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Antioxidant activity from non-conventional beverage plant sources in Argentina

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Abstract

The objective of this work was to evaluate the antioxidant activity of extracts from various ornamental and wild edible plants commonly found in urban parks. Antioxidant activities were assessed through ferric reducing antioxidant power (FRAP) and free radical scavenging assays (DPPH and ABTS) while the total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent. Eight commercial samples (ginger, turmeric, rosemary, thyme, rooibos, coriander, cloves, and drumstick) were analyzed under the same conditions. Clove extract exhibited the highest antioxidant activity among the commercial samples across all methods. The study further explored the pH, color characteristics, and antioxidant capacities of all the samples. The pH values of the extracts varied from slightly acidic (6.71) to alkaline (9.51), with coriander extract showing the highest pH. The color profiles ranged from green-yellowish tones in leaf extracts to brown tones in bark and pod extracts, and reddish tones in flower extracts. Notably, *Bougainvillea glabra* and *Callistemon citrinus* exhibited particularly high antioxidant activities in the FRAP and ABTS assays, respectively. Correlation analysis revealed significant relationships between antioxidant activity and specific color parameters, particularly at absorbance wavelengths of 490 and 550 nm. These findings underscore the potential of certain ornamental and commercial plant species to enhance the nutritional and sensory qualities of functional beverages, contributing valuable insights for the development of health-promoting products in the beverage industry.

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Introduction

In recent years, there has been a trend towards consuming products that provide health benefits. Honey and some teas are examples of natural products with a long history of medicinal use[1]. Spices have been used for a long time to enhance the aroma and flavor of food, as well as for their preservative and medicinal properties[2–4]. Herbs and spices are traditionally defined as any part of plants used in the diet for their aromatic properties. Herbs come from the leaves while spices come from any dried part of a plant, and both contain high levels of polyphenols and other physiologically active phytochemicals[5]. Wild edible plants (WEPs) as a source of nutrients, feeding livestock, or for therapeutic purposes have been highlighted in some studies[6–8]. Furthermore, WEPs tend to be resistant to extreme environmental conditions[9]. Depending on factors including the levels of inherent toxic compounds and/or those of fertilizers, herbicides, or pesticides, plants are classified as edible or inedible, depending on whether they may be dangerous to human health. The FDA has recognized more than 150 plants as GRAS, without any intake limitations. Coriander, cumin, anise, fennel, thyme, and oregano are found in this list[10]. Plants have numerous phytochemical substances that act as natural defenses protecting them from infections and guests giving them color, aroma, and flavor. The use of phytochemicals in food products has become an interesting area for the food industry to explore new sources of antioxidants to replace the current use of synthetic chemicals in the development of food products. The most important phytochemicals in plant foods are phenolics. Phenolic compounds are a heterogeneous group of products with more than 10,000 compounds, whose molecular structures contain at least one phenol group; an aromatic ring at least joined a hydroxyl group, and that plays a very heterogeneous range of roles in plants, defense, mechanical support, attract pollinators or absorbs ultraviolet radiation[11]. Some research on wild plants, from different geographical regions of the world, has shown that they contain strong antioxidant properties[6–9,12–15]. Antioxidants are molecules able to protect against free radicals and their damaging effects at multiple phases (prevention, interception, and repair) by donating hydrogen, reducing singlet oxygen, serving as chelators, and capturing free radicals through diverse methods[16]. Long-term consumption of diets rich in polyphenols would offer protection against the development of various chronic diseases, according to clinical and preclinical studies[17,18]. Polyphenols can be found in leaves, fruits, bark, or roots. Since ancient times, throughout various cultures, there are numerous examples of the use of plants for therapeutic purposes to treat diseases or ailments. The extract of the bark of *Pinus pinaster* is probably the most studied phenolic tree extract worldwide as a phytochemical remedy for various diseases, and having cardiovascular benefits[19,20]. The rhizomes of *Zinger* and *Curcuma* species have been exploited as spices and food preservatives, flavoring agents, and remedies for the treatment of many diseases, both have high antioxidant properties[10,21]. Other examples of samples with nutraceutical and pharmaceutical properties are thyme and rosemary[22], *Artemisia* species[23,24], *Moringa oleifera*[25,26], *Pelargonium* species[27], *Passiflora* species[28] and *Bauhinia forficata*[29,30]. Several flowers have been eaten in traditional country meal recipes and can also be consumed as tea[31]. Studies on edible flowers have been focused on their renewed popularity as a source of phytonutrients and antioxidants with a proven healthy effect[32]. *Tropaeolum majus* L. (Tropaeolaceae) is an example of a plant where all its parts are edible and highly nutritive[33]. Citrus represents one of the most important fruit crops cultivated worldwide, for alimentary or industrial purposes[34]. On the other hand, tomato is the world's largest vegetable crop, which can be consumed in both raw or processed forms[35]. Both fruit by-products are a source of bioactive compounds for example limonene and citral of lemon rind and lycopene from tomato[34,35].

Maceration, infusion, decoction, or Soxhlet extraction are examples of extraction methods applied for obtaining phytochemical compounds[11]. Based on sustainable development concepts and green chemistry, many efforts have been made to provide highly sensitive, efficient, and eco-friendly methods for the extraction of antioxidants from different sources[36]. The type and concentration of extraction solvent, extraction temperature, extraction time, and extraction pH are all factors affecting the extraction efficiency[37,38]. Polar solvents, such as ethanol or ethanol-containing aqueous mixtures, are frequently used for the recovery of phenolic compounds from plant tissues. However, these extracts may not accurately reflect the antioxidant levels found in infusions or other well-known beverages.

Therefore, the objective of this work was to compare the antioxidant activity of extracts from different plant samples harvested by hand from parks and green spaces in La Plata, Argentina using only warm water as an extraction solvent. DPPH, ABTS, FRAP, and Folin–Ciocalteu (FC) methods were adapted to a 96-well microplate format. Furthermore, in order to compare results, aqueous extracts from commercial herbs and spices were prepared and analyzed under the same conditions.

This study not only emphasizes the importance of local biodiversity but also promotes sustainable research practices, which could be extended to different areas. Collecting samples from green spaces also takes advantage of freely available resources and provides an opportunity to explore the antioxidant properties of often overlooked plant species. Additionally, the use of warm water as the extraction solvent is an environmentally friendly method, that simulates beverage preparation and preserves the natural properties of the plants.

Materials and methods

Reagents

Sodium carbonate was purchased from Biopack (Buenos Aires, Argentina). TPTZ reagent (2,4,6-tris(2-pyridyl)-s-triazine) was supplied by Fluka Chemicals (Barcelona, Spain). DPPH reagent(1,1-diphenyl-2-picrylhydrazyl), gallic acid, Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and ABTS reagent (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), $FeCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemical reagents were of analytical grade.

Plant materials

Thirty-one plant samples including leaves, flowers, bark, and fruits were hand-harvested in March 2023, in parks and green spaces of La Plata, Argentina, and eight samples were purchased in a marketplace (Figs 1–4). Samples were washed with chlorinated water (200 mg L^{-1} NaClO, for 15 min, and rinsed with tap water. The taxonomic recognition of samples was done with PlantNet app (https://plantnet.org/mobile-app-changelog/). Samples were dried in an oven at 60 °C and ground with a blender to a particle size lower than 1 mm (Liliana, Picatutto, AM910, China) before analysis.

Aqueous extraction of bioactive components

Ground samples were weighed and aqueous bioactive components were extracted (0.5% w/w) with warm filtered water (Chlorine off purifier, Hidrolit, Argentina) by stirring for 10 min at 600 rpm and 45 °C (Dragon Laboratory HCM100-Pro, Beijing, China). Then, after 15 min of resting time at room temperature, samples were centrifuged at 3500× g for 10 min (Giumelli Z-127-D Centrifuge, Argentina). The pH of each aqueous extract was measured (HI1330B, Hanna instruments) and samples were stored at −18 °C before use. The absorbance of 150 µL of extracts was determined using 490, 550, 600, 630, and 750 nm filters in a microplate reader (BioTek 800 TC, USA). The images of the obtained extracts can be found in Fig. 5.

Total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent using the micro method described by Attard[39]. This assay measures the ability of compounds in an alkaline medium to reduce the phosphomolybdic/phosphotungstic acid reagent to blue complexes which are detected spectrophotometrically at 760 nm. The Folin–Ciocalteu reagent was diluted 1:10 before

Fig. 1 Different samples analyzed: 1 *Laurus nobilis*; 2 *Mentha spicata*; 3 *Passiflora caerulea*; 4 *Eucalyptus globulus*; 5 *Eucalyptus gunnii*; 6 *Tropaeolum majus*; 7 *Bauhinia forficata*; 8 *Urtica urens*; 9 *Plantago major*; 10 *Echium vulgare*; 11 *Helichrysum thianschanicum*; 12 *Ruta graveolens*; 13 *Ruta chalepensis*; 14 *Pinus pinea* (tree bark). (a) Fresh or dried samples, (b) dried samples used to prepare the extracts.

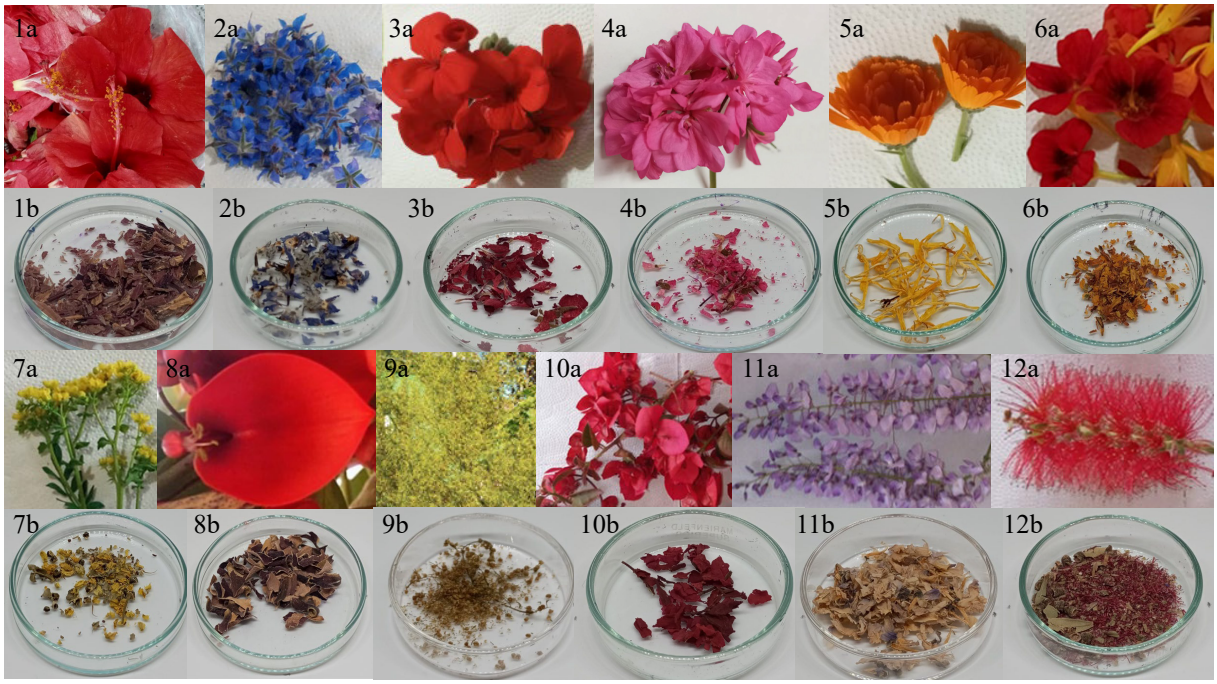

Fig. 2 Flower samples analyzed: 1 *Hibiscus rosa-sinensis*; 2 *Borago officinalis*; 3 *Pelargonium* × *hortorum* (red); 4 *Pelargonium* × *hortorum* (pink); 5 *Calendula officinalis*; 6 *Tropaeolum majus*; 7 *Ruta graveolens*; 8 *Erythrina crista-galli*; 9 *Artemisia annua* (plant); 10 *Bougainvillea glabra*; 11 *Wisteria sinensis*; 12 *Callistemon citrinu.* (a) Fresh samples, (b) dried samples used to prepare the extracts.

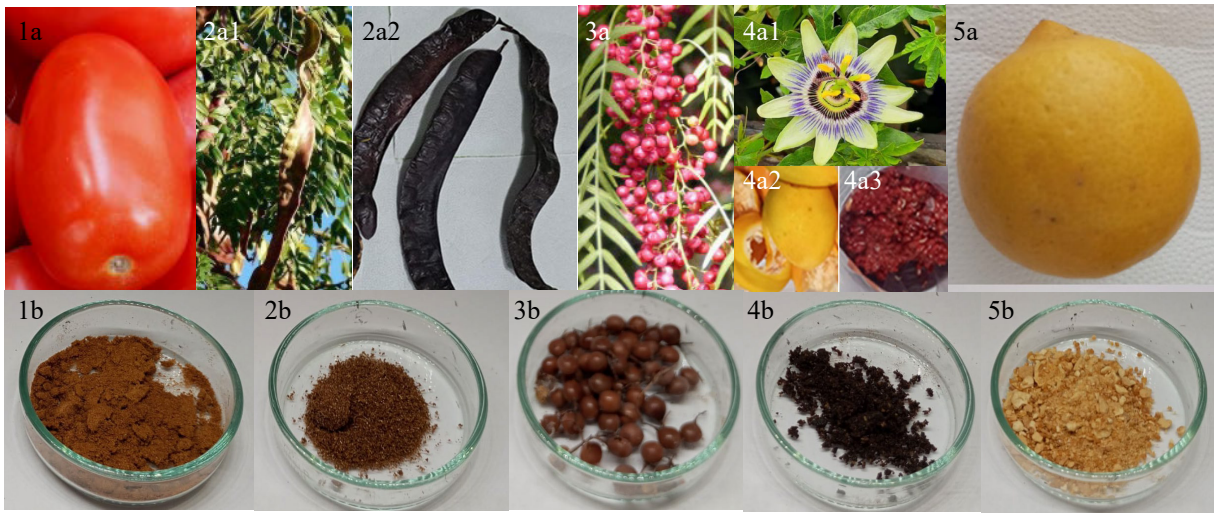

Fig. 3 Different fruits samples analyzed: 1 *Solanum lycopersicum*; 2 *Gleditsia triacanthos*; 3 *Schinus areira*; 4 *Passiflora caerulea*; 5 *Citrus* × *limón*. (a) Fresh samples, (b) dried samples used to prepare the extracts.

Fig. 4 Different commercial samples analyzed: 1 ginger; 2 turmeric; 3 rosemary; 4 thyme; 5 rooibos; 6 coriander; 7 clove; 8 drumstick.

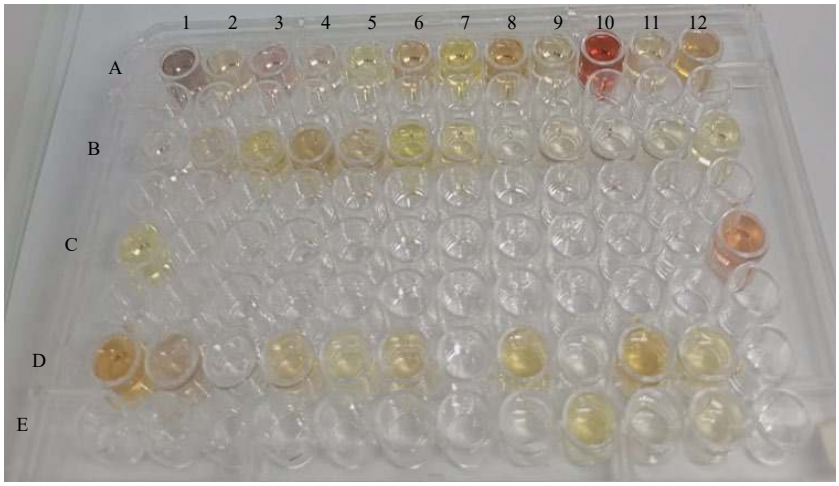

Fig. 5 Photographs of the analyzed extracts (300 µL). Row A: Flower extracts: 1 *Hibiscus rosa-sinensis*; 2 *Borago officinalis*; 3 *Pelargonium x hortorum* (red)*;* 4 *Pelargonium x hortorum* (pink)*;* 5 B *Calendula officinalis;* 6 *Tropaeolum majus;* 7 *Ruta graveolens*; 8 *Erythrina crista-galli*; 9 *Artemisia annua* (plant); 10 *Bougainvillea glabra*; 11 *Wisteria sinensis*; 12 *Callistemon citrinu.* Row B: Leaf extracts: 1 *Laurus nobilis;* 2 *Mentha spicata;* 3 *Passiflora caerulea;* 4 *Eucalyptus globulus*; 5 *Eucalyptus gunnii*; 6 *Tropaeolum majus*; 7 *Bauhinia forficata;*8 *Urtica urens*; 9 *Plantago major*; 10 *Echium vulgare*; 11 *Helichrysum thianschanicum*; 12 *Ruta graveolens.* Row C: 1 *Ruta chalepensis;* 12*: Pinus pinea* (tree bark). Row D: 1 *Solanum lycopersicum*; 2 *Gleditsia triacanthos*; 3 *Schinus areira*; 4 *Passiflora caerulea* ; 5 *Citrus* × *limón, 8* rooibos; 9 coriander; 10 clove; 11 drumstick. Row E: 8 ginger; 9 turmeric; 10 rosemary; 11 thyme.

use. Briefly, 10 µL of each extract, blank (water) or diluted standard was mixed with 100 µL Folin–Ciocalteu solution (FC 1:10 v/v) in a 96-well microplate. After 3 min, 80 µL sodium carbonate solution (20% prepared in NaOH 0.1 M) was added. The reaction was performed 1 h at room temperature in darkness. After that, the absorbance was measured at 750 nm in a microplate reader (BioTek 800 TC, USA). The results were expressed as milligrams of gallic acid equivalent per gram of sample (mgGAE/g) using a freshly prepared gallic acid solution to produce the calibration curve (Gallic acid 0–125 mg/L). The obtained curve was Abs 750 nm = 3.0436x + 0.1276 (R^2 = 0.9988).

Antioxidant activity by the Ferric reducing antioxidant power (FRAP) method

Ferric Reducing Antioxidant Power measures the reduction of ferric 2,4,6-tripyridyl-S-triazine (TPTZ) to a colored solution (blue). The FRAP method was performed using the method described by Prastiwi et al.[40], with slight modifications. Briefly, 30 µL of the extract, standard, or blank, was mixed with 270 µL of FRAP assay solution (20 mM ferric chloride solution, 10 mM TPTZ solution, and 0.3 M acetate buffer, pH 3.6). The absorbance was measured at 600 nm at room temperature (25 °C) in a microplate reader (BioTek 800 TC, USA), after 30 min of incubation. Trolox standard curve was run simultaneously (0–640 µg/mL). Antioxidant activity (AAFRAP) was expressed as mg Trolox equivalent per gram of sample (mgTE/g) using the equation Abs 600 nm = 0.0051x + 0.1822 (R^2 = 0.9939).

Antioxidant activity by the DPPH method

Free radical scavenging assay (DPPH assay) consists of the reduction of DPPH• radicals in the presence of hydrogen-donating antioxidant, and in the formation of the non-radical DPPH-H causing the solution decolorization[41]. The DPPH radical scavenging activity of all extracts was measured by DPPH described by Krošlák et al.[42] with slight modifications. In wells of a 96-well plate, 10 µL of extract, standard or blank was mixed with 300 µL of DPPH• in ethanol (40 µg/mL). After a 0.5 h reaction in the dark, the absorbance was measured at 490 nm in a microplate reader (BioTek 800 TC, USA) at room temperature (25 °C). A standard curve with Trolox (0–640 µg/mL) was run simultaneously. The antioxidant activity (AADPPH) was expressed as mg Trolox equivalent per gram of sample (mgTE/g) through the equation % Abs 490 nm reduction = 0.2281x – 8.77 (R^2 = 0.9946).

Antioxidant activity by the ABTS method

ABTS radical was prepared by reaction of 7 mmol/L ABTS with 2.45 mmol/L ammonium persulfate in the volume of 10 mL, followed by incubation in the dark for 16 h. To start the reaction, 10 µL of extract, standard or blank was mixed with 300 µL of ABTS reagent. After 6 min of incubation in the dark, absorbance was measured at 750 nm in a microplate reader (BioTek 800 TC, USA) at room temperature (25 °C). A standard curve with Trolox (0–640 µg/mL) was run simultaneously. The antioxidant activity (AAABTS) was expressed as mg Trolox equivalent per gram of sample (mg TE/g) by the equation % Abs 750 nm reduction = 0.2837x + 2.2372 (R^2 = 0.9944).

Data analysis

All analysis was performed at least in duplicate and were performed in an area protected from light. Analysis of variance (ANOVA) and Fisher tests were carried out to identify significant differences between different samples ($p < 0.05$). The antioxidant activity of each sample with different methods was transformed into a score value as (AAs – AAg) / SDg, where AAs is the antioxidant activity of the sample while AAg and SDg are the global mean and global standard deviation respectively. To summarise the high dimensional space of the data set, data were analyzed by a principal component analysis (PCA). Before the analysis, data were standardized and centered. Also, to evaluate the strength of the relations between variables, Pearson's correlation coefficients were calculated (InfoStat, 2012; Córdoba, Universidad Nacional de Córdoba, Argentina)

Results

The pH of a substance is known to affect the racemization of molecules, with different reactivity in the respective reagent[43]. The pH range values registered was 6.71–9.51 with only three extracts with pH values less than 7 (*Passiflora caerulea* fruit 6.71, *Erythrina crista-galli*, and *Callistemon citrinu* flowers with 6.78 and 6.94 respectively (Table 1).

Table 1. pH, total phenolic content (TPC), and antioxidant activity (AA) of aqueous extracts by different methods (FRAP, DPPH, and ABTS).

N°	M*	Raw material		Family	pH	TPC[1]	AA $_{FRAP}$[2]	AA $_{DPPH}$[2]	AA $_{ABTS}$[2]
1	C	Ginger	*Zingiber officinale*	Zingiberaceae	9.42^{d}	5.6^{ab}	26.4^{ab}	42.9^{ab}	43.6^{ab}
2	C	Turmeric	*Curcuma longa*	Zingiberaceae	8.90^{cd}	1.4^{a}	9.3^{a}	13.1^{ab}	7.5^{a}
3	C	Rosemary	*Rosmarinus officinalis*	Lamiaceae	8.86^{cd}	11.7^{ab}	23.3^{ab}	13.1^{ab}	14.4^{b}
4	C	Thyme	*Thymus vulgaris*	Lamiaceae	9.40^{d}	15.4^{b}	41.2^{ab}	14.9^{ab}	15.7^{ab}
5	C	Rooibos	*Aspalathus linearis*	Fabaceae	9.31^{d}	12.3^{b}	17.8^{ab}	12.4^{ab}	16.3^{ab}
6	C	Coriander	*Coriander sativum*	Apiaceae	9.51^{d}	2.5^{a}	8.0^{a}	13.6^{ab}	8.1^{a}
7	C	Clove	*Eugenia caryophyllata*	Myrtaceae	8.17^{bc}	43.9^{cd}	159.8^{d}	45.4^{b}	70.9^{b}
8	C	Drumstick	*Moringa oleifera*	Moringaceae	8.92^{cd}	16.0^{b}	37.6^{ab}	12.8^{ab}	21.0^{ab}
9	L	Bay	*Laurus nobilis*	Lauraceae	9.38^{d}	2.6^{a}	11.7^{ab}	30.2^{ab}	29.6^{ab}
10	L	Spearmint	*Mentha spicata*	Lamiaceae	9.28^{d}	2.9^{ab}	11.7^{ab}	13.8^{ab}	2.0^{a}
11	L	Mburucuya	*Passiflora caerulea*	Passifloraceae	7.44^{b}	10.6^{ab}	26.8^{ab}	19.7^{ab}	39.6^{ab}
12	L	Blue gum tree	*Eucalyptus globulus*	Myrtaceae	7.46^{b}	40.6^{cd}	140.5^{cd}	70.9^{c}	98.7^{bc}
13	L	Cider gum	*Eucalyptus gunnii*	Myrtaceae	7.06^{ab}	18.1^{b}	52.3^{b}	66.0^{bc}	36.3^{ab}
14	L	Garden nasturtium	*Tropaeolum majus*	Tropaeolaceae	8.87^{cd}	18.2^{b}	43.7^{ab}	47.7^{bc}	35.3^{ab}
15	L	Cow's foot	*Bauhinia forficata*	Fabaceae	7.62^{b}	15.0^{b}	35.9^{ab}	15.2^{ab}	25.8^{ab}
16	L	Dwarf nettle	*Urtica urens*	Urticaceae	9.32^{d}	1.8^{a}	7.8^{a}	8.5^{ab}	3.9^{a}
17	L	Greater plantain	*Plantago major*	Plantaginaceae	9.09^{cd}	6.1^{ab}	16.4^{ab}	9.2^{ab}	9.2^{a}
18	L	Blueweed	*Echium vulgare*	Boraginaceae	8.57^{c}	2.4^{a}	7.7^{a}	10.4^{ab}	3.3^{a}
19	L	Silver spike	*Helichrysum thianschanicum*	Asteraceae	8.58^{c}	4.2^{ab}	10.2^{ab}	12.8^{ab}	14.3^{a}
20	L	Garden rue	*Ruta graveolens*	Rutaceae	8.33^{c}	8.8^{ab}	23.7^{ab}	13.8^{ab}	25.7^{ab}
21	L	Rue fragrant	*Ruta chalepensis*	Rutaceae	8.66^{cd}	11.0^{ab}	28.1^{ab}	13.5^{ab}	26.7^{ab}
22	B	Stone pine nut	*Pinus pinea*	Pinaceae	7.35^{ab}	20.3^{b}	90.9^{bc}	62.6^{bc}	56.8^{ab}
23	F	Chinese rose	*Hibiscus rosa-sinensis*	Malvaceae	7.67^{bc}	46.3^{d}	134.9^{cd}	5.8^{a}	52.8^{ab}
24	F	Borage	*Borago officinalis*	Boraginaceae	9.02^{cd}	6.2^{ab}	17.7^{ab}	29.6^{ab}	37.1^{ab}
25	F	Geranium	*Pelargonium × hortorum* (red)	Geraniaceae	7.50^{b}	44.9^{cd}	114.3^{c}	75.9^{c}	128.5^{bc}
26	F	Geranium	*Pelargonium × hortorum* (pink)	Geraniaceae	7.30^{sb}	57.1^{e}	135.5^{cd}	73.1^{c}	126.5^{bc}
27	F	Pot marigold	*Calendula officinalis*	Asteraceae	9.18^{d}	6.2^{ab}	17.8^{ab}	13.7^{ab}	13.4^{a}
28	F	Nasturtium	*Tropaeolum majus*	Tropaeolaceae	8.51^{c}	13.6^{b}	26.0^{ab}	21.7^{ab}	44.9^{ab}
29	F	Garden rue	*Ruta graveolens*	Rutaceae	8.96^{cd}	14.4^{b}	46.1^{ab}	12.0^{ab}	35.6^{ab}
30	F	Coral tree	*Erythrina crista-galli.*	Fabaceae	6.78^{a}	34.3^{c}	69.1^{b}	17.1^{ab}	29.5^{ab}
31	F	Sweet wormwood	*Artemisia annua*	Asteraceae	7.25^{ab}	8.2^{ab}	24.0^{ab}	15.3^{ab}	31.8^{ab}
32	F	Buganvilla	*Bougainvillea glabra*	Nyctaginaceae	7.54^{b}	39.7^{cd}	272.1^{e}	20.4^{ab}	89.6^{bc}
33	F	Glycine floribunda	*Wisteria floribunda*	Fabaceae	7.80^{bc}	8.2^{ab}	34.6^{ab}	7.6^{ab}	26.8^{ab}
34	F	Bottlebrush	*Callistemon citrinu*	Myrtaceae	6.94^{ab}	49.6^{de}	145.9^{cd}	50.1^{bc}	138.4^{c}
35	Fr	Tomato	*Solanum lycopersicum*	Solanaceae	6.99^{ab}	10.7^{ab}	26.8^{ab}	55.6^{bc}	7.9^{a}
36	Fr	Honey locusts	*Gleditsia triacanthos*	Fabaceae	7.87^{bc}	14.2^{b}	87.4^{bc}	69.8^{bc}	43.0^{ab}
37	Fr	Rose pepper	*Schinus molle*	Anacardiaceae	8.14^{bc}	1.6^{a}	8.8^{a}	13.3^{ab}	6.2^{a}
38	Fr	Mburucuyá	*Passiflora caerulia* (pulp)	Passifloraceae	6.71^{a}	11.0^{ab}	15.1^{ab}	13.6^{ab}	26.0^{ab}
39	Fr	Lemon	*Citrus × limón* (rind)	Rutaceae	7.93^{bc}	9.4^{ab}	25.3^{ab}	16.0^{ab}	31.6^{ab}
$LSD_{0.05}$ least significant difference					0.68	9.7	42.8	38.7	56.4

* M: material samples. C: commercial samples. L: leaves. B: bark. F: flowers. Fr: fruits. Values in the same column followed by different superscript letters are significantly different ($p \leq 0.05$). Results are expressed as [1] mg GAE/g and [2] mg TE/g.

The extracts of commercial samples showed pH values higher than 8.0 being the highest pH value registered in extracts of coriander (9.51). The aqueous extracts presented a wide variety of colors, the leaf extracts with green-yellowish toned, brown tones in bark and pod extracts, while most of the flower extracts showed reddish tones. All extracts presented high absorbance values at 490 and 550 nm compared to those obtained at 750 nm. *Bougainvillea glabra* extract presented the highest absorbance values at 490 and 550 nm while *Hibiscus rosa-sinensis* showed the highest values at 600 and 630 nm (Fig. 6).

Antioxidant properties

Some authors reported that plant extracts showed a concentration-dependent manner antioxidant activity[44]. In the present study, water was used as an extraction solvent to extract the hydrophilic antioxidants present in the plant samples and all extracts were prepared in the same way (0.5% w/w, 45 °C). Furthermore, data obtained by different researchers are often extremely difficult to compare and interpret as they use different substrates, system compositions, and analytical methods in screening tests to evaluate antioxidant efficacy[45]. That is why in this work results were compared with samples widely known to have antioxidant activity (ginger, turmeric, rosemary, thyme, red tea, coriander, cloves, and drumstick).

DPPH, ABTS, and FRAP assays were used to assess the *in vitro* antioxidant capacities of samples by using microplate methods. Antiradical activity is usually defined as the amount of sample capable of consuming half the amount of free radicals (DPPH or ABTS) by using a dose-response curve[46].

A wide range of values were found with all methodologies (Table 1). TPC values ranged from 1.4 (turmeric) to 57.1 mg GAE/g (*Pelargonium × hortorum* (pink)). FRAP assay values ranged from 7.7 (*Echium vulgare*) to 272.1 mg TE/g (*Bougainvillea glabra*). The antioxidant activity of extracts by ABTS assay was between 2.0 (*Mentha spicata*) and 138.4 mg TE/g (*Callistemon citrinu*) which represents the major variation, of approximately 70-fold. Finally, the minor variation between samples was found by DPPH assay, values ranged from 5.8 (*Hibiscus rosa-sinensis*) to 75.9 mg TE/g (*Pelargonium ×*

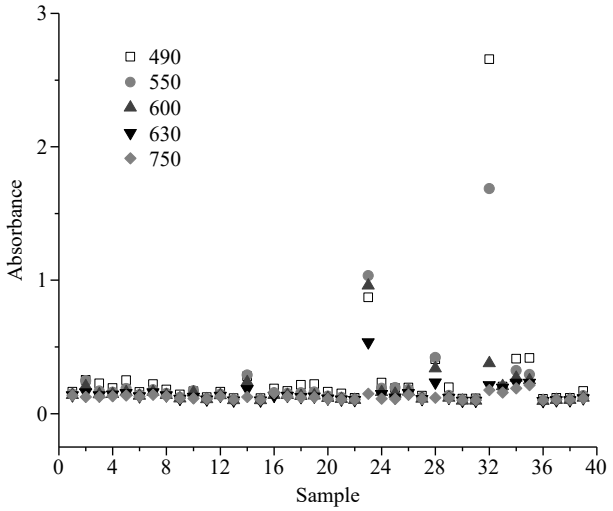

Fig. 6 Absorbance of extracts at different wavelengths: 490, 550, 600, 630, and 750 nm. Sample references. 1: *Zingiber officinale*; 2: *Curcuma longa*; 3: *Rosmarinus officinalis*; 4: *Thymus vulgaris*; 5: *Aspalathus linearis*; 6: *Coriander sativum*; 7: *Eugenia caryophyllata*; 8: *Moringa oleifera*; 9: *Laurus nobilis*; 10: *Mentha spicata*; 11: *Passiflora caerulea*; 12: *Eucalyptus globulus*; 13: *Eucalyptus gunnii*; 14: *Tropaeolum majus*; 15: *Bauhinia forficate*; 16: *Urtica urens*; 17: *Plantago major*; 18: *Echium vulgare*; 19: *Helichrysum thianschanicum*; 20: *Ruta graveolens*; 21: *Ruta chalepensis*; 22: *Pinus pinea*; 23: *Hibiscus rosa-sinensis*; 24: *Borago officinalis*; 25: *Pelargonium* × *hortorum* (red); 26: *Pelargonium* × *hortorum* (pink); 27: *Calendula officinalis*; 28: *Tropaeolum majus*; 29: *Ruta graveolens*; 30: *Erythrina crista-galli*; 31: *Artemisia annua*; 32: *Bougainvillea glabra*; 33: *Wisteria floribunda*; 34: *Callistemon citrinu*; 35: *Solanum lycopersicum*; 36: *Gleditsia triacanthos*; 37: *Schinus molle*; 38: *Passiflora caerulia* (pulp); 39: *Citrus* × *limón* (rind).

hortorum (pink)). Of the commercial samples clove extract presented the highest antioxidant activity with all methods analyzed. Other authors also reported that clove has the highest antioxidant activity among other herbs and spices[3,5]. The use of clove as an antioxidant in food is limited due to its strong flavor and aroma, thus is so important to look for other sources of natural antioxidants for use in foods. The *Pelargonium* × *hortorum* (pink) extract showed the biggest TPC value (57.1 mg GAE/g) and its value is greater than clove ($p < 0.05$). A similar TPC value in geranium extract was reported by Boukhris et al.[47] (60.7 ± 3 mg GAE/g dry weight in *Pelargonium graveolens*). Other authors reported higher values (136.5 mg of GAE/g) in extracts of *Pelargonium hortorum*, but they used stronger extraction conditions[27]. Six extracts showed TPC values similar to those of clove ($p > 0.05$), bottlebrush (*Callistemon citrinu*), Chinese rose (*Hibiscus rosa-sinensis*), red garden geranium (*Pelargonium* × *hortorum* (red), coral tree (*Erythrina crista-galli*), bougainvillea (*Bougainvillea glabra*), and blue gum tree (*Eucalyptus globulus*) with respectively, 49.6, 46.3, 44.9, 40.6, 39.7, and 34.3 mg GAE/g of dry sample. Among the selected plants, turmeric, *Urtica urens* and *Schinus molle* extracts showed a very low phenolic content (< 2 mg GAE/g).

Both free radical scavenging assays (DPPH and ABTS) are carried out at neutral pH, while the ferric-reducing potential (FRAP) assay is done under acidic pH (pH 3.6). Considering a value of 70 mg TE/g for high antioxidant activity, nine extracts showed high ferric-reducing potential activity, six extracts showed high ABTS values, and only three high antioxidant activity by the DPPH method (Table 1). The ABTS assay is particularly interesting in plant extracts because there is less color interference at 750 nm than at 600 or 490 nm (Fig. 6). Among extracts evaluated, *Bougainvillea glabra* by FRAP, *Callistemon citrinu* by ABTS, *Eucalyptus globulus*, and both *Pelargonium* × *hortorum* (red and pink) by DPPH, presented significantly greater antioxidant activity than clove ($p < 0.05$). The antioxidant score values by ABTS, DPPH, FRAP, TPC, and the average score value of samples are shown in Fig. 7.

Again, five extracts showed average score values higher than clove (0.78), *Eucalyptus globulus* (0.99), *Bougainvillea glabra* (1.03), *Pelargonium* × *hortorum* red (1.13), *Callistemon citrinu* (1.16), and *Pelargonium* × *hortorum* pink (1.35).

Multivariate statistical analysis

For a proper interpretation of the data set a PCA was performed with the samples that presented the highest antioxidant score according to Fig. 7. The results of the PCA can be seen in Fig. 8.

The first and second principal components described 44.9 and 22.8% of the variance, respectively. Principal component 1 (PC1) was better associated with the color of the samples (absorbance at 490, 550, 600, 630, and 750) while PC2 was mostly related to antioxidant parameters (ABTS, DPPH, and FRAP) and TPC. Results showed that extracts of *Eucalyptus gunnii* (13) and *Pinus pinea* (22) presented similar characteristics (Euclidean distance = 0.89) as well as *Pinus pinea* (22) and *Gleditsia triacanthos* (36) (Euclidean distance = 0.92), these samples displayed negative values of PC1 and could be associated with high values of antioxidant capacity determined by DPPH and low values of pH. Moreover, *Eucalyptus globulus* (12) and *Pelargonium* × *hortorum* (red) (25) presented positive values of PC2 and could be associated with high values of TPC and antioxidant capacity determined by ABTS. *Callistemon citrinu* (34) also presented positive values of PC2, but in this case its antioxidant capacity could be better related to the high values of FRAP and TPC. Finally, samples *Bougainvillea glabra* (32) and *Hibiscus rosa-sinensis* (23) could present high values of PC1 and they could be better associated with an important absorbance.

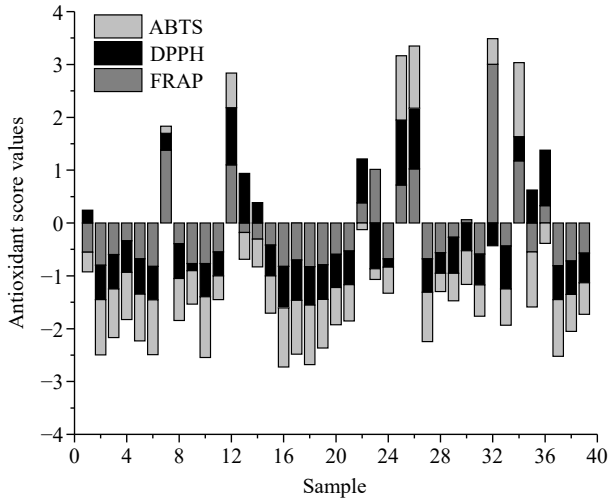

Fig. 7 Antioxidant score values. Sample references. 1: *Zingiber officinale*; 2: *Curcuma longa*; 3: *Rosmarinus officinalis*; 4: *Thymus vulgaris*; 5: *Aspalathus linearis*; 6: *Coriander sativum*; 7: *Eugenia caryophyllata*; 8: *Moringa oleifera*; 9: *Laurus nobilis*; 10: *Mentha spicata*; 11: *Passiflora caerulea*; 12: *Eucalyptus globulus*; 13: *Eucalyptus gunnii*; 14: *Tropaeolum majus*; 15: *Bauhinia forficate*; 16: *Urtica urens*; 17: *Plantago major*; 18: *Echium vulgare*; 19: *Helichrysum thianschanicum*; 20: *Ruta graveolens*; 21: *Ruta chalepensis*; 22: *Pinus pinea*; 23: *Hibiscus rosa-sinensis*; 24: *Borago officinalis*; 25: *Pelargonium* × *hortorum* (red); 26: *Pelargonium* × *hortorum* (pink); 27: *Calendula officinalis*; 28: *Tropaeolum majus*; 29: *Ruta graveolens*; 30: *Erythrina crista-galli*; 31: *Artemisia annua*; 32: *Bougainvillea glabra*; 33: *Wisteria floribunda*; 34: *Callistemon citrinu*; 35: *Solanum lycopersicum*; 36: *Gleditsia triacanthos*; 37: *Schinus molle*; 38: *Passiflora caerulia* (pulp); 39: *Citrus* × *limón* (rind).

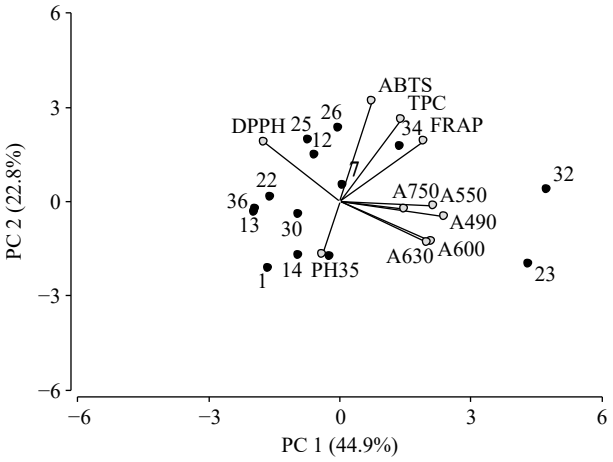

Fig. 8 PCA biplot of antioxidant capacity (determined by FRAP, ABTS, and DPPH); total phenolic content (TPC), pH and absorbance (A) at 490, 550, 600, 630, and 750 nm determined in 1: *Zingiber officinale*; 7: *Eugenia caryophyllata*; 12: *Eucalyptus globulus*; 13: *Eucalyptus gunnii*; 14: *Tropaeolum majus*; 22: *Pinus pinea*; 23: *Hibiscus rosa-sinensis*; 25: *Pelargonium* × *hortorum* (red); 26: *Pelargonium* × *hortorum* (pink); 30: *Erythrina crista-galli*; 32: *Bougainvillea glabra*; 34: *Callistemon citrinu*; 35: *Solanum lycopersicum*; 36: *Gleditsia triacanthos*.

Pearson's correlation coefficients were calculated to evaluate the strength of relations between the variables (Table 2).

Results showed significant linear correlations ($p < 0.05$; Pearson's correlation coefficients > 0.60) between the ABTS and TPC (correlation coefficient = 0.79); ABTS and FRAP (correlation coefficient = 0.62) and TPC and FRAP (correlation coefficient = 0.70). Furthermore, no significant correlation was observed between the pH and the antioxidant determinations ($p \geq 0.05$). This indicates that natural antioxidants from plants are not affected by the pH values. This is slightly contradictory to Bayliak et al.[48] who indicated that the antioxidant activity of plant extracts may be inhibited in an alkaline medium.

Significant correlations ($p > 0.65$) were found between the color of the samples at 490 and 550 nm and the antioxidant determined by FRAP (correlation coefficient = 0.74 and 0.7 respectively). This result suggests that samples that have blue or green pigments would present at high antioxidant capacity. This was also observed with the DPPH technique which showed a good correlation with the absorbance at 550 and 630 nm (correlation coefficient = 0.64 and 0.66 respectively). Therefore, it could be concluded that water-soluble pigments could be responsible for the high antioxidant capacity of the samples.

Discussion

In the present work, the average pH was 8.25 and it ranged from slightly acidic (6.71) to alkaline (9.51). According to Friedman & Jürgens[49], the pH strongly affects the stability of the phenolic compounds, as their structure may be modified by the different pH values. For example, anthocyanins are more stable at low pH which gives a red pigment. Meanwhile, higher pH value will provide a blue anthocyanin color. In the samples analyzed the pH did not significantly affect the color of the samples ($p \geq 0.05$), this indicates that in the natural pH values of the samples their color did not undergo significant changes. This stability suggests that the characteristic pH of the samples is within a range that does not trigger any structural modifications in the phenolic compounds, maintaining their natural characteristic pigmentation

The color of the extracts is the first characteristic that the consumer perceives. Before we drink, we will look at the beverage first, and signals are sent to the brain which expects a certain taste or flavor before we drink it[50]. Therefore, it is very important that the extracts present attractive colors that may catch the attention of consumers. In the present paper the most colored extracts were obtained from *Hibiscus rosa-sinensis, Tropaeolum majus, Bougainvillea glabra, Callistemon citrinu*, and *Solanum lycopersicum*. It is interesting to mention that sample *Bougainvillea glabra* also showed an important antioxidant activity and a high antioxidant score (Table 1 & Fig. 7). This plant has previously been used to prepare low-calorie beverages by Contreras-López et al.[51] who indicate that these formulations presented red and pink intense colors, due to their high content of more than 30 betanins which are also responsible for their medical properties. Moreover, this color positively influenced consumer acceptance of the product[51].

The color may be associated with the type and amount of phenolic compounds. In the present paper, a water extraction was performed, as this is the most common practice for preparing beverages. However, this may limit the extraction of some phenolic compounds as many have limited solubility in water[52], therefore some differences may be found in the antioxidant capacity determined by other authors. However, according to Wong et al.[53], plant extracts made with water are more nutritionally relevant than extracts prepared with organic solvents and have no ecological limitations.

The correlations between the color and the antioxidant power of samples have been previously described. According to Kandylis[54], anthocyanins which are responsible for the color of flowers have been correlated with plants with high antioxidant activity and therefore with high nutritional value. Besides, results by Benvenuti et al.[55] showed that varieties with red and blue flowers have the greatest antioxidant power, while the present results indicate that

Table 2. Pearson's correlation coefficients of antioxidant capacity (measured by DPPH, ABTS, and FRAP assays); TPC; pH and absorbance.

	A490	A550	A600	A630	A750	pH	TPC	FRAP	DPPH	ABTS
A490	1									
A550	0.96*	1								
A600	0.47	0.68*	1							
A630	0.38	0.60*	0.98*	1						
A750	0.47	0.46	0.38	0.50	1					
pH	−0.05	−0.02	0.01	0.0006	−0.15	1				
TPC	0.22	0.28	0.31	0.31	0.15	−0.39	1			
FRAP	0.74*	0.70*	0.31	0.24	0.27	−0.21	0.70*	1		
DPPH	−0.54	−0.64*	−0.66*	−0.62	−0.24	−0.07	−0.11	−0.25	1	
ABTS	0.14	0.11	−0.02	−0.02	0.13	−0.20	0.79*	0.62*	0.30	1

* Significant at $p \leq 0.05$ and Pearson's correlation coefficients > 0.60.

the antioxidant capacity was better associated with the blue, yellow, and green colors, and no significant association was found with the red color of samples with high antioxidant capacity.

Finally, according to the antioxidant score values, the highest antioxidant capacity was determined in *Pelargonium* × *hortorum* red and pink. Benvenuti et al.[55] studied the antioxidant content of this ornamental species, and tested determined its organoleptic characteristics. Their results indicate that *Pelargonium* × *hortorum* had a high content of anthocyanins (12.52 ± 1.1 cyanidin-3-glucoside per 100 g of fresh matter) a flavor similar to grapefruit and a good general product acceptability.

Therefore, because of their high antioxidant activity and attractive colors, the most recommended samples for beverage preparation would be *Pelargonium* × *hortorum* (red and pink) and *Bougainvillea glabra*.

Conclusions

In conclusion, this study underscores the potential of a wide range of plant extracts as natural sources of phytochemicals with significant antioxidant activity, particularly when prepared as aqueous extracts. The research highlights the diverse pH levels, color profiles, and antioxidant capacities of both ornamental and wild plant samples, emphasizing their applicability in food, beverage, cosmetics, and nutraceutical products. Among the commercial samples analyzed, clove extract exhibited the highest antioxidant activity across all methods. However, several extracts, including those from *Eucalyptus globulus* leaves and the flowers of *Bougainvillea glabra*, *Pelargonium* × *hortorum* (red and pink petals), and *Callistemon citrinus*, demonstrated even higher average antioxidant scores than clove.

Moreover, results indicate that because of their high antioxidant activity and color, *Pelargonium* × *hortorum* (red and pink) and *Bougainvillea glabra* are highly recommended for use in beverage preparation.

These findings suggest that such plant extracts, particularly those from flowers could be valuable not only for their antioxidant properties but also for their ability to enhance the visual appeal of beverages and foods through. This study provides a robust foundation for future research in beverage plant science, aiming to optimize the use of natural antioxidants in the food and beverage industry, ultimately contributing to the development of functional beverages with enhanced nutritional and sensory qualities.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Conforti PA; data collection: Conforti PA; analysis and interpretation of results: Conforti PA, Patrignani M; draft manuscript preparation: Conforti PA, Patrignani M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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Conflict of interest

The authors declare that they have no conflict of interest.

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