

Research Article

Bioaccessibility and Antioxidant Capacity of Ethanolic Extracts From *Tagetes patula* and *Zingiber officinale* During In Vitro Gastrointestinal Digestion

Yenni Leandra Rodríguez Ruiz ^{1,2,3} Rocio Campos-Vega ⁴
Nelsy Loango Chamorro ^{1,2,3} and Johanny Aguillón Osma ²

¹Cardiovascular and Metabolic Diseases Biochemistry Research Group, Faculty of Health Sciences, University of Quindío, Armenia, Colombia

²Research Group in Basic and Educational Sciences, Faculty of Basic Sciences and Technologies, University of Quindío, Armenia, Colombia

³PhD Program in Biomedical Sciences, Faculty of Health Sciences, University of Quindío, Armenia, Colombia

⁴Food Science Graduate Program, Faculty of Chemistry, Autonomous University of Querétaro, Santiago de Querétaro, Mexico

Correspondence should be addressed to Rocio Campos-Vega; chio_cve@yahoo.com.mx and Nelsy Loango Chamorro; neloango@uniquindio.edu.co

Received 7 April 2025; Revised 18 October 2025; Accepted 23 October 2025

Academic Editor: Charalampos Proestos

Copyright © 2025 Yenni Leandra Rodríguez Ruiz et al. Journal of Food Biochemistry published by John Wiley & Sons Ltd. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

This study evaluated the bioaccessibility and antioxidant capacity of the ethanolic extracts of *Tagetes patula* (ETP) and *Zingiber officinale* (EZO) during simulated in vitro gastrointestinal digestion. The phenolic content and antioxidant capacity were analyzed using the DPPH and FRAP methods at the oral, gastric, and intestinal stages. Simulated digestion led to a reduction in phenolic and flavonoid levels in both extracts. The *T. patula* extract retained 64.05 ± 4.63 mg GAE/g DE of phenolic compounds and 49.18 ± 7.97 mg RE/g DE of flavonoids in the UF. In contrast, *Z. officinale* exhibited higher susceptibility, with phenolic content decreasing from 130.13 ± 4.69 to 16.96 ± 0.14 mg GAE/g DE and flavonoid content from 64.97 ± 5.75 to 0.32 ± 0.09 mg RE/g DE. Nevertheless, during the final stage of digestion, an increase in DPPH radical scavenging capacity was observed compared to previous stages, reaching 23.29 ± 0.42 mg TE/g DE. Notably, high-performance liquid chromatography (HPLC) analysis revealed that *T. patula* maintained its free phenolic compounds throughout digestion, while *Z. officinale* showed enhanced release of these compounds.

Keywords: antioxidant capacity; bioaccessibility; in vitro gastrointestinal digestion; *Tagetes patula*; *Zingiber officinale*.

1. Introduction

Tagetes patula Linn, commonly known as French marigold, is an edible plant from the Asteraceae family. Traditionally, it has been used to treat colic, diarrhea, vomiting, fever, skin diseases, and liver conditions. This plant is renowned for its diverse biological properties, including nematicidal [1], antibacterial, antifungal [2, 3], anti-inflammatory, analgesic, and antioxidant activities [4]. Phytochemical studies have identified the presence of metabolites such as alkaloids,

thiophenes, terpenes, and flavonoids. Notably, these compounds exhibit inhibitory effects on cell growth; for instance, quercetin, rutin, and apigenin from *Brassica oleracea italica* *x* *alboglabra* have demonstrated activity against colon cancer (SW480) and liver cancer (HepG2). Similarly, kaempferol, isorhamnetin, and rutin found in *Chenopodium quinoa* have shown efficacy against prostate cancer in rats (AT-2) and human prostate cancer cells (MDA-MB-231). Additionally, flavonoids such as quercetin, apigenin, and kaempferol have also demonstrated antitumor activity in the xenograft mouse

model against human lung, colon, and osteosarcoma cancers, respectively [5, 6].

On the other hand, *Zingiber officinale* is a plant belonging to the Zingiberaceae family; it is a perennial herb and grows to a height of about 100 cm [7]. Due to its nutritional value, its rhizome is widely used for medicinal purposes around the world and is prescribed either alone or in combination for the treatment of infectious and non-infectious diseases. This plant has been of research interest mainly because of its multiple biological properties, such as antibacterial, antidiabetic, nephroprotective, hepatoprotective, larvicidal, analgesic, anti-inflammatory, immunomodulatory, antioxidant, and anticancer effects [8, 9], and due to the presence of metabolites such as quercetin and catechin, which have been shown to be effective against human breast cancer (MCF-7 y MDA-MB-231) [6].

The biological activities of plant extracts are closely linked to the presence of chemical compounds within their matrices, with phenolic compounds standing out as a group of metabolites with diverse functions [10]. However, for these compounds to exert their effects, they must be bioavailable, meaning that they need to undergo digestion, absorption, and utilization in normal metabolism. Several factors influence their bioavailability [11], including their bioaccessibility (the proportion of compounds released from the extract during digestion), their stability under biochemical conditions, and their subsequent intestinal absorption. Accordingly, the objective of this research was to evaluate the bioaccessibility and antioxidant capacity of the ethanolic extract of *T. patula* (ETP) and extract of *Z. officinale* (EZO) during simulated in vitro gastrointestinal digestion.

2. Methodology

2.1. Ethanolic Extract Preparation. The *T. patula* plants were collected from the production unit of the Universidad del Quindío (located at the geographical coordinates 4.552720° latitude and -75.66171721° longitude, at 1517.46 m.a.s.l.), once they had reached the flowering stage and it was confirmed that the plant material, specifically leaves and flowers, was under optimal phytosanitary conditions (without direct observation of bite lesions, discoloration, or wilting due to diseases). The rhizomes of *Z. officinale* were obtained from an organic farm in the department of Quindío, selected under the same phytosanitary criteria. The plant material was carefully washed and disinfected with a 1% hypochlorite solution and then dried in an oven at 35°C until it reached constant weight. Subsequently, both samples were pulverized and subjected to a leaching process for 10 days using 300 mL of 96% ethanol per 100 g of sample and continuously recirculated in a separatory funnel. The extracts obtained were concentrated by reduced pressure (60 mbar) at 40°C, using a Heidolph Rotary Evaporator, Laborota 4003, to eliminate the solvent. Finally, the extracts were dried in a hot air-drying oven at temperatures below 39°C and stored in airtight bags in refrigeration and darkness until used in biological assays.

2.2. Quantification of Total Free Phenols. The concentration of total phenolic (TP) compounds was determined using the Folin–Ciocalteu method [12, 13]. In a 96-well microplate, 30 µL of the extract (1 mg/mL) or gallic acid (used as standard) was mixed with 150 µL of Folin–Ciocalteu reagent (diluted 1:10 in distilled water) and 120 µL of 7.5% (w/v) sodium carbonate (Na_2CO_3). The mixture was homogenized and incubated at room temperature in the dark for 30 min. For the blank, a solution containing 120 µL of 7.5% Na_2CO_3 , 30 µL of distilled water, and 150 µL of Folin–Ciocalteu reagent was prepared. Absorbance was measured using a spectrophotometer at a wavelength of 765 nm. The results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g DE).

2.3. Quantification of Total Flavonoids. The determination of total flavonoids was carried out using an assay reported by Ref. [14] with some modifications. In a 96-well microplate, 50 µL of the extract, 180 µL of pure methanol, and 20 µL of a 1% (w/v) solution of 2-aminoethyl diphenylborinate were combined. The absorbance was measured immediately with a spectrophotometer at a wavelength of 404 nm. Rutin was used as the reference standard, and the results were expressed as milligrams of rutin equivalents per gram of dry extract (mg RE/g DE).

2.4. Determination of Antioxidant Capacity. The antioxidant capacity was determined using the DPPH method, following the methodology proposed by Fukumoto and Mazza [15]. For the control, 20 µL of methanol and 200 µL of DPPH solution (adjusted to an absorbance of 0.8–0.9) were used, while 220 µL of methanol was used as a blank. For the test samples, 20 µL of the extract or standard (Trolox at various concentrations to construct the calibration curve) was combined with 200 µL of DPPH solution. Absorbance readings were taken at different incubation times, ranging from 0 to 90 min, at a wavelength of 520 nm. The results were expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE/g DE).

The antioxidant capacity was also assessed using the FRAP methodology proposed by Benzie and Strain [16]. For this method, a solution was prepared consisting of acetate buffer (pH 3.6), TPTZ (10 mmol/L), and FeCl_3 (20 mmol/L) in a ratio of 10:1:1, respectively. A total of 20 µL of the sample, blank, or standard (Trolox was used as the reference) and 175 µL of the working solution were added to microplates. The plates were stirred at 15 rpm for 15 min at 37°C. Absorbance was measured at 595 nm, and the results were expressed as milligrams of Trolox equivalents per gram of dry extract (mg TE/g DE).

2.5. In Vitro Gastrointestinal Digestion. Human physiological conditions were simulated using the procedure described by Campos Vega et al. [17]. All participants provided written informed consent prior to the study. Subjects rinsed their mouths with 5 mL of sterile water for 30 s, and the rinsate was spat into a container containing an additional

5 mL of sterile water. This process was repeated four times. The collected saliva was mixed with the plant extracts (0.3 g/mL). A portion of this mixture was used to quantify secondary metabolites via high-performance liquid chromatography (HPLC) [18] as well as to determine antioxidant capacity and TP compounds.

Subsequently, the suspensions of each sample were aliquoted into 10-mL portions and adjusted to a pH of 2.0 using a 150 mM HCl solution. Pepsin (0.055 g), dissolved in 0.94 mL of a 20 mM HCl solution, was added to each aliquot. The samples were incubated for 2 hours at 37°C with constant agitation. A portion of these suspensions was stored for metabolite characterization, following the same procedure as in the oral stage. The intestinal extract was prepared 30 min before use by mixing 3 mg of gall ox (bile) and 2.6 mg of pancreatin in 5 mL of Krebs–Ringer buffer (NaCl 118 mM, KCl 4.7 mM, MgSO₄ 1.2 mM, KH₂PO₄ 1.2 mM, NaHCO₃ 25 mM, glucose 11 mM, and CaCl₂ 2.5 mM, pH 6.8), which was degassed to simulate anaerobic conditions. This solution (5 mL) was added to each sample, and the resulting 15 mL suspension was transferred to a glass test tube containing an inverted intestinal sac prepared from Wistar rats (250–300 g body weight, $n = 4$). These experimental animals were obtained from the animal facility of the Universidad Nacional Autónoma de México (UNAM), Juriquilla Campus.

Before the surgical procedure, the rats had *ad libitum* access to water for 16 to 20 h and were subjected to a solid fasting period of 12 h. Anesthesia was induced using sodium pentobarbital (60 mg/kg, i.p.). A midline abdominal incision was made to expose the intestines, and a 20–25-cm segment of the proximal jejunum was excised and placed in a gasified Krebs–Ringer buffer solution at 37°C. The intestine was rinsed with the same buffer, carefully inverted over a glass rod, and divided into segments of approximately 6 cm in length. The serosal side of each segment was filled with 1 mL of Krebs–Ringer buffer and tied, achieving a final length of approximately 4 cm. This procedure was performed with the intestinal segments submerged in Krebs–Ringer buffer to maintain tissue viability. The inverted intestinal bags, each containing 15 mL of the suspension, were incubated in a shaking water bath (80 cycles per minute) at 37°C for 2 h. After incubation, the bags were removed to collect the digested fraction (DF) and the sample from the mucosal side (external surface), referred to as the undigested fraction (UF). The same analyses performed for the oral and gastric stages were applied to these fractions. All experiments were conducted in triplicate, with a blank prepared using saliva from volunteers without extract, under identical conditions.

2.6. Identification and Quantification of Free Phenolic Compounds by HPLC-DAD. The identification and analysis of free phenolic compounds were performed following the methodology proposed by Sánchez-Quezada et al. [18]. The analysis was conducted using an Agilent Series 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. The compounds were separated using a ZORBAX Eclipse XDB-C18 guard column (4.6 × 12.5 mm, 5 µm, pressure limit of 400 bar) and

a ZORBAX Eclipse XDB-C18 analytical column (250 × 4.6 mm inner diameter; particle size 5 µm), with a flow rate of 0.750 mL/min. A 10 µL sample volume, previously filtered through 0.2-µm cellulose filters (Millipore, Bedford, MA, USA), was injected. The mobile phase consisted of two solvents: Solvent A (acidified water with formic acid) and Solvent B (acetonitrile). The linear gradient applied was as follows: 0 min [A:B, 100:0], 3 min [A:B, 95:5], 4 min [A:B, 88:12], 7 min [A:B, 80:20], 13 min [A:B, 78:22], 14 min [A:B, 77:23], 15 min [A:B, 75:25], 17 min [A:B, 74:26], 20 min [A:B, 72:28], 22 min [A:B, 70:30], 25 min [A:B, 50:50], 26 min [A:B, 25:75], 27 min [A:B, 0:100], followed by an equilibration step for 4 min. Detection was performed at wavelengths of 240, 280, and 360 nm. The area under the curve for the samples was interpolated using standard straight-line equations.

2.7. Statistical Analysis. The data are presented as the mean ± standard deviation, performed in triplicate for each independent assay. Statistical differences among three or more datasets, as well as simple regression models, were analyzed using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA) and Statgraphics Centurion for the analysis of variance (ANOVA). A p value of < 0.05 was considered statistically significant.

The spectrophotometric data obtained on the concentration of secondary metabolites at different stages of the simulated digestion process were analyzed using a one-way ANOVA to determine the existence of significant differences among the stages. Subsequently, a multiple range test was applied to identify which means differed significantly from each other. In cases where the data did not meet the assumptions of normality or homoscedasticity, the non-parametric Kruskal–Wallis test was used. All statistical analyses were performed using Statgraphics Centurion 19 software.

3. Results and Discussion

3.1. Phenolic Content and Antioxidant Capacity of Ethanolic Extracts of *T. patula* and *Z. officinale*. The methods used to quantify phenolic and flavonoid compounds in the extracts revealed a higher content of these compounds in the ETP compared to the EZO, with statistically significant differences between samples (Table 1). In the case of *T. patula* and *Z. officinale*, the TP content (TPC) was 177.83 and 130.13 mg GAE/g DE, respectively. In contrast, previous studies such as Moliner et al. [19] reported a TPC of 51.06 ± 0.24 and 54.08 ± 0.4 mg/g DE in extracts of orange and yellow flowers of *T. erecta*. These variations can be attributed to several factors, such as the specific part of the plant used. Some studies indicate that leaves tend to have a higher concentration of phenolic compounds compared to other plant structures [20], which could explain the results obtained in this study. Additionally, various factors, such as the extraction method, solvent type, environmental conditions, the presence of pathogens, storage stability, and differences between plant species, may also influence phenolic content

TABLE 1: Phytochemical characterization of ethanolic extracts using spectrophotometry.

	<i>Tagetes patula</i>	<i>Zingiber officinale</i>
Total phenols (mg GAE/g DE)	177.83 ± 5.10 ^a	130.13 ± 4.69 ^b
Total flavonoids (mg RE/g DE)	177.13 ± 12.92 ^a	64.97 ± 5.75 ^b
Antioxidant activity		
DPPH (mg TE/g DE)	201.02 ± 3.03 ^a	177.33 ± 1.94 ^b
FRAP (mg TE/g DE)	136.16 ± 3.14 ^a	154.96 ± 1.01 ^b

Note: Analysis of variance (ANOVA) was performed for the comparison of means. Means in a row with different letters are significantly different ($p < 0.05$). Concentrations of phenolic compounds are expressed as milligram equivalents of gallic acid and rutin, per gram of sample for phenolic compounds and flavonoids, respectively. Antioxidant activity is expressed as mg Trolox equivalent to per gram of sample. The results are the average of at least three independent experiments.

Abbreviations: DE, dry extract; GAE, gallic acid equivalent; RE, rutin equivalents; TE, Trolox equivalent.

[21, 22]. In studies such as that of Moliner et al. [19], lower concentrations of phenolic compounds have been reported in ethanolic extracts of *T. erecta*, a species phylogenetically related to *T. patula*, with values of 51.06 ± 0.24 and 54.08 ± 0.4 mg/g of extract for orange and yellow flowers, respectively. In contrast, ethanolic extracts of *Z. officinale* have been reported to contain a TPC of 10.099 mg GAE/g ED [23].

The antioxidant capacity, determined using the DPPH method, was significantly higher in ETP (201.02 ± 3.03 mg TE/g DE), whereas the antioxidant capacity assessed by the FRAP method was higher in EZO (154.96 ± 1.01 mg TE/g DE) (Table 1). Previous studies have reported an antioxidant capacity of $87.60 \mu\text{mol TE/g}$ using the DPPH method in essential oils of *T. patula* [24] and, in the case of ginger, an antioxidant capacity ranging from 15.279 to 234.65 mg Fe (II)/g extract using the FRAP method [25]. These findings suggest that the analyzed extracts contain antioxidant compounds capable of neutralizing reactive oxygen species (ROS) [10], which may contribute to the protection of essential biomolecules such as DNA, lipids, and proteins against oxidative stress. This mechanism is of particular interest due to its potential role in the prevention of chronic diseases, including cancer [26].

3.2. Identification and Quantification of Phenolic Compounds by HPLC. HPLC analysis allowed identification and quantification of the phenolic compounds present in the extracts (Table 2). Trigonelline was identified in both extracts, with a concentrations of $33.5 \mu\text{g E/g DE}$ in the EZO and $39.32 \mu\text{g E/g DE}$ in the ETP, this being the only compound detected in the EZO with the method used. These results suggest that certain factors may have influenced the detection of other compounds in this matrix, such as low solubility in the mobile phase due to the formation of complexes with other molecules of the plant cell wall, such as cellulose [27], which does not necessarily imply their absence. As will be discussed later, the digestion process favored the release of other phenolic compounds, thus facilitating their detection.

TABLE 2: Free phenolic compounds from the ethanolic extracts identified and quantified by HPLC.

Phenolic compounds	<i>Tagetes patula</i> Concentration ($\mu\text{g E/g DE}$)	<i>Zingiber officinale</i>
Trigonelline	39.33 ± 0.18	33.5 ± 0.08
Sinapic acid	36.24 ± 6.52	n.d
Gallic acid	538.46 ± 5.96	n.d
Dihydroxybenzoic acid	4321.53 ± 110.94	n.d
Catechin	740.68 ± 47.79	n.d
Syringic acid	6.98 ± 0.10	n.d
Coumaric acid	316.39 ± 51.34	n.d
Apigenin	166.36 ± 11.37	n.d
Rutin	5846.98 ± 148.25	n.d
Ellagic acid	541.37 ± 0.33	n.d

Note: Concentration is presented as μg equivalent of each compound per gram of dry extract. The values represent the mean $\pm$ standard deviation of independent duplicate assays.

Abbreviation: n.d., not detected.

Pirpour Tazehkand et al. [28] evaluated the therapeutic potential of trigonelline in cancer treatment, highlighting its ability to induce apoptosis and inhibit cell invasion and migration through the suppression of nuclear factor erythroid Type 2 (Nrf2) in oxaliplatin-resistant SW480 colorectal cancer cells. The results showed a significant decrease in the IC50 (down to $15 \pm 3 \mu\text{M}$) following pretreatment with trigonelline-loaded micelles, suggesting an enhancement in oxaliplatin efficacy. Nrf2 is a transcription factor that is overexpressed in various types of cancer and plays a crucial role in promoting tumor cell growth and chemotherapy resistance [29]. Therefore, trigonelline, along with other polyphenols present in ETP, represents a promising avenue for research due to its multiple biological properties.

Among these compounds, rutin, a flavonoid present in ETP at a concentration of $9.58 \pm 0.2 \mu\text{M}$, has demonstrated cytotoxic activity against SW480 colorectal cancer cells ($\text{IC}_{50} = 125 \pm 11.3 \mu\text{M}$). Additionally, studies in murine models indicated that rutin inhibits tumor growth by 58% at a dose of 20 mg/kg , without affecting the relative weight of vital organs (spleen, liver, heart, and kidney) or overall body weight. Interestingly, comparable effects were observed with cisplatin, a chemotherapeutic agent widely used to treat various types of cancer [30].

3.3. TPC and Flavonoid Content During Simulated Digestion. The analyses revealed significant differences in the TPC across the different stages of digestion (Figure 1). In the ETP, the total polyphenol content showed a progressive decrease throughout the digestive process, likely due to the instability of certain compounds in response to pH changes (Figure 1(a)). For example, several phenolic compounds, such as anthocyanins and phenolic acids, are known to degrade under alkaline conditions in the small intestine [31–33].

In contrast, the EZO extract exhibited a significant decrease during the oral phase, followed by stabilization (Figure 1(b)). According to [34], this reduction may be associated with the interaction between polyphenols and salivary proteins through hydrogen bonding between the hydroxyl groups of polyphenols and the carbonyl groups of

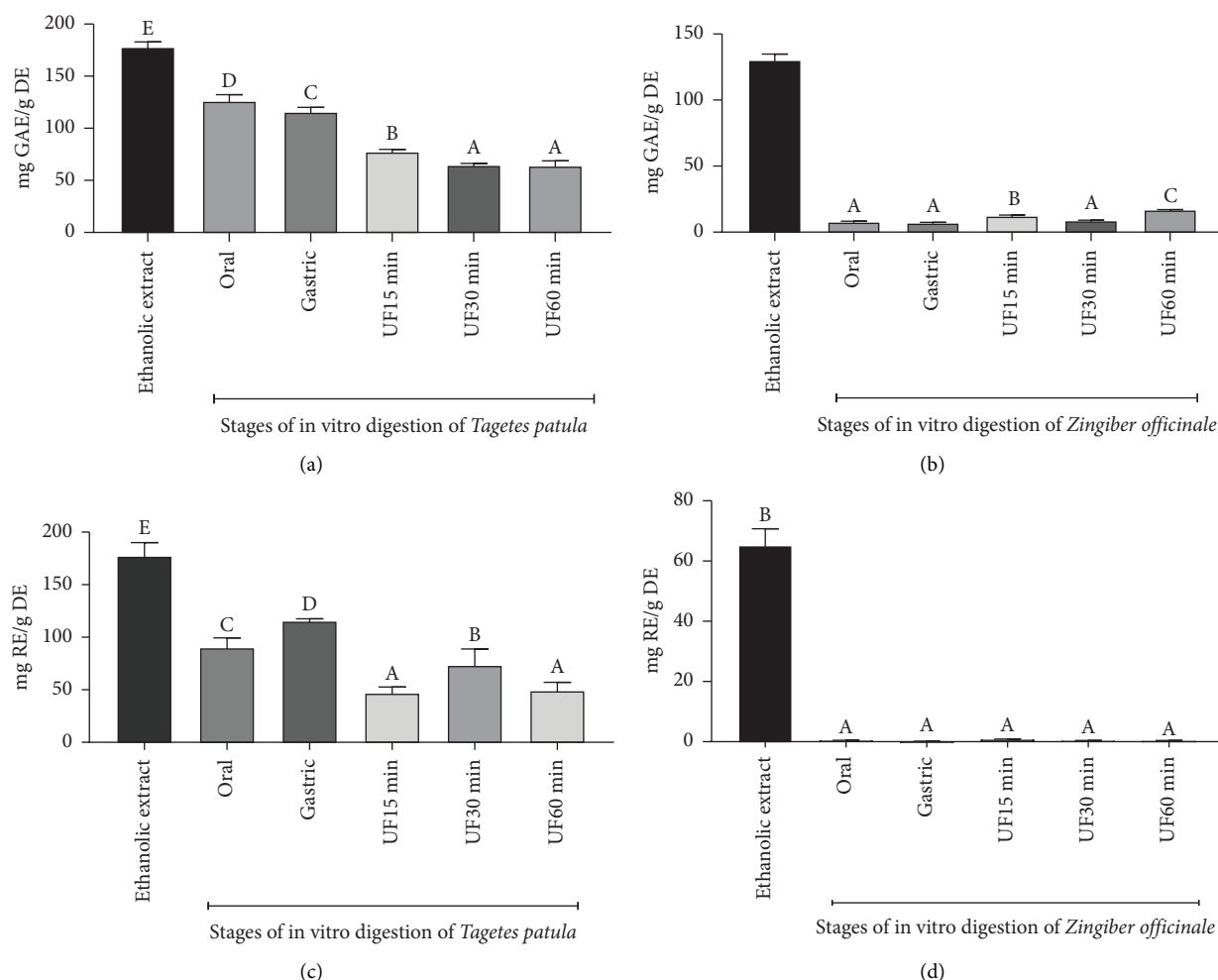


FIGURE 1: Phenolic changes during simulated digestion. (a) Total phenolic content of the ETP. (b) Total phenolic content of the EZO. (c) Flavonoid content of the ETP. (d) Flavonoid content of the EZO. UF: undigested fraction (apical small intestine). Different letters in each stage and for each extract indicate statistically significant differences at $p < 0.05$.

proteins, which could lead to the precipitation of the polyphenol–protein complex and consequently interfere with their identification using spectrophotometric techniques. However, at the end of digestion, a higher phenolic content was observed compared to the oral phase (Figure 1(b)). Similarly, a previous study on the bioaccessibility of antioxidant compounds in different varieties of beans (*Phaseolus vulgaris* L.), using an in vitro gastrointestinal system, reported an increase in the phenolic content in all samples. This phenomenon was attributed to the digestive process facilitating the release of polyphenols trapped in the food matrix or in the form of complexes with other molecules, thereby enhancing their bioaccessibility [35].

The flavonoid content also exhibited significant changes in concentration during digestion in both extracts. In the ETP, the highest flavonoid content was observed during the gastric stage, followed by a decrease in the intestinal stage (Figure 1(c)). However, the overall concentration of these compounds was significantly higher compared to the EZO, where a greater content was detected at the beginning of intestinal digestion, remaining unchanged in the subsequent

stages (Figure 1(c)). Although a decrease in both phenolic and flavonoid content was observed by the end of digestion, phenolic compounds persisted in the UF. These compounds could undergo fermentation and metabolism in the colon, thereby exerting their biological activity in this portion.

3.4. Bioaccessibility of Phenolic Compounds During Simulated Gastrointestinal Digestion. The effect of digestion on the phenolic content and profile, as well as the antioxidant capacity of the ethanolic extracts, was evaluated at three distinct stages: mouth, stomach, and intestine. This evaluation considered the portion of compounds absorbed in the intestine (digestible fraction) and those that were not absorbed (UF). Table 3 shows the bioaccessibility of free phenolic compounds from EPT during simulated digestion. Sixteen compounds were identified in the oral phase, of which 15 remained bioaccessible in the UFs until the intestinal phase, potentially being targeted and metabolized in the colon. In contrast, eight compounds successfully crossed the intestinal barrier and were identified in the digestible fraction, making them potentially available for absorption in

TABLE 3: Bioaccessibility of free phenolic compounds during the simulated digestion process of the ethanolic extract of *Tagetes patula*.

Phenolic compounds	Oral	Gastric	Stages during simulated digestion					
			Indigestible fraction (UF) at different incubation times		Digestible fraction (DF) at different incubation times			
			15 min	30 min	60 min	15 min	30 min	60 min
Trigonelline	2.13 ± 0.09	1.09 ± 0.08	0.98 ± 0.01	1.22 ± 0.00	1.08 ± 0.00	1.60 ± 0.12	2.17 ± 0.14	0.49 ± 0.01
Sinapic acid	239.63 ± 20.16	172.42 ± 74.89	117.06 ± 5.69	124.13 ± 0.33	124.46 ± 0.41	6.20 ± 0.13	20.61 ± 0.79	2.63 ± 0.19
Gallic acid	647.42 ± 38.12	345.61 ± 49.19	117.40 ± 0.07	104.97 ± 0.00	37.46 ± 5.58	n.d	n.d	n.d
Dihydroxybenzoic acid	2008.60 ± 73.25	1917.16 ± 56.07	748.83 ± 11.77	604.69 ± 4.92	393.76 ± 0.39	n.d	n.d	n.d
Caffeine	14.03 ± 0.41	9.90 ± 5.43	1.56 ± 0.39	2.12 ± 0.03	2.39 ± 0.01	n.d	n.d	n.d
Catechin	4622.22 ± 158.45	3334.50 ± 1662.67	573.64 ± 0.97	651.56 ± 1.73	724.19 ± 1.36	n.d	n.d	n.d
Epicatechin	2316.08 ± 60.21	1669.79 ± 853.77	661.42 ± 59.07	631.90 ± 50.94	627.93 ± 3.12	16.44 ± 1.43	123.32 ± 14.58	108.01 ± 18.74
Syringic acid	7.90 ± 0.61	5.61 ± 2.63	4.07 ± 0.05	4.39 ± 0.01	4.17 ± 0.00	0.51 ± 0.00	1.25 ± 0.01	0.15 ± 0.00
Coumaric acid	601.15 ± 75.20	430.68 ± 165.88	251.55 ± 11.19	277.02 ± 0.15	281.31 ± 0.27	18.56 ± 0.87	25.19 ± 2.07	9.59 ± 0.27
Caffeic acid	835.38 ± 66.29	609.32 ± 253.41	358.49 ± 0.41	400.85 ± 0.90	395.61 ± 0.89	n.d	47.82 ± 0.55	25.64 ± 0.32
Ferulic acid	83.06 ± 9.32	47.78 ± 40.57	2.40 ± 0.52	6.11 ± 0.03	4.99 ± 0.08	n.d	n.d	n.d
Apigenin	219.88 ± 12.44	273.88 ± 88.79	80.73 ± 6.01	91.46 ± 0.15	89.07 ± 0.40	7.95 ± 0.09	16.92 ± 0.77	12.69 ± 0.05
Rutin	140.84 ± 22.85	103.89 ± 29.41	2097.22 ± 25.46	2444.77 ± 7.19	2308.86 ± 1.41	281.45 ± 25.63	596.74 ± 6.61	336.74 ± 0.79
Ellagic acid	285.24 ± 13.04	238.59 ± 52.93	225.80 ± 0.39	240.28 ± 0.19	250.84 ± 0.32	26.29 ± 0.19	68.04 ± 0.47	41.38 ± 0.18
Morin	17.90 ± 0.21	13.18 ± 6.46	n.d	n.d	n.d	n.d	n.d	n.d
Quercetin	2221.33 ± 52.05	1627.11 ± 788.03	538.03 ± 1.65	609.57 ± 0.29	622.13 ± 0.89	n.d	n.d	n.d

Note: The values represent the mean ± standard deviation of independent assays. The concentration is expressed in µg equivalents of each compound/g dry extract. UF: undigested fraction (apical small intestine).
 DF: Digestible fraction (basolateral small intestine).

Abbreviation: n.d., not detected.

TABLE 4: Bioaccessibility of free phenolic compounds during the simulated digestion process of the ethanolic extract of *Zingiber officinale*.

Phenolic compounds	Oral	Gastric	Stages during simulated digestion					
			Indigestible fraction (UF) at different incubation times			Digestible fraction (DF) at different incubation times		
			15 min	30 min	60 min	15 min	30 min	60 min
Trigonelline	1.466 ± 0.21	1.342 ± 0.00	0.769 ± 1.09	1.073 ± 0.61	0.513 ± 0.04	1570 ± 0.00	1495 ± 0.06	1503 ± 0.09
Sinapic acid	13.358 ± 0.05	10.857 ± 0.08	n.d	18.560 ± 0.21	11.848 ± 16.76	14.468 ± 0.09	6343 ± 0.11	25,240 ± 0.23
Gallic acid	65.735 ± 1.18	107.117 ± 0.03	111.519 ± 0.29	78.287 ± 0.41	63.942 ± 1.89	n.d	n.d	114,018 ± 0.01
Dihydroxybenzoic acid	87.454 ± 0.28	98.109 ± 2.15	n.d	n.d	274.155 ± 1.44	n.d	n.d	1,626,045 ± 1.69
Catechin	24.816 ± 1.69	112.534 ± 1.13	83.675 ± 0.85	67.088 ± 1.65	71.893 ± 1.27	n.d	n.d	n.d
Epicatechin	n.d	25.484 ± 0.35	n.d	n.d	n.d	2.160 ± 0.12	n.d	n.d
Syringic acid	0.249 ± 0.00	0.064 ± 0.01	0.087 ± 0.00	0.368 ± 0.00	0.423 ± 0.00	0.328 ± 0.00	0.287 ± 0.00	0.463 ± 0.00
Coumaric acid	n.d	5.568 ± 0.27	n.d	n.d	n. d	n.d	n.d	n.d
Ellagic acid	0.234 ± 0.00	n.d	n.d	0.062 ± 0.00	n.d	n.d	n.d	n.d

Note: The values represent the mean ± standard deviation of independent assays. The concentration is expressed in µg equivalents of each compound/g dry extract. UF: undigested fraction (apical small intestine). DF: Digestible fraction (basolateral small intestine). Abbreviation: n.d., not detected.

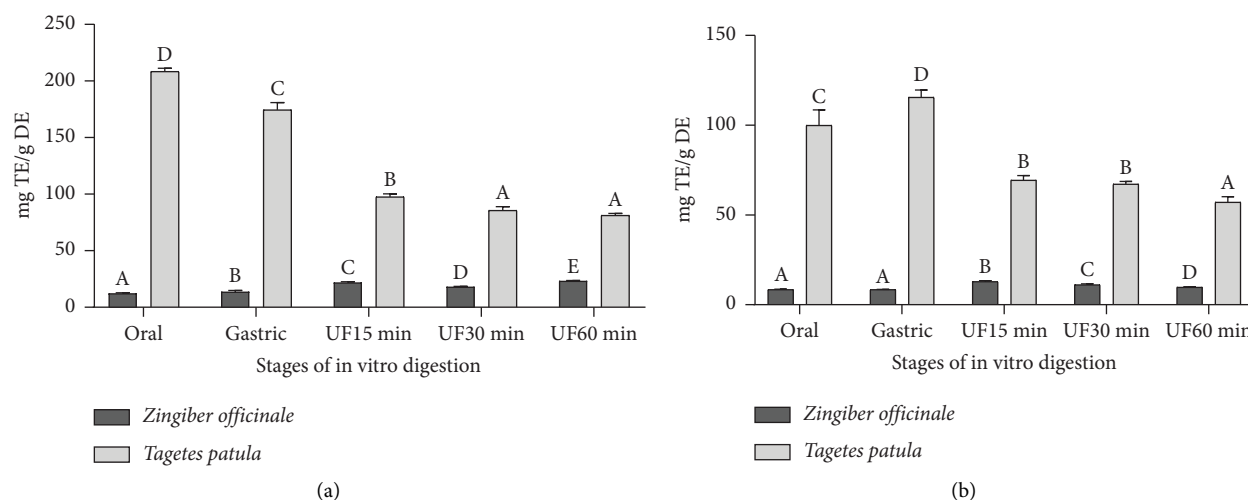


FIGURE 2: Antioxidant capacity of the extracts during simulated digestion. (a) Determination of the antioxidant capacity by the DPPH method at each stage of the digestion for the ethanol extract of *Tagetes patula* and *Zingiber officinale*. (b) Determination of the antioxidant capacity by the FRAP method in each of the digestion steps for the ethanol extract of *Tagetes patula* and *Zingiber officinale*. Different letters in each stage indicate the statistically significant differences at $p < 0.05$.

vivo and thus influencing cellular activity. Among the most abundant compounds that did not cross the intestinal barrier but exhibit various biological activities, such as antioxidant capacity, catechin, dihydroxybenzoic acid, epicatechin, and rutin stand out. Rutin is considered a multi-specific nutraceutical agent with various pharmacological activities, among which its antioxidant properties and ability to eliminate ROS stand out. In addition, it exhibits antibacterial, anti-inflammatory, and antimutagenic effects [36], along with numerous health benefits, including protection against cardiovascular diseases and diabetes mellitus, as well as the reduction of inflammation, oxidative damage, and apoptosis [37, 38].

The bioaccessibility of phenolic compounds from the EZO is presented in Table 4. Nine compounds were identified, six of which remained bioaccessible in the UFs until the end of digestion (60 min). The presence of these compounds varied between the matrices studied. However, in the case of EZO, the digestion process facilitated the release of certain compounds that could not be detected in the extract prior to digestion using the employed method. This behavior may be attributed to the activity of gastrointestinal digestive enzymes, which stimulate the release of phenolic compounds bound to carbohydrates, thereby increasing their concentration following digestion. [39–41].

3.5. Antioxidant Capacity During the Simulation of Oral–Gastric Digestion. The changes in antioxidant capacity during simulated digestion are shown in Figure 2. In the ETP, the antioxidant activity measured by the DPPH method (Figure 2(a)) progressively decreased, stabilizing in the final stages of the intestinal process. In contrast, the EZO extract showed a significant increase in this antioxidant capacity toward the end of digestion. According to Ref. [42], these variations may be attributed to modifications in the chemical structure of phenolic compounds and flavonoids,

as well as changes in their solubility or interactions with other components. A previous study [43] indicated that these compounds may be present in free and soluble form or bound to proteins, polysaccharides, or low-solubility mineral complexes, which could influence their antioxidant activity throughout digestion. A similar trend was observed for both extracts when antioxidant capacity was assessed using the FRAP method (Figure 2(b)). To evaluate the relationship between antioxidant capacity and phenolic content, a simple regression analysis was performed. A significant correlation was found between TPC and antioxidant capacity measured by the DPPH method for both extracts. The fitted model equation was $\text{antioxidant capacity} = (3.30562 + 0.0872422 \times \text{Phenols})^2$, with a correlation coefficient of 0.99 and a 95% confidence interval.

Correlation analyses confirm the relationship between TPC and antioxidant activity measured using the DPPH assay in both extracts; however, it is essential to highlight that biological activities do not depend solely on metabolite concentration, as the ability of polyphenols to scavenge or neutralize free radicals is strongly influenced by pH. This influence arises from the transitions from the oral to the intestinal phase, which can induce structural modifications in the compounds [44]. Given that these compounds contain multiple dissociable hydroxyl (–OH) groups in their chemical structure, it can be inferred that pH affects their dissociation rate. Previous studies have shown that in hydroxyflavones and anthocyanins, pH modulates the dissociation of oxygen-containing functional groups, suggesting a similar effect on polyphenols [45].

This study demonstrated that despite the observed decrease in vitro antioxidant capacity, the UFs retain this property. This suggests that these compounds may reach the colon, where they can be metabolized by the intestinal microbiota, generating metabolites with potential health benefits. Therefore, further research on the antioxidant properties of these fractions at the cellular level and *in vivo*

models is recommended to better understand their impact on human health and their potential application in the development of functional products.

4. Conclusion

The evaluated extracts exhibited variations in their antioxidant capacity, the concentration of identified metabolites, and the phenolic compound content. However, in both cases, the digestion process preserved their antioxidant activity, suggesting that these compounds may retain their functionality even after undergoing modifications in the gastrointestinal tract. Despite changes in the phenolic profile during digestion, the UFs retained a significant amount of these compounds, opening new perspectives for future research on their biological potential at the colonic level.

Data Availability Statement

Data will be available on reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

The authors are grateful for the funding provided by the University of Quindío under Grant Number 1116, to MINCIENCIAS (call for proposals 15, 2021-2022 biennium), and the Autonomous University of Querétaro (AUQ).

Acknowledgments

The authors are grateful for the funding provided by the University of Quindío (100016837) under Grant Number 1116, to MINCIENCIAS (call for proposals 15, 2021-2022 biennium), and the Autonomous University of Querétaro (AUQ).

References

- [1] S. Faizi, A. Dar, H. Siddiqi, et al., "Bioassay-Guided Isolation of Antioxidant Agents With Analgesic Properties From Flowers of *Tagetes Patula*," *Pharmaceutical Biology* 49, no. 5 (2011): 516–525, <https://doi.org/10.3109/13880209.2010.523006>.
- [2] S. Sukhikh, S. Ivanova, O. Babich, E. Shepel, and O. Kriger, "Study of Biological Activity of Aqueous Extracts of *Tagetes patula* L. Flowers," *Fitoterapia* 185 (2025): 106712, <https://doi.org/10.1016/j.fitote.2025.106712>.
- [3] S. Faizi, H. Siddiqi, S. Bano, et al., "Antibacterial and Antifungal Activities of Different Parts of *Tagetes patula*: Preparation of Patuletin Derivatives," *Pharmaceutical Biology* 46, no. 5 (2008): 309–320, <https://doi.org/10.1080/13880200801887476>.
- [4] S. Faizi, S. Fayyaz, S. Bano, E. Yawar Iqbal, H. Siddiqi, and A. Naz, "Isolation of Nematicidal Compounds From *Tagetes patula* L. Yellow Flowers: Structure–Activity Relationship Studies Against Cyst Nematode *Heterodera Zeae* Infective Stage Larvae," *Journal of Agricultural and Food Chemistry* 59, no. 17 (2011): 9080–9093, <https://doi.org/10.1021/jf201611b>.
- [5] J. Dai, P. G. Van Wie, L. Y. Fai, et al., "Downregulation of NEDD9 by Apigenin Suppresses Migration, Invasion, and Metastasis of Colorectal Cancer Cells," *Toxicology and Applied Pharmacology* 311 (2016): 106–112, <https://doi.org/10.1016/j.taap.2016.09.016>.
- [6] M. Kashif, S. Bano, S. Naqvi, et al., "Cytotoxic and Antioxidant Properties of Phenolic Compounds From *Tagetes patula* Flower," *Pharmaceutical Biology* 53, no. 5 (2015): 672–681, <https://doi.org/10.3109/13880209.2014.936471>.
- [7] M. Aleem, M. I. Khan, F. A. Shakshaz, N. Akbari, and D. Anwar, "Botany, Phytochemistry and Antimicrobial Activity of Ginger (*Zingiber officinale*): A Review," *International Journal of Herbal Medicine* 8, no. 6 (2020): 36–49, <https://doi.org/10.22271/flora.2020.v8.i6a.705>.
- [8] K. Gamage, C. Dissanayake, W. Angoda, L. Chandrasiri Waliwita, and R. P. Liyanage, "A Review on Medicinal Uses of *Zingiber officinale* (Ginger)," *International Journal of Health Sciences & Research* 10, no. 6 (2020): 142, <https://www.ijhsr.org>.
- [9] S. Hafuth and S. Randhawa, *Investigating the Anti-Cancer Properties of 6-Shogaol in Zingiber officinale* (Begell House, 2022).
- [10] E. Mfotie Njoya, L. J. McGaw, and T. J. Makhafola, "Investigating the Phytochemical Composition, Antioxidant, and Anti-Inflammatory Potentials of *Cassinopsis ilicifolia* (Hochst.) Kuntze Extract Against Some Oxidative Stress and Inflammation Molecular Markers," *Current Issues in Molecular Biology* 46, no. 9 (2024): 9639–9658, <https://doi.org/10.3390/cimb46090573>.
- [11] D. G. Bortolini, L. Barros, G. M. Maciel, et al., "Bioactive Profile of Edible Nasturtium and Rose Flowers During Simulated Gastrointestinal Digestion," *Food Chemistry* 381 (2022): 132267, <https://doi.org/10.1016/j.foodchem.2022.132267>.
- [12] O. Folin and V. Ciocalteu, "On Tyrosine and Tryptophane Determinations in Proteins," *Journal of Biological Chemistry* 73, no. 2 (1927): 627–650, [https://doi.org/10.1016/S0021-9258\(18\)84277-6](https://doi.org/10.1016/S0021-9258(18)84277-6).
- [13] Ó. A. Muñoz-Bernal, G. A. Torres Aguirre, J. A. Núñez-Gastélum, et al., "Nuevo Acercamiento a la Interacción del Reactivo de Folin-Ciocalteu Con Azúcares Durante la Cuantificación de Polifenoles Totales," *TIP* 20, no. 2 (2017): 23–28, <https://doi.org/10.1016/j.recqb.2017.04.003>.
- [14] B. Boua Boua, L. Traoré, D. C. Ouattara, J.-A. Mamyrbekova-Békro, and Y.-A. Békro, "The Changes in the Contents of Polyphenolic Compounds of Taro (*Colocasia esculenta*) Upon Domestic Processing," *World Journal of Pharmaceutical Research SJIF Impact Factor* 10, no. 11 (2021): 102–111, <https://doi.org/10.20959/wjpr202111-21306>.
- [15] L. R. Fukumoto and G. Mazza, "Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds," *Journal of Agricultural and Food Chemistry* 48, no. 8 (2000): 3597–3604, <https://doi.org/10.1021/jf000220w>.
- [16] I. F. F. Benzie and J. J. Strain, "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay," *Analytical Biochemistry* 239, no. 1 (1996): 70–76, <https://doi.org/10.1006/abio.1996.0292>.
- [17] R. Campos Vega, K. Vázquez-Sánchez, D. López-Barrera, G. Loarca-Piña, S. Mendoza-Díaz, and B. D. Oomah, "Simulated Gastrointestinal Digestion and *In Vitro* Colonic Fermentation of Spent Coffee (*Coffea arabica* L.): Bioaccessibility and Intestinal Permeability," *Food Research International* 77 (2015): 156–161, <https://doi.org/10.1016/j.foodres.2015.07.024>.
- [18] V. Sánchez-Quezada, M. Gaytán-Martínez, I. Recio, and G. Loarca-Piña, "Avocado Seed by-Product Uses in Emulsion-

- Type Ingredients With Nutraceutical Value: Stability, Cytotoxicity, Nutraceutical Properties, and Assessment of *in Vitro* Oral-Gastric Digestion,” *Food Chemistry* 421 (2023): 136118, <https://doi.org/10.1016/j.foodchem.2023.136118>.
- [19] C. Moliner, L. Barros, M. I. Dias, et al., “Edible Flowers of *Tagetes erecta* L. as Functional Ingredients: Phenolic Composition, Antioxidant and Protective Effects on *Caenorhabditis elegans*,” *Nutrients* 10, no. 12 (2018): 2002, <https://doi.org/10.3390/nu10122002>.
 - [20] J. Brozdowski, B. Waliszewska, S. Gacnik, M. Hudina, R. Veberic, and M. Mikulic-Petkovsek, “Phenolic Composition of Leaf and Flower Extracts of Black Cherry (*Prunus serotina* Ehrh.),” *Annals of Forest Science* 78, no. 3 (2021): 66, <https://doi.org/10.1007/s13595-021-01089-6>.
 - [21] E. S. Savitri, K. Holil, R. S. Resmisari, U. Syarifah, and S. Munawaroh, “Effect of Extraction Solvent on Total Phenol, Total Flavonoid Content and Antioxidant Activities of Extract Plants *Punica granatum*, *Vitis vinifera* L, *Ficus Carica* L. and *Olea europea*,” in *AIP Conference Proceedings*, 2120 (2019), 030034, <https://doi.org/10.1063/1.5115638>.
 - [22] J. Yang, J. Guo, and J. Yuan, “*In Vitro* Antioxidant Properties of Rutin,” *LWT-Food Science and Technology* 41, no. 6 (2008): 1060–1066, <https://doi.org/10.1016/j.lwt.2007.06.010>.
 - [23] D. Ezez and M. Tefera, “Effects of Solvents on Total Phenolic Content and Antioxidant Activity of Ginger Extracts,” *Journal of Chemistry* 2021 (2021): 1–5, <https://doi.org/10.1155/2021/6635199>.
 - [24] V. M. López Zambrano, A. A. Dueñas Rivadeneira, J. G. Cuenca Nevárez, and J. M. Rodríguez Díaz, “Phytochemical Characterization, Antioxidant and Antibacterial Activity of Essential Oil and Extracts of *Tagetes patula* on *Staphylococcus aureus*,” *Revista de La Facultad de Agronomía* 37, no. 4 (2020): 347–367, [https://doi.org/10.47280/RevFacAgron\(LUZ\).v37.n4.02](https://doi.org/10.47280/RevFacAgron(LUZ).v37.n4.02).
 - [25] I. Mustafa and N. L. Chin, “Antioxidant Properties of Dried Ginger (*Zingiber officinale* Roscoe) Var. Bentong,” *Foods* 12, no. 1 (2023): 178, <https://doi.org/10.3390/foods12010178>.
 - [26] L. A. Pham Huy, H. He, and C. Pham-Huyc, “Free Radicals, Antioxidants in Disease and Health,” *International Journal of Biomedical Sciences* 4, no. 2 (2008): 89–96, <https://doi.org/10.59566/ijbs.2008.4089>.
 - [27] F. Shahidi and J. D. Yeo, “Insoluble-Bound Phenolics in Food,” in *Molecules*, 21, no. 9 (MDPI AG, 2016), 1216, <https://doi.org/10.3390/molecules21091216>.
 - [28] A. Pirpour Tazehkand, R. Salehi, K. Velaie, and N. Samadi, “The Potential Impact of Trigonelline Loaded Micelles on Nrf2 Suppression to Overcome Oxaliplatin Resistance in Colon Cancer Cells,” *Molecular Biology Reports* 47, no. 8 (2020): 5817–5829, <https://doi.org/10.1007/s11033-020-05650-w>.
 - [29] X. J. Wang, Y. Li, L. Luo, et al., “Oxaliplatin Activates the Keap1/Nrf2 Antioxidant System Conferring Protection Against the Cytotoxicity of Anticancer Drugs,” *Free Radical Biology and Medicine* 70 (2014): 68–77, <https://doi.org/10.1016/j.freeradbiomed.2014.02.010>.
 - [30] A. J. Alonso Castro, F. Domínguez, and A. García-Carrancá, “Rutin Exerts Antitumor Effects on Nude Mice Bearing SW480 Tumor,” *Archives of Medical Research* 44, no. 5 (2013): 346–351, <https://doi.org/10.1016/j.arcmed.2013.06.002>.
 - [31] A. C. Holanda Nunes, L. da Silva Freire, G. R. Reis de Alencar, R. Carvalho de Moura, and A. de Lima, “Bioaccessibilidade *in Vitro* de Polifenóis Naturalmente Presentes em Frutos,” *Revista Brasileira de Plantas Medicinais* 21 (2019): 132–139, https://doi.org/10.1590/1983-084X/20_02_020.
 - [32] L. Liang, X. Wu, T. Zhao, et al., “*In Vitro* Bioaccessibility and Antioxidant Activity of Anthocyanins From Mulberry (*Morus atropurpurea* Roxb.) Following Simulated Gastro-Intestinal Digestion,” *Food Research International* 46, no. 1 (2012): 76–82, <https://doi.org/10.1016/j.foodres.2011.11.024>.
 - [33] D. Tagliazucchi, E. Verzelloni, D. Bertolini, and A. Conte, “*In Vitro* Bio-Accessibility and Antioxidant Activity of Grape Polyphenols,” *Food Chemistry* 120, no. 2 (2010): 599–606, <https://doi.org/10.1016/j.foodchem.2009.10.030>.
 - [34] S. Soares, A. Sousa, N. Mateus, and V. De Freitas, “Effect of Condensed Tannins Addition on the Astringency of Red Wines,” *Chemical Senses* 37, no. 2 (2012): 191–198, <https://doi.org/10.1093/chemse/bjr092>.
 - [35] L. M. Perez Perez, C. L. Del Toro Sanchez, E. Sánchez Chávez, et al., “Bioaccessibilidad de Compuestos Antioxidantes de Diferentes Variedades de Frijol (*Phaseolus vulgaris* L.) En México, Mediante Un Sistema Gastrointestinal *in Vitro*,” *Revista de Ciencias Biológicas y de la Salud* 22 (2019): <http://biotecnia.unison.mx>.
 - [36] I. Pinzaru, R. Chioibas, I. Marcovici, et al., “Rutin Exerts Cytotoxic and Senescence-Inducing Properties in Human Melanoma Cells,” *Toxics* 9, no. 9 (2021): 226, <https://doi.org/10.3390/toxics9090226>.
 - [37] Z. Nouri, S. Fakhri, K. Nouri, C. E. Wallace, M. H. Farzaei, and A. Bishayee, “Targeting Multiple Signaling Pathways in Cancer: The Rutin Therapeutic Approach,” in *Cancers*, 12, no. 8 (MDPI AG, 2020), 2276, <https://doi.org/10.3390/cancers12082276>.
 - [38] N. Muvhulawa, P. V. Dlodla, K. Ziqubu, et al., “Rutin Ameliorates Inflammation and Improves Metabolic Function: A Comprehensive Analysis of Scientific Literature,” *Pharmacological Research* 178 (2022): 106163, <https://doi.org/10.1016/j.phrs.2022.106163>.
 - [39] H. F. Alhuthayli, M. Ahmed, N. A. Al Kharashi, F. M. Al-Jasass, and M. A. Osman, “Correlation of *in Vitro* Digestibility With Chemical Composition and Bioaccessibility of Phenolic Compounds in Date Pomace and Antioxidant Potential,” *CyTA-Journal of Food* 22, no. 1 (2024): 2405631, <https://doi.org/10.1080/19476337.2024.2405631>.
 - [40] J. Bouayed, L. Hoffmann, and T. Bohn, “Total Phenolics, Flavonoids, Anthocyanins and Antioxidant Activity Following Simulated Gastro-Intestinal Digestion and Dialysis of Apple Varieties: Bioaccessibility and Potential Uptake,” *Food Chemistry* 128, no. 1 (2011): 14–21, <https://doi.org/10.1016/j.foodchem.2011.02.052>.
 - [41] G. J. McDougall, S. Fyffe, P. Dobson, and D. Stewart, “Anthocyanins From Red wine—Their Stability Under Simulated Gastrointestinal Digestion,” *Phytochemistry* 66, no. 21 (2005): 2540–2548, <https://doi.org/10.1016/j.phytochem.2005.09.003>.
 - [42] R. A. Soriano Sancho, V. Pavan, and G. M. Pastore, “Effect of *in Vitro* Digestion on Bioactive Compounds and Antioxidant Activity of Common Bean Seed Coats,” *Food Research International* 76 (2015): 74–78, <https://doi.org/10.1016/j.foodres.2014.11.042>.
 - [43] A. Scalbert, C. Morand, C. Manach, and C. Rémésy, “Absorption and Metabolism of Polyphenols in the Gut and Impact on Health,” *Biomedicine & Pharmacotherapy* 56, no. 6 (2002): 276–282, [https://doi.org/10.1016/s0753-3322\(02\)00205-6](https://doi.org/10.1016/s0753-3322(02)00205-6).
 - [44] K. Kahle, M. Kempf, P. Schreier, et al., “Intestinal Transit and Systemic Metabolism of Apple Polyphenols,” *European Journal of Nutrition* 50, no. 7 (2011): 507–522, <https://doi.org/10.1007/s00394-010-0157-0>.
 - [45] M. Muzolf, H. Szymusiak, A. Gliszczynska-Świgło, I. M. C. M. Rietjens, and B. Tyrakowska, “pH-Dependent Radical Scavenging Capacity of Green Tea Catechins,” *Journal of Agricultural and Food Chemistry* 56, no. 3 (2008): 816–823, <https://doi.org/10.1021/jf0712189>.