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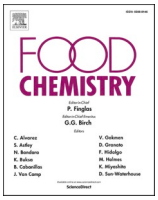

Bioactive profile of edible nasturtium and rose flowers during simulated gastrointestinal digestion

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ABSTRACT

Rose and nasturtium are common ornamental edible flowers rich in phytochemicals whose application as food is not widely explored. The gastrointestinal environment can modify these compounds, resulting in new combinations with different bioactivity. This study aimed to evaluate the effects of simulated gastrointestinal digestion (SGD) on rose and nasturtium flower extracts. Using UPLC-HRMS, 38 phenolic compounds were identified, and the SGD caused significant changes, mainly in the glycosylated phenolic. Furthermore, antioxidant activity was correlated with the increase in the concentrations of some polyphenols. Tested Gram-negative bacteria showed sensitivity to the flower extracts; their growth was inhibited by up to 82.7%. SGD interrupted the bacterial growth inhibition power of the rose extracts. On the other hand, an increase in inhibition ranging from 52.25 to 54.72% was found for nasturtium extracts, correlated to the behavior of some bioactive. Hence, SGD resulted in significant changes in phenolic profiles of the edible flowers, increasing antioxidant activity and changing antimicrobial effects.

1. Introduction

Ornamental flowers are often used to decorate gardens and aromatize rooms. However, the consumption of edible flowers is an ancient human habit. Nowadays, fresh and organic flowers have been considered unconventional food plants (UFP). However, their potential to be used as food is not widely explored (Matyjaszczyk & Śmiechowska, 2019). Chefs worldwide have utilized edible flowers in making salads, garnishes, and beverages, improving their sensorial (Pires et al., 2018) and nutritional (Matyjaszczyk & Śmiechowska, 2019) properties. Thus, edible flowers may be used strategically, resulting in different products, contributing to their market growth.

Nasturtium (*Tropaeolum majus* L.) is an ornamental medicinal plant native from Bolivia and Colombia, specifically from the Andes region. The flower's diameter may range from 2.5 to 6 cm, and each flower displays five petals, varying from yellow and orange to red, dark purple, or spotted colors (Garzón, Manns, Riedl, Schwartz, & Padilla-Zakour, 2015). *Rosa* sp. is a popular ornamental flower usually found in parks and gardens. Roses are deciduous shrubs that grow upright, and the stems have dense prickles. The fragrant flowers may grow as solitary or in clusters, and the most common colors are red, pink, yellow, and white (Zhang et al., 2018).

Phenolic compounds are the main bioactive compounds found in these edible flowers, which are involved in plant defense and reproduction mechanisms. Their daily ingestion has been associated with benefits for consumers' health (Garzón & Wrolstad, 2009). Among them, anthocyanins are water-soluble vacuolar pigments responsible for the many colors of these plant matrices (Pires et al., 2018). Other phenolic compounds are also found, including hydroxycinnamic and hydroxybenzoic acids, flavanols, flavonols, and other flavonoids. These compounds contribute considerably to the antioxidant and antimicrobial properties of edible flowers. Furthermore, the leading health benefits of

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edible flowers include their anti-inflammatory and antitumor properties and prevention of cognitive decline, diabetes, and cardiovascular disease (Fernandes, Casal, Pereira, Saraiva, & Ramalhosa, 2017).

However, these bioactive substances are sensitive to the gastrointestinal environment, especially to the pH variation, enzymes, biliary salts, and fermentation through gut microbiota, which modifies the phenolic structures and biological characteristics (Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009).

Recently, de Morais et al. (2020) reported on the effect of in vitro gastrointestinal digestion on antioxidant activity of some edible flowers. Despite this, most studies only use colorimetric assays to collect information on the bioaccessibility of phenolic compounds from edible flowers (Martin et al., 2016; Ozkan et al., 2021). Though the colorimetric technique is the traditional method for estimating bioaccessibility due to its simplicity and speed, interferences from other natural non-phenolic reducing compounds of food matrices, such as sugars, alcohols, organic acids, and amino acids, play an important role in the results (Rover & Brown, 2013).

In this study, ultra-performance liquid chromatography combined with high-resolution mass spectrometry analysis (UPLC-HRMS) was used to identify and monitor the phenolic compounds in edible rose (*Rosa* spp.) and nasturtium (*Tropaeolum majus* L.) flowers during simulated in vitro gastrointestinal digestion. The bioaccessibility and major changes in the chemical structure of each compound identified through chromatographic analysis were studied. Furthermore, the effect of the digestive process on the antioxidant and antimicrobial activities of edible flowers was investigated.

2. Material and methods

2.1. Plant material, standards, and reagents

Rose (*Rosa* spp.) samples were kindly provided by Lapinha Spa (Lapa, Parana, Brazil), and nasturtium (*Tropaeolum majus* L.) samples were obtained from Casa da Videira (Curitiba, Parana, Brazil). Edible flowers were harvested and immediately taken to the biotechnology laboratory of the Universidade Tecnologica Federal do Paraná (LaBiotec – UTFPR), where they were washed, frozen at −85 °C, and freeze-dried (Liotop, L101, SP, Brazil).

The Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ, (2,4,6-Tris(2-pyridyl)-s-triazine), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), the phenolic standards (purity > 97%) of quercetin-3-rutinoside, (+)-catechin, (-)-epicatechin, and cyanidin-3-glucoside, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma-Aldrich (São Paulo, SP, Brazil). Acetonitrile and methanol of HPLC grade were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). Gallic acid and solvents such as ethanol and glacial acetic acid were of analytical grade and purchased from Neon (Suzano, SP, Brazil).

2.2. Preparation of edible flower extracts

Edible flower samples were reduced to around 0.5 × 1 cm pieces and submitted to extraction according to Yang and Shin (2017), with modifications. The extraction was performed by stirring (120 rpm) the sample with 80% ethanol acidified with 1% glacial acetic acid at 40 ± 2 °C for 10 min, at a solid/liquid ratio of 1:20 w/v. Then, the liquid phase was recovered after filtration and kept at −20 ± 1 °C until further analyses.

2.3. Simulated gastrointestinal digestion assay

The simulated gastrointestinal digestion was carried out according to the methodology previously described by Gawlik-Dziki et al. (2009), with minor changes. Briefly, the gastric fluid was prepared using pepsin (3.2 g/L) diluted in 0.03 mol/L NaCl, acidified to pH 2.0 using 5 mol/L HCl. The intestinal fluid was prepared with pancreatin (1.42 g/L) and biliary salts (8.57 g/L) diluted in 0.1 mol/L $NaCO_3$, adjusted to pH 6.0 using 1 mol/L $NaHCO_3$. Edible flower extracts (5 mL) were added to 15 mL of gastric fluid and maintained in the dark for 2 h at 37 °C and 150 rpm. Then, an aliquot of the gastric samples was centrifuged (14000×*g*) and the supernatant was recovered and frozen until further analyses. The digestion process continued by adding 15 mL of intestinal fluid, 2.5 mL of 120 mmol/L NaCl, and 2.5 mL of 5 mmol/L KCl to 20 mL of previously digested samples in the gastric phase. The mixtures (in darkness) were shaken at 150 rpm for 1 h at 37 °C. After the intestinal digestion process, samples were centrifuged, and the supernatant was frozen at −20 ± 1 °C. The bioaccessibility of total phenolic compounds, total flavonoid compounds, total monomeric anthocyanins, and antioxidant activity was estimated according to Eq. (1), where B is the bioaccessibility (%), *C* is the remaining concentration (mg/g), and C_0 is the initial concentration (mg/g).

$$B(\%) = \left(\frac{C}{C_0}\right) \times 100 \quad (1)$$

2.4. Measurement of phenolic contents

Total phenolic compounds (TPC) were measured according to Singleton and Rossi (1965), with some changes. Folin-Ciocalteu reagent (150 μL) was mixed with 2.205 mL of distilled water and 45 μL of previously diluted extracts. After 3 min, 450 μL of 15% $CaCO_3$ was added, being vortexed again. The reaction occurred in the dark for 1 h, and the absorbance was measured at 765 nm. Gallic acid (ranging from 100 to 1000 mg/L) was used as a standard to plot the calibration curve, and the results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g dw).

Total flavonoid compounds (TFC) were determined as described by Zishen, Mengcheng, and Jianming (1999), with modifications. In test tubes, 1.525 mL of distilled water was mixed with 250 μL of the samples, 75 μL of 5% $NaNO_2$, 150 μL of 10% $AlCl_3$, and 500 μL of 1 M NaOH. The solutions were shaken, and the absorbance was measured at 510 nm. The concentration was calculated from the calibration curve obtained with (+)-catechin (100–1000 mg/L), and the results were expressed as milligram of catechin equivalents (CE) per gram of dry weight (mg CE/g dw).

Total monomeric anthocyanins (TMA) were measured through the pH differential method described by Giusti and Wrolstad (2001). Samples (250 μL) were put in contact with 1 mL of buffer solution pH 1.0 and another buffer solution pH 4.5. The change of pH modifies the color of the anthocyanins of the samples, which were measured at 510 and 700 nm. First, the absorbance value (A) was calculated using Eq. (2). Then, the TMA content was estimated using Eq. (3), where TMA means total monomeric anthocyanins, *A* is absorbance calculated on Eq. (2), *MW* is the molecular weight of cyanidin-3-*O*-glucoside (449.2 g/mol), *D* means dilution factor, ε is molar absorptivity coefficient of cyanidin-3-*O*-glucoside (26900 L/mol.cm), and λ is the optical length (1 cm). The result was expressed as milligrams of cyanidin-3-glucoside equivalents (CYA) per gram of dry weight (mg CYA/g dw).

$$A = \left[\left(A_{510nm} - A_{700nm}\right)_{pH1.0} - \left(A_{510nm} - A_{700nm}\right)_{pH4.5}\right] \quad (2)$$

$$TMA = \frac{A \times MW \times D \times 1000}{\varepsilon \times \lambda} \quad (3)$$

2.5. Antioxidant activity evaluation

The antioxidant activity by scavenging ABTS free radicals was evaluated according to the method described by of Re et al. (1999), with modifications. Firstly, the two stock solutions of potassium persulfate (2.45 mmol/L) and ABTS (7 mmol/L) were prepared, mixed, and allowed to react in the dark for 16 h. Next, a 4.0–4.5 mL aliquot of this

solution was further diluted in 250 mL of distilled water, obtaining an absorbance of 0.7, measured at 734 nm. Next, 2 mL of ABTS solution was added to 20 μL of the samples, vortexed, and kept in the dark for 30 min until the absorbance measurement (734 nm).

The antioxidant activity by the DPPH assay was assessed accordingly to the method previously described by Brand-Williams, Cuvelier, and Berset (1995), with minor modifications. The DPPH solution (0.0025 mol/L) was prepared in ethanol and added to the samples. The mixtures were shaken and kept in the dark for 30 min. The absorbances were measured at 517 nm.

The antioxidant activity by the ferric reducing ability of plasma (FRAP) was carried out by the method described by Benzie and Strain (1996). FRAP reagent was prepared using buffered solution (pH 3.6), TPTZ (0.032 g/mL) diluted in 40 mM HCl, and $FeCl_3$ (0.0054 g/mL). FRAP reagent (1.5 mL) was added to 50 μL of samples. The absorbance was measured at 593 nm.

All the antioxidant assays used Trolox as standard (ranging from 100 to 1000 μmol/L) to plot the calibration curve, and the results were expressed as micromoles of Trolox equivalent (TE) per gram of dry weight (μmol TE/g dw).

2.6. Antimicrobial activity evaluation

Edible flower extracts were prepared as previously described (Section 2.2). After removing the ethanol from the extracts at 40 ± 2 °C. The residue was resuspended with ultrapure water to the concentration of 50 g of edible flower per L of solvent. The solutions were filtered through 0.22 μm filters and kept in amber flasks at −20 °C until analysis.

Bacterial strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538), and *Salmonella* sp. were grown in Muller Hinton Agar (MHA) medium at 35 °C for 24 h.

The microdilution method described by Wiegand, Hilpert, and Hancock (2008) was used to determine the inhibitory effect of the flower extracts against the selected bacteria. The antimicrobial assay was performed in 96-well microplates, with 100 μL of sample extracts serially diluted in Mueller Hinton Broth (MHB). Growth and sterility controls were run in parallel to the tests. All analyses were carried out in duplicate. The incubation was carried out at 37 ± 2 °C/20 h. Amoxicillin (64 mg/L – initial concentration) was the antibiotic standard used as a reference. Antimicrobial activity was evaluated by measuring turbidity in a 96-wells microplate at 625 nm. The results were expressed as percentage growth inhibition compared to the growth control.

2.7. Analysis of phenolic compounds

2.7.1. Sample preparation

UPLC-HR was used to analyze the extracts obtained in Section 2.2. Before that, proteins were removed from samples by adding a solution of 50% methanol and 50% acetonitrile at the ratio of 1:1 (*v/v*). The mixture was centrifuged at 1500×g for 10 min at 4 °C, and the supernatant was recovered. All samples were diluted to 1.25 mg/mL using ultrapure water and filtered through a 0.22 μm syringe filter into amber glass vials. To eliminate interferences of the digestion methodology, control of the digested samples was prepared using water and the gastrointestinal digestion reagents.

2.7.2. UPLC-HRMS analysis

Chromatographic analysis was performed according to Ribeiro, Maciel, et al. (2019), with minor changes. Briefly, the phenolic compounds were identified using an Acquity UPLC H-Class system (Waters Corp., Milford, USA) equipped with a stationary phase C18 column (50 mm × 2.1 mm, 1.7 μm) kept at 35 °C.

Mass spectrometry analysis was performed on a XevoG2-S mass spectrometer (Waters Co., Milford, USA) equipped with a quadrupole time of flight hybrid (Q-TOF) with electrospray ionization (ESI) source operating in both positive and negative mode.

The quantification of +(−)catechin, cyanidin-3-glucoside, (−)-epicatechin, and quercetin-3-rutinoside was performed using authentic standards and validated based on the ICH Guideline Q2 (Guideline, 2005). In turn, for the other compounds identified but for which no authentic standards were available, the peak area was used to assess the impact of the digestion process and determine their bioavailability.

2.8. Statistical analysis

Data were obtained in triplicate, and the results of simulated gastrointestinal digestion of the same sample were analyzed by ANOVA test, which evaluates the variance of the data set ($p \leq 0.05$). The two edible flower samples were analyzed by a Student *t*-test ($p < 0.05$). Statistical analysis was carried out through the software Statistica 8.0 (StatSoft Inc., South America, Tulsa, OK, USA).

3. Results and discussion

3.1. Bioaccessibility of phenolic compounds in edible flower extracts

The phenolic compounds tentatively identified in the edible flower extracts are shown in Table 1. The flavanols (+)-catechin and (−)-epicatechin, as well as the flavonol, quercetin-3-rutinoside, and the anthocyanin cyanidin-3-glucoside, were identified by chromatographic comparison with analytical standards. The other phenolic compounds detected were identified through molecular mass and confirmed by theoretical mass and their respective fragment ions.

Thirty-eight different phenolic compounds were tentatively identified by UPLC-HRMS analysis, including 10 phenolic acids, 13 anthocyanins, 2 flavanols, 1 flavone, 8 flavonols, 1 flavononol, 2 gallotannins, and 1 cinnamic acid-derived lactone were found (Table 1 and Supplementary material Table S1). The bioaccessibility (B) was determined as the remaining percentage of the bioactive compound after gastrointestinal digestion. Bioactive compounds such as (+)-catechin, (−)-epicatechin, quercetin-3-rutinoside, and cyanidin-3-glucoside were quantified using analytical standard calibration curves (Table 2). However, other bioactive compounds were evaluated without a calibration curve (Table 3). Bioaccessibility of the identified phenolic compounds calculated by the percentage of increasing or decreasing the area of the peaks is presented in Table 3. The comparison of the area of the peaks was possible because the dilution was standardized to the final concentration of 1.25 mg of edible flower /mL of solvent for all experimental steps.

The concentration of some compounds, such as (+)-catechin, (−)-epicatechin, cyanidin-3-glucoside, and quercetin-3-rutinoside, was determined before and after simulated gastrointestinal digestion through standard calibration curves (Table 2). The edible flower samples showed different phenolic profiles, and all bioactive compounds were found on undigested samples. Gastrointestinal digestion occurs with modification of pH and presence of digestive enzymes, which may modify chemically molecular structures of phenolic compounds, obtaining new compounds with different bioaccessibility (Gawlik-Dziki et al., 2009). The monitoring of phenolic compounds during gastric and intestinal digestion phases showed modification of chemical structures. Nasturtium extracts showed flavanols concentrations lower than LOQ (limit of quantification; $LOQ_{catechin} = 0.04$ mg/g and $LOQ_{epicatechin} = 0.4$ mg/g). On these samples, quercetin-3-rutinoside was highlighted among other bioactive compounds, whereas in the rose extracts, (−)-epicatechin was detected in the highest concentration.

The bioaccessibility (considered the concentration available for absorption after simulated gastrointestinal digestion) of cyanidin-3-glucoside was up to 96% for both edible flowers. In rose extracts, this anthocyanin concentration was maintained stable during simulated digestion phases ($p > 0.05$); however, in nasturtium, this compound had a discrete increase during the gastric phase and a significant reduction

Table 1
Phenolic compounds tentatively identified in the edible flower extracts.

Phenolic compound	Molecular formula	Theoretical mass $[M + H]^+$	Found mass $[M + H]^+$	Fragments	RT (min)
(+)-Catechin*	$C_{15}H_{14}O_6$	291.0868	291.0872		4.15
Cyanidin-3-glucoside*	$C_{21}H_{21}O_{11}^+$	449.1078	449.1073		4.18
(−)-Epicatechin*	$C_{15}H_{14}O_6$	291.0868	291.0876		2.61
Quercetin-3-rutinoside*	$C_{27}H_{30}O_{16}$	611.1612	611.1619		4.35
Cyanidin	$C_{15}H_{11}O_6^+$	287.0556	287.0559	–	4.63
Cyanidin hexosyl deoxyhexoside	$C_{27}H_{31}O_{15}^+$	595.1663	595.1662	287.0554 449.1097	4.43
Cyanidin malonylhexoside	$C_{24}H_{23}O_{14}^+$	535.1088	535.1100	287.0555	4.56
Cyanidin hexoside	$C_{21}H_{20}O_{11}^+$	449.1084	449.1087	287.0554	4.48
Delphinidin	$C_{15}H_{11}O_7^+$	303.0499	303.0505	287.0557	4.39
Hydroxycoumarin	$C_9H_6O_3$	163.0394	163.0389	133.0296 105.0340	5.094
Ellagic acid pentoside	$C_{19}H_{14}O_{12}$	435.0563	435.0561	–	4.26
Cyanidin pentoside	$C_{20}H_{19}O_{10}^+$	419.0978	419.0982	287.0553	4.58
Pelargonidin hexoside	$C_{21}H_{21}O_{10}^+$	433.1135	433.1136	287.0560	4.63
Delphinidin hexoside	$C_{21}H_{21}O_{12}^+$	465.1033	465.1021	303.0156	4.40
Delphinidin dihexoside	$C_{27}H_{31}O_{17}^+$	627.1561	627.1550	303.0501 465.0862	4.24
Cyanidin (malonylhexosyl)-hexoside	$C_{30}H_{33}O_{19}^+$	697.1616	697.1609	287.0555	4.37
Trigalloylhexoside	$C_{27}H_{24}O_{18}$	659.2895	659.2795	–	7.12
Cyanidin galloylhexoside	$C_{28}H_{25}O_{1'5}^+$	601.1193	601.1194	287.0550	4.45
Phenolic compound	**Molecular formula**	**Theoretical mass $[M-H]^-$**	**Found mass $[M-H]^-$**	**Fragments**	**RT (min)**
Quinic acid	$C_7H_{12}O_6$	191.0548	191.0556	173.0082 111.0091 85.0301	0.34
Kaempferol	$C_{15}H_{10}O_6$	285.0395	285.0399	–	4.63
Ellagic acid	$C_{14}H_6O_8$	300.9981	300.9984	123.0077 117.0339	4.35
Coumaric acid hexoside	$C_{15}H_{18}O_8$	325.0923	325.0925	169.0140	4.29
Gallic acid hexoside	$C_{13}H_{16}O_{10}$	331.0648	331.0655	–	0.34
Coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0918	337.0918	–	4.03
Caffeoylquinic acid	$C_{16}H_{18}O_9$	353.0872	353.08	191.0410 179.0391	2.98
Apigenin hexoside	$C_{21}H_{20}O_{10}$	431.0990	431.0990	269.0459	4.62
Kaempferol pentoside	$C_{20}H_{18}O_{10}$	417.0829	417.0835	285.0388	4.55
Quercetin pentoside	$C_{20}H_{18}O_{10}$	433.2446	433.2435	301.0352	4.17
Taxifolin pentoside	$C_{20}H_{20}O_{11}$	435	435.1297	303.0505	4.60
Kaempferol hexoside	$C_{21}H_{20}O_{11}$	447,0928	447.0935	285.0391	4.49
Quercetin hexoside	$C_{21}H_{20}O_{12}$	463.0877	463.0856	301.0529	4.39
Digalloylshikimic acid	$C_{21}H_{18}O_{13}$	477.0229	477.0226	–	4.41
Kaempferol acetyl-hexoside	$C_{23}H_{22}O_{12}$	489.1033	489.1028	285.0399	4.55
Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1194	515.1189	173.0500 135.0461	5.51
Kaempferol hexosyl deoxyhexoside	$C_{27}H_{30}O_{15}$	593.1511	593.1512	447.0915 285.0399	4.44
Cyanidin dihexoside	$C_{27}H_{31}O_{16}^+$	611.1607	611.1607	285.0401	4.31
Tricaffeoylquinic acid	$C_{34}H_{30}O_{15}$	677.1506	677.1506	515.1166	4.34
HHDP-galloyl-hexoside	$C_{27}H_{22}O_{18}$	633.3791	633.3791	–	6.20

*Samples identified and quantified via analytical standard. HHDP: hexahydroxydiphenol. RT: Retention time. $[M + H]^+$: Molecular mass of the compound summed mass of 1 hydrogen. $[M-H]^-$: Molecular mass of the compound less mass of 1 hydrogen. Hydrogen mass: 1.0078 g.

after intestinal digestion ($p \leq 0.05$). On the other hand, the quercetin-3-rutinoside concentration decreased under LOQ after simulated gastrointestinal digestion of rose extracts. In nasturtium samples, this flavonol was gradually reduced during gastric and intestinal phases (B = 58.46%).

Catechins degradation has been related to the action of biliary salts and pancreatin present on the intestinal fluid. The same catechin profile behavior was observed in other edible flowers, such as marigold (*Tagetes patula* L.) and mini daisy (*Bellis annua* L.) (de Morais et al., 2020). The catechin's isomeric form, (−)-epicatechin, showed an increase after simulated gastrointestinal digestion of rose extracts, reaching a bioaccessibility of 132.24%. The increase in the levels of this flavonoid after gastrointestinal digestion may be associated with the degradation of polymeric compounds such as procyanidins and gallate epicatechin forms (Mendes et al., 2019).

The bioaccessibility of flavonols such as (+)-catechin and (−)-epicatechin was undetermined in samples that showed concentrations under LOQ (at least one of the monitored steps). Nasturtium extracts showed a concentration of flavanols lower than LOQ. Catechin was found in the rose sample. However, its bioaccessibility has not been determined for both edible flowers. For rose extracts, the concentration decreased until lower than LOQ after gastric digestion, indicating a degradation or possible modification on the chemical structure of these bioactive compounds. Simultaneously, the concentration of epicatechin increased after rose extract digestion, meaning possible biotransformation among flavanols during simulated gastrointestinal digestion.

The modifications of cyanidin 3-glucosides on the gastric phase are mainly related to the extreme reduction of pH (pH 2.0) associated with temperature (37 °C). The drastic decrease of pH may increase the flavylium cation concentration (Pérez-Vicente, Gil-Izquierdo, & García-Viguera, 2002), as observed in the gastric phase of nasturtium. In general, anthocyanins showed reduced concentration after the intestinal phase. However, the elevated bioaccessibility of this compound observed during the digestive stages is influenced by many factors. The presence of glucosidases, probably from the enzymatic mix used to prepare gastric and intestinal fluids, may modify glycosylated phenolic profiles. All anthocyanins showed bioaccessibility higher than 100% on rose extract (Table 3). On nasturtium extracts, the bioaccessibility of

Table 2
Effect of simulated gastrointestinal digestion on the content and bioaccessibility of phenolic compounds from the two edible flowers.

	Rose			
	Undigested extract	Gastric phase	Intestinal phase	Bioaccessibility (%)
(+)-Catechin (mg/g dw)	0.044 ± 0.001	< LOQ	< LOQ	ND
Cyanidin-3-glucoside (mg/g dw)	0.062 ± 0.001 [a]	0.062 ± 0.001 [a]	0.061 ± 0.001 [a]	98.38
(-)-Epicatechin (mg/g dw)	11.9 ± 0.8[b]	16 ± 2 [a]	15.8 ± 0.9 [a]	132.24
Quercetin-3-rutinoside (mg/g dw)	0.43 ± 0.01	< LOQ	< LOQ	ND
	Nasturtium			
	Undigested extract	**Gastric phase**	**Intestinal phase**	**Bioaccessibility (%)**
(+)-Catechin (mg/g dw)	< LOQ	< LOQ	< LOQ	ND
Cyanidin-3-glucoside (mg/g dw)	0.055 ± 0.001 [ab]	0.056 ± 0.001 [a]	0.053 ± 0.002[b]	96.36
(−)-Epicatechin (mg/g dw)	< LOQ	< LOQ	< LOQ	ND
Quercetin-3-rutinoside (mg/g dw)	11.7 ± 0.7 [a]	9.91 ± 0.05[b]	6.84 ± 0.44[c]	58.46

dw: dry weight. ND: not determined. [abc]: different letters in the same line mean significant difference among the gastrointestinal digestion phases.

anthocyanins ranged from 8.09% (delphinidin hexoside) to 204% (cyanidin hexosyl deoxyhexoside). Furthermore, synergistic and antagonistic effects may occur among different unidentified phenolic compounds, implying a phenolic profile modification.

The same effect was observed in other glycosylated phenolic compounds. Quercetin 3-rutinoside content also decreased after simulated gastrointestinal digestion (Table 2), whereas the bioaccessibility of other glycosylated flavonols (quercetins and kaempferols) increased (Table 3). In both edible flowers, quercetin pentoside was degraded during the digestive process. Quercetin hexoside showed bioaccessibility of 67.10 % and 168% in rose and nasturtium extracts, respectively. The increasing bioaccessibility of quercetin hexoside observed on nasturtium may be associated with partial hydrolysis of rutinoside radical, which may lose a rhamnose molecule, maintaining just a hexose molecule, probably linked on 3C position. Gastric and intestinal fluids were prepared with enzymes and bile salts extracted from digestive organs from mammal animals such as pigs or cattle (Ahmed, Nasim, Batool, & Bibi, 2017). These organs contain glucosidases and various other enzymes. Thus, radical glycosyl modification may occur during the gastric phase due to the pH challenges (acid hydrolysis) and, during the intestinal phase, due to the presence of glucosidases.

Other bioactive compounds were identified through mass spectrometry, and bioaccessibility was estimated through the area of the peaks, as previously mentioned. In these cases, bioaccessibility was considered not determined in cases of undetected peaks in all experimental steps, and zero when the peak was found on the undigested samples and not found after the intestinal phase. Individual compounds from rose and nasturtium edible flowers showed bioaccessibility up to 325.87% and 465.38%, respectively (Table 3).

As shown in Table 3, the bioaccessibility of phenolic acids in the rose extracts varied from 0 (dicaffeoylquinic and digalloylshikimic acids) to 193.39% (ellagic acid). In nasturtium extracts, the high increase in the bioaccessibility of caffeoylquinic acid (B = 465.38%) may be related to the degradation of di- and tricaffeoylquinic acids, which were not found after the digestion process. The increased bioaccessibility of coumaroylquinic acid may also be associated with the reduction in quinic acid levels after all digestion steps. The high bioaccessibility of ellagic acid may be related to the reduced bioaccessibility of ellagic acid pentoside.

Gallotannins and their derivatives have positive effects on human health, being involved in the reduction of obesity (Fang et al., 2018). Among gallotannins, tri-galloyl-hexoside showed bioaccessibility varying from 10.45% (rose) to 30.02% (nasturtium). The other compound, HHDP-galloyl-hexoside, was degraded after in vitro digestion, which showed its greater susceptibility to gastrointestinal digestion.

Hydroxycoumarin, a non-phenolic bioactive compound, was found in rose extracts and had a bioaccessibility of 73.06%. This lactone is derived from o-hydroxycinnamic acid and has application in the pharmaceutical industry due to its anticoagulant and antitumorigenic effects (Au & Rettie, 2008; Jung, Lee, Oh, Lee, & Park, 2004).

Although phenolic compounds generally show low stability during gastrointestinal digestion, this research showed exciting results, mainly for glycosylated compounds such as quercetin, kaempferols, and anthocyanins. This effect may be associated with the presence of glucosidases, enzymes that can catalyze the hydrolysis of sugar bonds, and the switch of glycosyl radicals. Furthermore, the results obtained through UPLC-HRMS analyses were consistent with those of the colorimetric determinations (Table 4).

3.2. Bioaccessible phenolic content and antioxidant activity

Colorimetric analyses were used as rapidly assays to estimate the concentrations of some constituents of the edible flowers extract samples, such as total phenolic content (TPC), total flavonoid compounds (TFC), total monomeric anthocyanins (TMA), and the in vitro antioxidant activity (ABTS, DPPH, and FRAP assays). The colorimetric analyses were carried out before and after each simulated gastrointestinal digestion phase in rose and nasturtium edible flowers (Table 4). TPC ranged from 12.33 to 24.26 mg GAE/g dw and had a positive correlation with antioxidant activity ($r > 0.99$), which means that phenolic compounds are the main ones responsible for the antioxidant properties of the edible flowers. The TPC was significantly different for the two edible flowers ($p < 0.0001$). The rose extract had TPC contents about 2-fold higher than the nasturtium. The main phenolic compounds found in edible flowers belong to the classes of phenolic acids and flavonoids. The TFC showed no significant difference for the rose and nasturtium edible flowers ($p > 0.05$), which had around 5 mg CE/g dw in both samples (Table 4).

The range of TFC was narrow between the studied edible flowers, from 4.92 to 5.05 mg CE/g dw. Although the undigested nasturtium samples had a lower TPC than rose, the TFC levels were comparable. The TFC in the rose extract represented about 20% of the TPC, while, in the nasturtium extract, the TPC represented 41% of TFC. Flavonoids have shown antioxidant, anti-inflammatory, antiproliferative, and anticancer properties (Xiao et al., 2011). Therefore, the extraction or isolation of flavonoid compounds from edible nasturtium flowers could improve these bioactive properties.

TMA concentration varied from 0.66 to 3.79 mg CYA/g dw. Anthocyanins are a type of flavonoid responsible for the attractive color and antioxidant activity of edible flowers (Benvenuti, Bortolotti, & Maggini, 2016). Although with a lower TPC concentration, nasturtium stood out in the TMA concentration ($p = 0.049$), as shown in Table 4. The higher TMA content in nasturtium probably may be related to their intense color (yellowness, redness, orangeness, and spots) (Garzón et al., 2015). As natural colorants of edible flowers, anthocyanins have been used as alternatives to artificial colorants used in manufacturing food products (Pires et al., 2018). Besides, these pigments are known for the health benefits (e.g., reduction of glycemia) promoted by the regular consumption of anthocyanin-containing foods (Sasaki et al., 2007).

Edible flowers have high concentrations of bioactive compounds and, therefore, can be considered as natural sources of antioxidants, as

Table 3
Effect of simulated gastrointestinal digestion on the bioaccessibility of the phenolic compounds identified in the two edible flowers.

	Rose				Nasturtium			
	Undigested extract	Gastric phase	Intestinal phase	Bioaccessibility (%)	Undigested extract	Gastric phase	Intestinal phase	Bioaccessibility (%)
Phenolic acids								
Caffeoylquinic acid	–	–	–	ND	780	654	3630	465.38
Coumaroylquinic acid	–	–	–	ND	293	481	902	307.84
Dicaffeoylquinic acid	49	20	–	0	106	39	–	0
Digalloylshikimic acid	155	–	–	0	–	–	–	ND
Ellagic acid	1679	1237	3247	193.39	–	–	–	ND
Ellagic acid pentoside	304	199	134	44.07	–	–	–	ND
Gallic acid hexoside	581	699	884	152.15	70	68	–	0
Coumaric acid hexoside	589	583	857	145.50	–	–	–	ND
Quinic acid	30,767	10,515	4627	15.04	654	274	–	0
Tricaffeoylquinic acid	–	–	–	ND	456	163	–	0
Anthocyanins								
Cyanidin	30,144	29,989	41,655	138.18	33,497	31,499	25,337	74.63
Cyanidin dihexoside	–	–	–	ND	1712	2158	1198	69.98
Cyanidin galloyl hexoside	754	414	1007	133.55	–	–	–	ND
Cyanidin hexoside	3245	2982	3943	121.51	1495	1547	1064	71.17
Cyanidin hexosyl deoxyhexoside	512	481	809	158	29,202	38,455	59,686	204
Cyanidin malonylhexoside	64	62	102	159	674	722	896	132.94
Cyanidin malonylhexosyl-hexoside	–	–	–	ND	3645	3614	2303	63.18
Cyanidin pentoside	1165	1099	1564	134.25	–	–	–	ND
Delphinidin	621	580	837	134.78	530	556	631	119.05
Delphinidin dihexoside	–	–	–	ND	445	390	36	8.09
Delphinidin hexoside	67	65	101	150.75	32	31	23	71.87
Pelargonidin hexoside	4009	3839	4374	109.10	–	–	–	ND
Flavone								
Apigenin hexoside	19,227	16,644	16,149	83.99	–	–	–	ND
Flavonols								
Kaempferol	1031	857	893	86.61	155	218	–	0
Kaempferol acetyl- hexoside	140	116	176	125.71	471	843	1451	308.07
Kaempferol hexoside	2087	7493	6801	325.87	3391	4933	3211	94.69
Kaempferol hexosyl deoxyhexoside	1485	1259	1203	81.01	4993	6382	4537	90.87
Kaempferol pentoside	4613	3827	3432	74.40	–	–	–	ND
Quercetina hexoside	310	253	208	67.10	73	115	123	168
Quercetina pentoside	1168	1600	–	0	128	–	–	0
Flavononol								
Taxifolin pentoside	6403	5579	6474	101.11	–	–	–	ND
Gallotannins								
HHDP-galloyl-hexoside	227	–	–	0	–	–	–	ND
Tri-galloyl-hexopiranose	1397	697	146	10.45	1066	545	320	30.02
Lactone from o-hydroxycinnamic acid								
Hydroxycumarin	193	165	141	73.06	–	–	–	ND

-: not detected; ND: not determined. The values correspond to the peak areas of the phenolic compounds identified in the undigested and digested extracts of the two edible flowers, used to determine its bioaccessibility.

can be observed in Table 4. The antioxidant activity of rose was superior ($p < 0.001$) to that of nasturtium for the three tests applied. Cyanidin 3-glucoside, (+)-catechin, and (−)-epicatechin were correlated to the antioxidant activity of the edible flowers ($r > 0.99$). In turn, quercetin 3-rutinoside had a negative correlation ($0 > r \geq -1$). The sample with the highest antioxidant activity (rose) had the lowest concentration of this compound. The presence of these constituents is important to the antioxidant activity because phenolic compounds may act as primary or secondary antioxidants accordingly to their chemical structure (Pannala, Chan, O'Brien, & Rice-Evans, 2001). The presence of flavonoids with conjugation on the C ring (for example, quercetins and cyanidins) increases the antioxidant activity through the ABTS assay (Rice-Evans, Miller, & Paganga, 1996). Furthermore, each method has shown a particular mechanism; the FRAP assay is known by the single electron transfer (ET) mechanism, whereas DPPH and ABTS assays may occur both by ET and hydrogen atom transfer (HAT) mechanisms (Apak, Ozyürek, Güçlü, & Çapanoglu, 2016). Therefore, both the characteristics of the phenolic structure and the method used to assess the antioxidant activity can influence the results of antioxidant assays.

Phenolic compounds from both edible flowers were modified during gastrointestinal digestion (Table 4). In nasturtium, the values of TPC, TFC, and antioxidant activity by FRAP translated a significant reduction after simulated gastrointestinal digestion ($3.11\% \leq B \leq 85.48\%$). In Rose extracts, the same results were observed. Additionally, a decrease in the antioxidant activity by DPPH assay was observed ($1.23\% \leq B \leq 81.64\%$). On the other hand, TMA concentration and antioxidant activity by ABTS increased after gastrointestinal digestion in both edible flowers ($119.78\% \leq B \leq 1073.91\%$). The same effect was observed on antioxidant activity through DPPH scavenging activity on nasturtium extracts ($B = 382.38\%$). The variations observed in phenolic compounds and antioxidant activity, evaluated through colorimetric assays, suggested that some phenolic classes are more sensitive to gastric liquid (pepsin solution, pH 2.0) and more stable to intestinal digestion than others. Furthermore, the bioaccessibility of TPC with values higher than 81% for both samples meant that phenolic compounds present in edible flowers are more stable than those determined in teas (Ribeiro et al.,

Table 4
Phenolic content and antioxidant activity of the edible flower extracts during simulated gastrointestinal digestion.

	Rose			
	Undigested extract	Gastric phase	Intestinal phase	Bioaccessibility (%)
TPC (mg GAE/g dw)	24 ± 1 [a]	24.6 ± 0.9 [a]	19.8 ± 0.4[b]	81.64
TFC (mg CAE/g dw)	4.9 ± 0.3 [a]	3.73 ± 0.02[b]	3.3 ± 0.2[b]	66.86
TMA (mg CYA/g dw)	0.66 ± 0.04[c]	1.9 ± 0.2[b]	4.6 ± 0.1 [a]	695.45
ABTS (μmol TE/g dw)	3.2 ± 0.2[b]	1.63 ± 0.02[c]	22.6 ± 0.5 [a]	696.3
DPPH (μmol TE/g dw)	145.4 ± 0.5 [a]	109.7 ± 0.2[b]	109.05 ± 0.05 [a]	74.99
FRAP (μmol TE/g dw)	771 ± 10 [a]	14.5 ± 0.6[b]	9.5 ± 0.8[b]	1.23
	Nasturtium			
	Undigested extract	**Gastric phase**	**Intestinal phase**	**Bioaccessibility (%)**
TPC (mg GAE/g dw)	12.3 ± 0.6[b]	14.3 ± 0.7 [a]	10.5 ± 0.7[c]	85.48
TFC (mg CE/g dw)	5.1 ± 0.2 [a]	2.5 ± 0.1[b]	1.8 ± 0.2[c]	36.43
TMA (mg CYA/g dw)	3.8 ± 0.1[b]	10.8 ± 0.8 [a]	4.5 ± 0.4[b]	119.78
ABTS (μmol TE/g dw)	1.38 ± 0.07[b]	1.03 ± 0.07[b]	15 ± 2 [a]	1073.91
DPPH (μmol TE/g dw)	28.34 ± 0.08[c]	50.2 ± 0.1[b]	108.5 ± 0.1 [a]	382.38
FRAP (μmol TE/g dw)	212 ± 5 [a]	6.26 ± 0.04[b]	6.6 ± 0.6[b]	3.11

TPC: Total phenolic content. GAE: Gallic acid equivalent. TFC: Total flavonoid compounds. CAT: (+)-Catechin equivalent. TMA: Total monomeric anthocyanins. CYA: Cyanidin-3-glucoside equivalent. TE: Trolox equivalent. [abc] Different letters in the same line mean significant differences ($p < 0.05$) between samples.

2021) and fruit purees (Stafussa et al., 2021). Therefore, technologies have been developed to preserve sensible phenolic compounds during gastrointestinal digestion, such as encapsulation of phenolic compounds (Piovesana & Noreña, 2018) and biosorption of bioactive compounds in *Saccharomyces cerevisiae* (Ribeiro, Fernandes, et al., 2019).

As shown in Table 4, nasturtium had a reduction ($p \leq 0.05$) of some parameters tested after simulated gastric digestion. However, after in vitro intestinal digestion, the antioxidant activity increased compared to the gastric phase (FRAP) and undigested samples (DPPH and ABTS). Some glycosylated compounds undergo hydrolysis before intestinal absorption, resulting in structural modifications and bioactive changes. Flavonoids, such as quercetins and anthocyanins, may show aglycone forms or glycosyl radicals at the C3 and C4 positions, respectively (Xiao et al., 2011). The losses of sugar molecules attached at this position could generate a proportionally available new hydroxyl radical. Therefore, the loss of glycosylated molecules during gastrointestinal digestion may have positively impacted the antioxidant activity of edible flowers. The different behaviors observed in the antioxidant activity through the FRAP assay may be explained through its mechanism of action.

3.3. Effects of edible flower extracts on bacterial growth

The antibacterial effects of rose and nasturtium edible flower extracts were determined against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* sp.) and Gram-positive (*Staphyloccocus aureus*) strains. The extracts inhibited bacteria growth in different levels, suggesting a sensitivity of the microorganisms to the edible flower extracts (Table 5). S. aureus was the only strain that was not sensitive to phenolic extracts from edible flowers. This result, repeated twice in duplicate and confirmed, indicates that the compounds present in these matrices may be selective for Gram-negative bacteria.

The antimicrobial effects of plant extracts depend on many factors, such as the species and variety, maturation degree, and the classes of phenolic compounds present in the extracts, as well as the resistance of microorganisms. Furthermore, the composition of the cell wall contributes to the reduction of bacterial sensitivity. For example, Gram-positive bacteria have a thick layer of peptidoglycans, whereas the cell wall of Gram-negative bacteria has a thin layer of peptidoglycans, periplasmic space, and an outer membrane of a lipopolysaccharide moiety (Masi, Réfregiers, Pos, & Pagès, 2017). Disruption of the bacterial membrane structure is not the most common factor associated with antimicrobial properties. However, some phenolic compounds such as gallic and ferulic acids may modify the hydrophobicity and hydrophilicity of Gram-positive and Gram-negative strains, respectively (Borges, Ferreira, Saavedra, & Simões, 2013). Furthermore, the growth inhibition of bacterial strains may result from different factors, such as interference on cell wall synthesis, inhibition of protein or nucleic acid synthesis, and inhibition of metabolic pathways. Besides, "quorum sensing," a gene regulation that bacteria cells use to detect and respond to cell density (Hossain et al., 2017), and enzymes inhibition also contribute to the detrimental effect on bacterial growth.

The gastrointestinal digestion process of bioactive compounds from the selected edible flowers modified the antimicrobial activity. After the digestive process, bacterial strains lost their sensitivity to rose extracts, whereas, on nasturtium extracts, the sensitivity increased almost 4-fold for *E. coli* and 13-fold for *Salmonella* sp. Thus, the peak area of the

Table 5
Bacterial strains' sensitivity to the edible flowers extracts during simulated gastrointestinal digestion.

	Rose		
	Undigested extract (% of growth inhibition)	Gastric phase (% of growth inhibition)	Intestinal phase (% of growth inhibition)
Escherichia coli	83 ± 2 [a]	85 ± 3 [a]	–
Salmonella sp.	62 ± 2 [a]	63 ± 2 [a]	–
Pseudomonas aeruginosa	78 ± 2	–	–
Staphylococcus aureus	–	–	–
	Nasturtium		
	Undigested extract (% of growth inhibition)	**Gastric phase (% of growth inhibition)**	**Intestinal phase (% of growth inhibition)**
Escherichia coli	17.6 ± 0.5[c]	46 ± 1[b]	70 ± 2 [a]
Salmonella sp.	4.3 ± 0.1[c]	8 ± 1[b]	59.0 ± 0.4 [a]
Pseudomonas aeruginosa	57 ± 2	–	–
Staphylococcus aureus	–	–	–

[abc]: different letters in the same line mean significant differences ($p < 0.05$) between samples. -: no growth inhibition.

individual compounds determined by UPLC-HRMS at each step of the gastrointestinal digestion (Table 2) was correlated with the sensitivity of each bacterial strain (Table 5). These results imply that some phenolic compounds on the edible flowers may have influenced bacterial growth (Table S2). In rose extracts, ellagic acid pentoside, dicaffeoylquinic acid, hydroxycoumarin, tri-galloyl-hexoside, kaempferol pentoside, and quercetin hexoside were the main phenolic compounds correlated with the growth inhibition of *E. coli*, *Salmonella* sp., and *P. aeruginosa*. Furthermore, the studied bacteria showed sensitivity for some identified polyphenols. The sensitivity of *E. coli* and *Salmonella* sp. was correlated to quercetin pentoside, but pelargonidin hexoside also affected the inhibition of *Salmonella* sp. The use of rose extracts to inhibit *P. aeruginosa* was also correlated with quercetin 3-rutinoside and other compounds such as kaempferol, caffeoylquinic acid, digalloylshikimic acid, kaempferol hexosyl deoxyhexoside, tri galloyl hexoside, HHDP-galloyl-hexoside (Table S2). Among the compounds found in nasturtium extracts, cyanidin hexosyl deoxyhexoside, delphinidin, coumaroylquinic acid, caffeoylquinic acid, quercetin hexoside, and kaempferol acetyl hexoside were correlated with the sensitivity observed for *E. coli* and *Salmonella* sp. Cyanidin malonylhexoside was also related to *Salmonella* sp. inhibition. In turn, the inhibition of *P. aeruginosa* was correlated to quercetin 3-rutinoside, cyanidin, kaempferol, quinic acids, dicaffeoylquinic, and tricaffeoylquinic acid.

Since some microorganisms considered probiotics (such as *Lactobacillus* spp.) are Gram-positive bacteria (Hill et al., 2018), the selectivity observed in these extracts, where the antimicrobial effect was identified only in Gram-negative bacteria and even enhanced after digestion, the tested extracts could be an alternative to non-selective antimicrobials. These compounds can be explored for synergistic use with other antimicrobials, promoting activity against potentially pathogenic Gram-negative bacteria found in the intestinal tract without harming the growth of beneficial bacteria such as Gram-positive.

Edible flower extracts obtained from acidified hydroalcoholic extracts did not show antimicrobial properties. However, the Gram-negative strains used in this study showed sensitivity to their components. Thus, future studies may focus on modifying the extraction method or purifying specific compounds responsible for the antimicrobial effect.

4. Conclusion

Flavonoids and phenolic acids were the major classes of phenolic compounds detected in rose and nasturtium edible flowers, in which up to 38 different structures were identified by UPLC coupled with mass spectrometry. These compounds, specifically the glycosylated polyphenols, underwent biochemical modification during the simulated gastrointestinal digestion process. Thus, greater bioaccessibility of some compounds was associated with reduced bioaccessibility of others. Furthermore, the antioxidant activity through ABTS was increased after simulated digestion. Gram-negative bacterial strains showed sensitivity to the edible flower extracts, which was positively correlated with individual phenolic compounds. Overall, rose and nasturtium show potential as gourmet foods for human consumption, as they present some phenolic compounds with high bioaccessibility and preserve some bioactive properties after simulated gastrointestinal digestion.

CRediT authorship contribution statement

Débora Gonçalves Bortolini: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Lillian Barros:** Writing – review & editing, Supervision, Funding acquisition. **Giselle Maria Maciel:** Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Tatiane Brugnari:** Conceptualization, Investigation, Data curation, Writing – original draft. **Tatiani Andressa Modkovski:** Formal analysis, Investigation, Writing – original draft. **Mariana Millan Fachi:** Formal analysis, Data curation, Writing – original draft. **Roberto Pontarolo:** Formal analysis, Resources, Supervision. **José Pinela:** Writing – review & editing, Funding acquisition. **Isabel C.F.R. Ferreira:** Writing – review & editing, Funding acquisition. **Charles Windson Isidoro Haminiuk:** Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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