forms is the most effective way to benefit from phenolics, flavonoids, and antioxidant capacity. However, further analysis is needed to identify the primary components responsible for the juice's antioxidant capacity.

Authors' contribution

Conceptualization: Maycon Hudson Contador; Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Data curation:** Maycon Hudson Contador; **Formal analysis:** Maycon Hudson Contador; **Funding acquisition:** Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Investigation:** Maycon Hudson Contador; **Methodology:** Maycon Hudson Contador; Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Project administration:** Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Resources:** Not applicable; **Software:** Not applicable; **Supervision:** Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Validation:** Maycon Hudson Contador; Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Visualization:** Maycon Hudson Contador; Jacqueline Aparecida Marques; Romaiana Picada Pereira; **Writing – original draft:** Maycon Hudson Contador; **Writing – review & editing:** Maycon Hudson Contador; Jacqueline Aparecida Marques; Romaiana Picada Pereira.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All data sets were generated or analyzed in the current study.

Artificial Intelligence usage statement

The authors did not use Artificial Intelligence tools at any stage of the preparation, correction, or evaluation of this work.

Funding

The financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Grant No: 0462/2021 process number: 88881.648449/2021-01; Fundação de Apoio ao Desenvolvimento Científico e Tecnológico de Paraná – Fundação Araucária, Grant No: 009/2017.

Acknowledgments

Complexo de Laboratórios Multiusuário (C-LABMU) da UEPG and all members from the research group who, in any capacity, contributed to the completion of this paper.

References

Amorim, J.; Simas, D.; Pinheiro, M.; Moreno, D.; Alviano, C.; da Silva, A.; Dias Fernandes, P. Anti-Inflammatory Properties and Chemical Characterization of the Essential Oils of Four Citrus Species. *PLoS One.* **2016**, *11*, e0153643. https://doi.org/10.1371/journal.pone.0153643

Apak, R.; Güçlü, K.; Özyürek, M.; Çelik, S. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchim. Acta.* **2007**, *160*, 413–419. https://doi.org/10.1007/s00604-007-0777-0

Berker, K.; Güçlü, K.; Tor, İ.; Apak, R. Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta.* **2007**, *72*, 1157–1165. https://doi.org/10.1016/j.talanta.2007.01.019

Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* **1995**, *28*, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cavalcanti, E.; Morais, S.; Lima, M.; Santana, E. Larvicidal Activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem. Inst. Oswaldo Cruz.* **2004**, *99*, 541–544. https://doi.org/10.1590/S0074-02762004000500015

Chatzianagnostou, K.; Del Turco, S.; Pingitore, A.; Sabatino, L.; Vassalle, C. The Mediterranean Lifestyle as a Non-Pharmacological and Natural Antioxidant for Healthy Aging. *Antioxidants.* **2015**, *4*, 719–736. https://doi.org/10.3390/antiox4040719

Chen, Y.; Xiao, H.; Zheng, J.; Liang, G. Structure-Thermodynamics-Antioxidant Activity Relationships of Selected Natural Phenolic Acids and Derivatives: An Experimental and Theoretical Evaluation. *PLoS One.* **2015**, *10*, 1–20. https://doi.org/10.1371/journal.pone.0121276

Couto, M. A. L.; Canniatti-Brazaca, S. G. Quantificação de vitamina C e capacidade antioxidante de variedades cítricas. *Ciênc. Tecnol. Aliment.* **2010**, *30* (Suppl. 1), 15-19. https://doi.org/10.1590/S0101-20612010000500003

Curk, F.; Ollitrault, F.; Garcia-Lor, A.; Luro, F.; Navarro, L.; Ollitrault, P. Phylogenetic origin of limes and lemons revealed by cytoplasmic and nuclear markers. *Ann. Bot.* **2016**, *117*, 565-583. https://doi.org/10.1093/aob/mcw005

Dong, X.; Hu, Y.; Li, Y.; Zhou, Z. The maturity degree, phenolic compounds and antioxidant activity of Eureka lemon [Citrus limon (L.) Burm. f.]: A negative correlation between total phenolic content, antioxidant capacity and soluble solid content. *Sci. Hortic.* **2019**, *243*, 281–289. https://doi.org/10.1016/j.scienta.2018.08.036

Estevam, E. B. B.; Miranda, M. L. D.; Alves, J. M.; Egea, M. B.; Pereira, P. S.; Martins, C. H. G.; Esperandim, V. R.; Magalhães, L. G.; Bolela, A. C.; Cazal, C. M.; Souza, A. F.; Alves, C. C. F. Chemical Composition and Biological Activities of the Essential Oils from the Fresh Leaves of *Citrus li*monia Osbeck and *Citrus latifolia* Tanaka (Rutaceae). *Rev. Virtual Quím.* **2016**, *8* (6), 1842–1854. http://doi.org/10.21577/1984-6835.20160124

Fagodia, S.; Singh, H.; Batish, D.; Kohli, R. Phytotoxicity and cytotoxicity of *Citrus aurantiifolia* essential oil and its major constituents: Limonene and

citral. *Ind. Crops Prod.* **2017**, *108*, 708–715. http://doi.org/10.1016/j.indcrop.2017.07.005

Fancello, F.; Petretto, G.; Zara, S.; Sanna, M.; Addis, R.; Maldini, M.; Foddai, M.; Rourke, J.; Chessa, M.; Pintore, G. Chemical characterization, antioxidant capacity and antimicrobial activity against food-related microorganisms of Citrus limon var. pompia leaf essential oil. *LWT - Food Sci. Technol.* **2016**, *69*, 579–585. https://doi.org/10.1016/j.lwt.2016.02.018

Food and Agriculture Organization of the United Nations (FAOSTAT). Crops and livestock products. http://www.fao.org/faostat/en/#data/QC (accessed Mar 1, 2020).

Floegel, A.; Kim, D.; Chung, S.; Koo, S.; Chun, O. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* **2011**, *24*, 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008

Fotakis, C.; Tsigrimani, D.; Tsiaka, T.; Lantzouraki, D.; Strati, I.; Makris, C.; Tagkouli, D.; Proestos, C.; Sinanoglou, V.; Zoumpoulakis, P. Metabolic and antioxidant profiles of herbal infusions and decoctions. *Food Chem.* **2016**, *211*, 963–971. https://doi.org/10.1016/j.foodchem.2016.05.124

Freitas, A.; Coelho, M.; Pereira, Y.; Freitas Neto, E.; Azevedo, R. Diversidade e usos de plantas medicinais nos quintais da comunidade de São João da Várzea em Mossoró, RN. *Rev. Bras. Plantas Med.* **2015**, *17*, 845–856. https://doi.org/10.1590/1983-084X/14_080

Gattuso, G.; Barreca, D.; Gargiulli, C.; Leuzzi, U.; Caristi, C. Flavonoid Composition of Citrus Juices. *Molecules.* **2007**, *12*, 1641–1673. http://doi.org/10.3390/12081641

Geronikaki, A.; Gavalas, A. Antioxidants and Inflammatory Disease: Synthetic and Natural Antioxidants with Anti-Inflammatory Activity. *Comb. Chem. High Throughput Screen.* **2006**, *9*, 425–442. https://doi.org/10.2174/138620706777698481

González-Mas, M. C.; Rambla, J. L.; López-Gresa, M. P.; Blázquez, M. A.; Granell A. Volatile Compounds in Citrus Essential Oils: A Comprehensive Review. *Front. Plant Sci.* **2019**, *10*, 1–18. https://doi.org/10.3389/fpls.2019.00012

Huang, D.; Ou, B.; Prior, R. The Chemistry behind Antioxidant Capacity Assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. https://doi.org/10.1021/jf030723c

Huynh, D. T.; Vo, M. T. N.; Kha, T. C. Enriching the Bioactive Components and Antioxidant Capacity of Concentrated Lime Juices Prepared by Cryogenic and Vacuum Processes. *Processes.* **2023**, *11*, 1883. https://doi.org/ 10.3390/pr11071883

Jeong, S.; Kim, S.; Kim, D.; Jo, S.; Nam, K.; Ahn, D.; Lee, S. Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels. *J. Agric. Food Chem.* **2004**, *52*, 3389–3393. https://doi.org/10.1021/jf049899k

Kimball, D. *Citrus Processing*; Springer Netherlands: Dordrecht, 1991; pp. 117–125. https://doi.org/10.1007/978-94-011-3700-3_8

Lagha-Benamrouche, S.; Madani, K. Phenolic contents and antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Algeria: Peels and leaves. *Ind. Crops Prod.* **2013**, *50*, 723–730. http://dx.doi.org/10.1016/j.indcrop.2013.07.048

Letaief, H.; Zemni, H.; Mliki, A.; Chebil, S. Composition of Citrus sinensis (L.) Osbeck cv «Maltaise demi-sanguine» juice. A comparison between organic and conventional farming. *Food Chem.* **2016**, *194*, 290–295. https://doi.org/10.1016/j.foodchem.2015.08.025

Loizzo, M.; Tundis, R.; Bonesi, M.; Menichini, F.; De Luca, D.; Colica, C.; Menichini, F. Evaluation of Citrus aurantifolia peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. *J. Sci. Food Agric.* **2012**, *92*, 2960–2967. https://doi.org/10.1002/jsfa.5708

Martins, M.; Fracarolli, L.; Vieira, T.; Dias, H.; Cruz, M.; Deus, C.; Nicolella, H.; Stefani, R.; Rodrigues, V.; Tavares, D.; Magalhães, L.; Crotti, A. Schistosomicidal Effects of the Essential Oils of Citrus limonia and Citrus reticulata Against *Schistosoma mansoni*. *Chem. Biodivers.* **2016**, *14*, e1600194. https://doi.org/10.1002/cbdv.201600194

Mcharek, N.; Hanchi, B. Maturational effects on phenolic constituents, antioxidant activities and LC-MS / MS profiles of lemon Citrus limon peels. *J. Appl. Bot. Food Qual.* **2017**, *90*, 1–9. https://doi.org/10.5073/JABFQ.2017.090.001

Mehmood, T.; Afzal, A.; Anwar, F.; Memon, N.; Memon, A. A.; Qadir, R. Variation in phenolic acids and antibacterial attributes of peel extracts from ripe and unripe [Citrus limon (L.) Osbeck] fruit. *J. Food Meas. Charact.* **2020**, *14*,1325–1332. https://doi.org/10.1007/s11694-020-00380-w

Millezi, A.; Baptista, N.; Caixeta, D.; Rossoni, D.; Cardoso, M.; Piccoli, R. Caracterização química e atividade antibacteriana de óleos essenciais de plantas condimentares e medicinais contra *Staphylococcus aureus* e *Escherichia coli*. *Rev. Bras. Plantas Med.* **2014**, *16*, 18–24. https://doi.org/10.1590/S1516-05722014000100003

Moriguchi, T.; Kita, M.; Tomono, Y.; Endo-Inagaki, T.; Omura, M. Gene expression in flavonoid biosynthesis: Correlation with flavonoid accumulation in developing citrus fruit. *Physiol. Plant.* **2001**, *111*, 66–74. https://doi.org/10.1034/j.1399-3054.2001.1110109.x

Muthiah, P.; Umamaheswari, M.; Asokkumar, K. In vitro antioxidant activities of leaves, fruits and peel extracts of citrus. *Int. J. Phytopharm.* **2012**, *2*, 13–20. https://doi.org/10.7439/ijpp.v2i1.293

Nogata, Y.; Sakamoto, K.; Shiratsuchi, H.; Ishii, T.; Yano, M.; Ohta, H. Flavonoid Composition of Fruit Tissues of Citrus Species. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 178–192. https://doi.org/10.1271/bbb.70.178

Othman, S.; Hassan, M.; Nahar, L.; Basar, N.; Jamil, S.; Sarker, S. Essential Oils from the Malaysian Citrus (Rutaceae) Medicinal Plants. *Medicin.* **2016**, *3*, 13. https://doi.org/10.3390/medicines3020013

Panahi, Y.; Rajaee, S.; Johnston, T.; Sahebkar, A. Neuroprotective effects of antioxidants in the management of neurodegenerative disorders: A literature review. *J. Cell. Biochem.* **2018**, *120*, 2742–2748. https://doi.org/10.1002/jcb.26536

Pękal, A.; Pyrzynska, K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Anal. Methods.* **2014**, *7*, 1776–1782. https://doi.org/10.1007/s12161-014-9814-x

Penjor, T.; Yamamoto, M.; Uehara, M.; Ide, M.; Matsumoto, N.; Matsumoto, R.; Nagano, Y. Phylogenetic Relationships of Citrus and Its Relatives Based on matK Gene Sequences. *PLoS One.* **2013**, *8*, e62574. https://doi.org/10.1371/journal.pone.0062574

Rafiq, S.; Kaul, R.; Sofi, S.; Bashir, N.; Nazir, F.; Ahmad Nayik, G. Citrus peel as a source of functional ingredient: A review. *J. Saudi Soc. Agric. Sci.* **2018**, *17*, 351–358. https://doi.org/10.1016/j.jssas.2016.07.006

Rahmiati, N.; Sari, R.; Wahyuni, S. T.; Lestari, M. L. A. D. Enhancing antioxidant properties of lime juice powder through polyelectrolyte microparticles of chitosan-alginate: Formulation, characterization and stability study. *J. Adv. Pharm. Technol. Res.* **2024**, *15*, 231-236. https://doi.org/10.4103/JAPTR.JAPTR_556_23

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rowell, K.; Winter, D. Quantitative Determination of Flavanones in Citrus Bioflavonoids by Potassium Borohydride Reduction. *J. Am. Pharm. Assoc.* **1959**, *48*, 746–749. https://doi.org/10.1002/jps.3030481214

Sanches, V. L.; Cunha, T. A.; Viganó, J.; Mesquita, L. M. S.; Faccioli, L. H.; Breitkreitz, M. C.; Rostagno, M. A. Comprehensive analysis of phenolics compounds in citrus fruits peels by UPLC-PDA and UPLC-Q/TOF MS using a fused-core column. *Food Chem.* **2022**, *14*, 1–9. https://doi.org/10.1016/j.fochx.2022.100262

Schäfer, G.; Bastianel, M.; Dornelles, A. Porta-enxertos utilizados na citricultura. *Ciência Rural.* **2001**, *31*, 723–733. http://hdl.handle.net/10183/22374

Schaich, K.; Tian, X.; Xie, J. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. *J. Funct. Foods*. **2015**, 14, 111–125. https://doi.org/10.1016/j.jff.2015.01.043

Sentkowska, A.; Biesaga, M.; Pyrzynska, K. Effects of brewing process on phenolic compounds and antioxidant activity of herbs. *Food Sci. Biotechnol.* **2016**, *25*, 965–970. https://doi.org/10.1080/10942912.2014.960932

Sentkowska, A.; Biesaga, M.; Pyrzynska, K. Polyphenolic Composition and Antioxidative Properties of Lemon Balm (*Melissa officinalis* L.) Extract Affected by Different Brewing Processes. *Int. J. Food Prop.* **2014**, *18*, 2009–2014. https://doi.org/10.1007/s10068-016-0157-9

Siddhartha, A.; Kaur, H.; Bains, K.; Kaur S. Comparative Analysis of Bioactive Compounds and Antioxidant Potential Among Various Citrus Varieties and Their Juices. *Appl. Fruit Sci.* **2025**, *67*, 31. https://doi.org/10.1007/s10341-024-01251-0

Synowiec-Wojtarowicz, A.; Kiselova, Y.; Yanov, D.; Ivanova, D.; Bialik, W.; Kimsa-Dudek, M.; Pawłowska–Góral, K. Effects of polyphenol supplementation to in vitro antioxidant potential of citrus juices. *Scripta Sci. Pharm.* **2018**, *5*, 28–32. http://doi.org/10.14748/ssp.v1i1.5036

Wang, H.; Chen, G.; Guo, X.; Abbasi, A.; Liu, R. Influence of the stage of ripeness on the phytochemical profiles, antioxidant and antiproliferative activities in different parts of Citrus reticulata Blanco cv. Chachiensis. *LWT - Food Sci. Technol.* **2016**, *69*, 67–75. https://doi.org/10.1016/j.lwt.2016.01.021

Yoo, K.; Lee, K.; Park, J.; Lee, H.; Hwang, I. Variation in Major Antioxidants and Total Antioxidant Activity of Yuzu (Citrus junosSieb ex Tanaka) during Maturation and between Cultivars. *J. Agric. Food Chem.* **2004**, *52*, 5907–5913. https://doi.org/10.1021/jf0498158

Zhang, Y.; Gan, R.; Li, S.; Zhou, Y.; Li, A.; Xu, D.; Li, H. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules.* **2015**, *20*, 21138–21156. https://doi.org/10.3390/molecules201219753

Zhang, Y.; Sun, Y.; Xi, W.; Shen, Y.; Qiao, L.; Zhong, L.; Ye, X.; Zhou, Z. Phenolic compositions and antioxidant capacities of Chinese wild mandarin (Citrus reticulata Blanco) fruits. *Food Chem.* **2014**, *145*, 674-680. https://doi.org/10.3390/molecules201219753

Zou, Z.; Xi, W.; Hu, Y.; Nie, C.; Zhou, Z. Antioxidant activity of Citrus fruits. *Food Chem.* **2016**, *196*, 885–896. https://doi.org/10.1016/j.foodchem.2015.09.072