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Effect of Drying on Bioaccessibility of Phenolic Compounds and Antioxidant Capacity of Indonesian Bay Leaf Decoction during Simulated Gastrointestinal Digestion

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ABSTRACT

Indonesian bay leaf (*Syzygium polyanthum* Wight) is commonly consumed in Indonesia as a decoction, but the bioaccessibility of its bioactive compounds following gastrointestinal digestion remains poorly understood. This study evaluated changes in antioxidant capacity (DPPH, FRAP, and ABTS methods), total phenolic content (TPC), total flavonoid content (TFC), and bioaccessibility index (BI) in decoctions prepared from fresh Indonesian bay leaves (FBLD) and leaves dried using a fluidized-bed dryer at 45–50 °C (DBLD) following simulated *in vitro* gastrointestinal digestion. Before digestion, DPPH antioxidant capacity, TPC, and TFC were comparable between FBLD and DBLD, whereas drying reduced FRAP and ABTS antioxidant capacities by 21 and 19%, respectively. After intestinal digestion, DPPH and ABTS antioxidant capacities and TPC in DBLD were 55, 72, and 42% lower, respectively, than those in FBLD. The intestinal-phase BI (BI PI) for ABTS antioxidant capacity in FBLD and for TPC and TFC in both decoctions exceeded 100%, indicating increased recovery of assay-responsive components after digestion. In contrast, BI PI values for DPPH antioxidant capacity in DBLD and FRAP antioxidant capacity in both decoctions were below 37%. FBLD also exhibited higher BI PI values for TPC and DPPH and ABTS antioxidant capacities than DBLD. Overall, drying and gastrointestinal digestion differentially affected the antioxidant properties and bioaccessibility of phenolic- and flavonoid-related compounds in Indonesian bay leaf decoctions. These findings highlight the importance of optimizing drying conditions to balance raw material stability and the retention of bioaccessible compounds. Further studies using intestinal absorption models and *in vivo* validation are needed to establish their bioavailability and biological effects.

Keywords: antioxidant, bioaccessibility, flavonoid, Indonesian bay leaf, phenolic

INTRODUCTION

Indonesian bay leaf (*Syzygium polyanthum* Wight) is a herbal plant commonly used in Indonesian cuisine. This leaf is also widely utilized in traditional medicine for treating conditions such as diabetes mellitus, hypertension, gastritis, cholesterol, and diarrhea (Kurniawan *et al*., 2023). A previous study by Bahriul *et al.* (2014) reported that the antioxidant content in Indonesian bay leaf extract, indicate by IC_{50} value exhibited very high antioxidant activity, with values of 37.441 ppm in young leaves, 14.889 ppm in half-old leaves, and 11.001 ppm in old leaves. These findings indicate that the older the age of the leaves, the stronger the antioxidant activity. As a medicinal plant, bay leaves are commonly consumed in the form of boiled water. Indonesian people used bay leaves by boiling them until a-half of initial water volume (Syabana *et al.*, 2022). Previous studies have suggested that boiled bay leaf preparations may reduce blood glucose levels, potentially related to the presence of flavonoid and tannin compounds. Plant-derived polyphenols, including flavonoids and tannins, are known to exhibit antioxidant activity and to inhibit carbohydrate-hydrolyzing enzymes. In particular, many polyphenolic compounds act as α-glucosidase inhibitors, thereby slowing carbohydrate digestion and reducing postprandial hyperglycemia (Hossain *et al*., 2020).

Heat treatment such as boiling can affect the bioactive properties of food, the chemical structure of bioactive compounds, and changes in compound composition (Grigoriou and Pinakoulaki, 2021; Syabana *et al*., 2022). Thermal treatment can trigger changes in soluble compounds, especially carbohydrates and phenolic compounds, which will affect the nutrient content and antioxidant capacity. A study also stated that leaves with antioxidant potential

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do not always produce effective antioxidants after consumption. The compounds in these ingredients must be able to maintain their antioxidant activity during cooking, gastrointestinal digestion, intestinal absorption, and subsequent transport to target tissues (Mawalagedera *et al*., 2016; Prangdimurti *et al*., 2025).

Bioaccessibility refers to portion released from food and potentially available for absorption. Furthermore, bioavailability refers to portion absorbed and available to body tissues (Zahir *et al*., 2026). The extent of bioaccessibility is governed by multiple factors, including the molecular structure and complexity of the compound, its efficiency of release from the food matrix, and its interactions with other dietary components such as dietary fiber, proteins, and lipids, which may either enhance or limit its availability for intestinal uptake. In addition, hydrolytic enzymes and the pH conditions of the digestive tract also affect its bioaccessibility (González-Aguilar *et al*., 2017). To evaluate the quantity of bioaccessible compounds, an *in vitro* digestion simulation can be used. These simulations are designed to mimic physiological digestive conditions by incorporating key parameters, including digestive enzymes, pH variations, digestion time, and salt concentration, in accordance with the INFOGEST protocol of Minekus *et al.* (2014). Fluidized-bed drying was selected as the drying method because it enables efficient heat and mass transfer, resulting in rapid and relatively uniform moisture removal from plant materials (Muhandri *et al*., 2023). Such controlled and relatively short drying conditions may influence the retention, release, and transformation of phenolic compounds and, consequently, the antioxidant capacity of bay leaves. Therefore, fluidized-bed drying was employed to investigate how the drying process affects the physicochemical properties, phenolic composition, and antioxidant capacity of bay leaf decoction before and after simulated gastrointestinal digestion.

Despite these findings, little information is available regarding how drying affects gastrointestinal bioaccessibility and metabolite transformation of bay leaf decoction. This study aimed to investigate the impact of drying on the antioxidant properties, phenolic and flavonoid contents, and bioaccessibility of fresh and dried bay leaf decoctions (FBLD and DBLD) under simulated gastrointestinal digestion, together with the characterization of digestion-induced compound transformations using UHPLC-HRMS (Ultra-High-Performance Liquid Chromatography Coupled with High-Resolution Mass Spectrometry).

MATERIALS AND METHOD

Materials

Indonesian bay leaves were collected directly from trees growing in a garden on the IPB University Dramaga campus, Bogor Regency, West Java, Indonesia. The plant was identified as *S. polyanthum* Wight based on its morphological characteristics. Materials used in the simulation of *in vitro* digestion include, pepsin enzyme P7000, pancreatin enzyme P7545, bile salts B8631 (Sigma-Aldrich Chemical Co., MO, USA), $MgCl_2(H_2O)_6$, KH_2PO_4, KCl, $CaCl_2$, $NaHCO_3$, NaCl, $(NH_4)CO_3$, HCl, dialysis membrane (cellulose, 12 kDa molecular weight cut-off (MWCO), 1.6 cm inner diameter) from Sigma-Aldrich, and NaOH. Materials for analysis included distilled water, Folin-Ciocalteu reagent (Merck, Germany), 95% ethanol, Na_2CO_3, gallic acid (Merck), ascorbic acid, trolox, $AlCl_3$, quercetin, $FeCl_3.6H_2O$, $FeSO_4.7H_2O$, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetate buffer pH 3.6, TPTZ (2,4,6-tripyridyl-s-triazine), methanol p.a (Merck), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), oxidant potassium persulfate, acetonitrile and formic acid (UHPLC-analytical grade).

Preparation of bay leaf decoction

Indonesian bay leaves were collected by selecting from the third to seventh leaf positions from the tip of each branch, with only healthy, undamaged, and intact leaves included. The initial moisture content of the fresh leaves was 52.9±0.01% (wet basis, wb). They were thoroughly rinsed under running water and dried using a laboratory-scale fluidized-bed dryer (custom-built by faculty members of IPB University, Bogor, Indonesia) for 4 h at 45–50 °C. The dried leaves had a moisture content of 9.2±0.02% wb and were subsequently chopped into small pieces. For decoction preparation, both fresh and dried bay leaves were weighed (±14 g on a dry basis or dry matter weight) and decocted in 400 mL boiling water (98.6–98.8 °C) using a hot plate (Thermo Cimarec SP131635, Germany) until the volume was reduced to half. The resulting extracts FBLD and DBLD were then diluted back to a final volume of 200 mL in a volumetric flask.

Simulated digestion of bay leaf decoction

Digestion simulation was carried out according to standardized INFOGEST protocol developed by Minekus *et al.* (2014). Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) freshly prepared from stock solutions in accordance with the protocols and maintained at 37 °C. For the gastric phase, 20 mL of bay leaf decoction was combined with 3.2 mL of pepsin solution (25,000 U/mL), 15 mL of SGF, 10 μL of $CaCl_2$ (0.3 M), and 1.39 mL of distilled water. The pH of the mixture was adjusted to 3.0 using 1 N HCl. The digestion solution was subsequently incubated at 37 °C in a shaking water-bath shaker (LSB-030s Lab Tech, USA) set at 90 rpm for 2 h. After incubation, the gastric chyme was immediately placed on ice to halt enzymatic activity. A 20 mL aliquot of the gastric

chyme was subsequently used for the intestinal phase. The remaining gastric mixture was centrifuged for 60 min at 1,409 × g, and the resulting supernatant representing the post-gastric digestion (PG) fraction was stored at −20 °C for further analyses. During the intestinal phase, the collected gastric chyme (20 mL) was adjusted to pH 7.0 using 1 M NaOH. Subsequently, 11 mL of SIF, 2.5 mL of bile extract (160 mM), 5 mL of pancreatin solution (800 U/mL, trypsin activity), 40 μL of $CaCl_2$ (0.3 M), and 1.31 mL of distilled water were added. The mixture was then incubated at 37 °C in a shaking water bath set for 2 h at 90 rpm.

After the intestinal phase, 20 mL of the intestinal solution was transferred into a flask. A dialysis membrane with MWCO of 12 kDa, containing 10 mL of sodium bicarbonate ($NaHCO_3$) solution (0.1 mol/L, pH 7.5), was immersed in the chyme and incubated at 37 °C in a shaking water bath at 90 rpm for 16 h. After dialysis, the dialysate was centrifuged at 1,917 × g for 30 mins. The remaining intestinal chyme was also centrifuged under the same conditions. The resulting supernatants representing the post-gastrointestinal digestion (PI) fraction and the dialysate were stored at −20 °C for subsequent analyses, including antioxidant assays (DPPH, FRAP, ABTS), total phenolic content (TPC), and total flavonoid content (TFC).

Total phenolic content assay

Quantification of phenolic compounds was performed using the Folin-Ciocalteu reagent described in Prangdimurti *et al.* (2025). A 0.5 mL sample of pre-digestion (PD), post-gastric (PG), post-gastrointestinal (PI), or dialysate of bay leaves was reacted with 0.5 mL of 95% ethanol, 2.5 mL of purified water, and 2.5 mL Folin-Ciocalteu (0.2 N). The reaction mixture was homogenized and kept at rest for 5 min. After that, 0.5 mL of 5% Na_2CO_3 solution was included, homogenized using a vortex, and incubated for 60 min. Measurement of changes in absorbance of the solution was performed using a UV-Vis spectrophotometer (Thermo Scientific Genesys 20, Germany) at a wavelength of 725 nm. Gallic acid was utilized as the standard, diluted to concentrations of 0, 20, 40, 60, 80, and 100 mg/L. Total phenolics were reported as mg GAE/g dry matter.

Total flavonoid content assay

The total flavonoid determination method was derived from Prangdimurti *et al.* (2025) which uses $AlCl_3$ reagent. Samples of PD, PG, PI, or dialysate of bay leaves, up to 5 mL were mixed with 5 mL of 2% $AlCl_3$ in methanol p.a. The sample mixture was vortex mixed until homogeneous and incubated for 10 min in the dim lights. Absorbance measurements were performed at a wavelength of 415 nm. The standard solution used was quercetin with concentration variations of 0, 10, 20, 30, and 40 mg/L. The results of the sample were reported as mg QE/g dry matter.

DPPH radical scavenging activity assay

The DPPH radical scavenging capacity test was performed by adapting Prangdimurti *et al.* (2025). At the sample testing stage, 0.1 mL of PD, PG, PI, or dialysate of bay leaves were reacted with 2.9 mL of 0.05 mM DPPH. The mixture of sample and DPPH reagent was homogenized with a vortex, followed by incubation in the dark for 30 min. Observations were made at a wavelength of 517 nm. A standard solution (ascorbic acid) was diluted to a concentration series of 0, 20, 40, 80, and 100 mg/L. The results of the sample were reported as mg AEAC/g dry matter.

Ferric-ion (Fe^{3+}) reducing antioxidant power (FRAP) assay

The antioxidant capacity testing of FRAP assay method began with the preparation of FRAP reagent by reacting 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3.6H_2O$, and 10 mM TPTZ solution in 40 mM HCl in a 10:1:1 (v/v/v) ratio and incubated for 30 min. Sample testing was conducted by mixing 25 μL sample, 1 mL of FRAP reagent, and 1 mL of distilled water. The sample was then incubated for 4 min at 37 °C under dark conditions. The solution was subsequently analyzed by measuring its absorbance at 593 nm. In the FRAP method, $FeSO_4.7H_2O$ was used as a standard diluted at concentrations of 0, 200, 400, 600, 800, and 1000 μmol/L, and the results of the sample was reported in μmol $FeSO_4$ equivalent/g dry matter (Prangdimurti *et al.,* 2025).

ABTS radical cation scavenging activity antioxidant assay

The antioxidant activity assay used ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) reagent and was conducted according to Prangdimurti *et al.* (2025). Reagent preparation began with the formation of ABTS cation radicals by mixing 7 mM ABTS and 2.45 mM potassium persulfate (1:1, v/v). The mixture was incubated at ambient temperature in the dark for 12 h. After that, the ABTS reagent was adjusted using distilled water to obtain an absorbance of approximately 0.70±0.02 at a wavelength of 734 nm. Testing of bay leaves samples was conducted by mixing 300 μL of sample with 2.7 mL of ABTS reagent, then incubated at 30 °C for 30 min. Spectrophotometric measurements were conducted at 734 nm. The standard used was trolox diluted at concentrations of 0, 12.5, 25, 50, 62.5, 75, 87.5, 100 mg/L.

Phytochemical profiling of Indonesian bay leaf extract using UHPLC-HRMS

The analysis method was based on Nora *et al.* (2025) which uses a UHPLC-HRMS instrument. A total of 10 mL of PD, PG, or PI of the bay leaves were concentrated to approximately 1 mL under reduced pressure using a rotary evaporator at 60 °C. The concentrated aliquots will be dissolved in methanol (1:10, v/v) and filtered using a 0.2 *μ*m nylon membrane. Then, 2 *μ*L of the sample were injected into UHPLC-HRMS (Thermo Scientific, Germany) using a C18 column (100 × 2.1 mm, 1.5 *μ*m) at a flow rate of 0.2 mL/min. The elution solvent used were water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), with a gradient of 0–1 min (5%B), 1–25 min (5-95%B), 25–28 min (95%B), 28–33 min (5%B). The analysis was performed at a column temperature of 30 °C and an injection volume of 2.0 *μ*L. The detector used was an MS/MS detector (ESI-Q Orbitrap) with ion mass scan range of 100–1500 m/z at negative ionization modes. Compounds were identified through comparison of their fragmentation patterns of ions detected in the chromatogram of the analysis results with databases from ChemSpider, mzCloud, and compared to online database such as PubChem and FoodDB. Final data presented in relative peak area.

Bioaccessibility index (BI)

Evaluation of the changes experienced by phenolic compounds, flavonoids, and antioxidants throughout simulated digestion was performed by calculating the post-gastric bioaccessibility index (BI PG) and post-gastrointestinal bioaccessibility index (BI PI) calculated according to Equations 1 and 2 as follows.

$$\text{BI PG (\%)} = \frac{B}{A} \times 100 \quad \ldots\ldots\ldots\

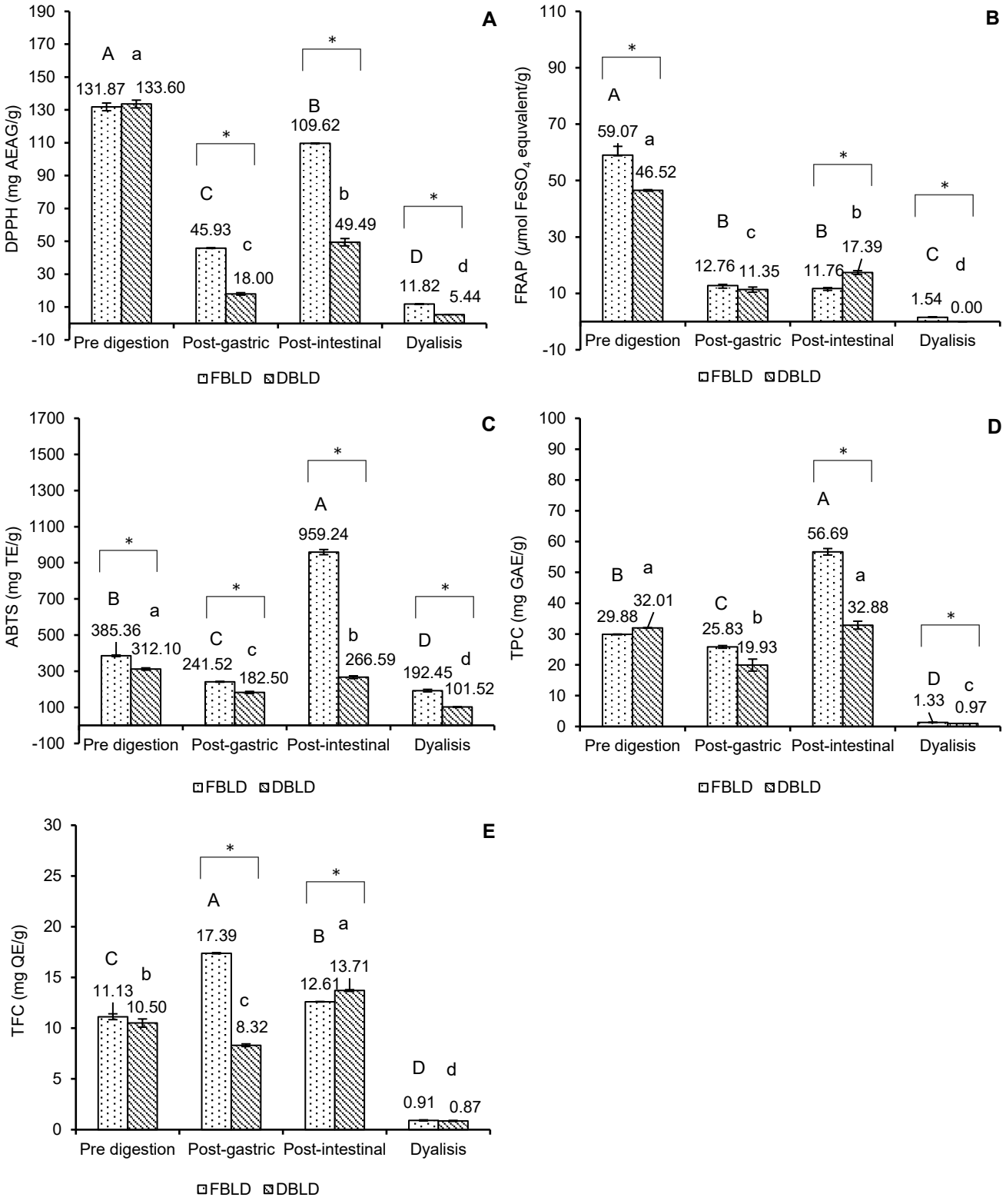


Figure 1. Antioxidant capacity (DPPH, FRAP, and ABTS), total phenolic content (TPC), and total flavonoid content (TFC) of fresh (FBLD) and dried bay leaf decoctions (DBLD) before and after *in vitro* digestion. Different uppercase and lowercase letters indicate significant differences among digestion phases for FBLD and DBLD, respectively, while an asterisk (*) indicates a significant difference between FBLD and DBLD within the same phase (p<0.05). Results are expressed on a dry basis. Error bars represent ± standard deviations

During drying, the reduction in moisture content can alter plant tissue structures and the interactions between phenolic compounds and cell wall components. Such changes may facilitate the release of certain phenolic compounds, while simultaneous heat exposure can lead to the degradation or transformation of heat-sensitive compounds. Consequently, the final phenolic content reflects a balance between release and degradation processes during drying.

The use of moderate drying temperatures and the characteristics of fluidized-bed drying, which facilitate efficient heat and mass transfer, likely limited excessive heat exposure, thereby minimizing compound degradation. Furthermore, the reduction in water activity during drying may inhibit enzymatic reactions that could otherwise lead to the degradation of phenolic compounds. These conditions likely contributed to the relative preservation of TPC and TFC values in dried bay leaf decoctions compared to fresh bay leaves. Nevertheless, the decrease in FRAP and ABTS values in dried samples suggests that drying can affect specific antioxidant components or alter the ability of these compounds to donate electrons and scavenge free radicals, even though such changes are not reflected in the total TPC and TFC values.

Pujiastuti and Saputri (2019) reported that drying at around 50 °C yields relatively high phenolic content, although this is not always directly associated with antioxidant capacity. In contrast, prolonged drying at lower temperatures (28–30 °C) may induce enzymatic degradation of phenolic compounds. Fluidized bed drying is a technique that utilizes a stream of hot air to create fluid-like movement of the material, allowing more uniform and efficient drying (Manfaati *et al*., 2019; Mardiah *et al.*, 2012). This method enables relatively rapid and homogeneous heat transfer to the leaves, even at moderate temperatures. These findings indicate that most phenolic and flavonoid compounds in bay leaf decoctions remain stable under drying treatment. However, certain non phenolic antioxidant components that are more sensitive to heat may experience structural modifications, which could contribute to a reduction in the overall antioxidant capacity. At the same time, the application of thermal treatment at this temperature may lead to the inactivation of polyphenol oxidase, thereby preventing the enzymatic oxidation of phenolic compounds during the drying process (Gu *et al.* 2018). Bernard *et al.* (2014) reported that sun-drying significantly reduced the total phenolic and flavonoid contents of Ceylon cinnamon (*Cinnamomum zeylanicum*) compared with fresh samples.

Antioxidant capacity, total phenolic content (TPC), and total flavonoid content (TFC) after simulated digestion

After simulated gastrointestinal digestion, the antioxidant capacity of FBLD decreased, as indicated by DPPH (109.62 mg AEAC/g; 17% reduction) and FRAP (11.76 μmol $FeSO_4$ equivalent/g; 80% reduction) (Figure 1). In DBLD, all measures of antioxidant capacity decreased, with reductions ranging from 15 to 63%. In contrast, the ABTS antioxidant capacity of FBLD increased markedly after digestion, reaching almost 2.5-fold the value observed before digestion. The TPC and TFC of both FBLD and DBLD did not decrease after simulated digestion. The ABTS assay provides a broad measure of antioxidant capacity and can respond to both hydrophilic and lipophilic antioxidants. The marked decrease in FRAP may be associated with changes in the chemical structure or micro-environment of antioxidant compounds, resulting in a reduced ability to donate electrons.

The results showed that drying affected antioxidant capacity and TPC after simulated gastrointestinal digestion (Figure 1). Following the gastric and intestinal digestion phases, decoctions prepared from FBLD generally exhibited higher DPPH antioxidant activity (109.62 vs. 49.49 mg AEAC/g; 55% higher) and ABTS antioxidant capacity (959.24 vs. 266.59 mg TE/g; 72% higher) than those prepared from DBLD. FBLD also showed a higher TPC than DBLD (56.69 vs. 32.88 mg GAE/g; 42% higher). However, different trends were observed for FRAP and TFC after gastrointestinal digestion, with DBLD exhibiting higher values than FBLD by 48 and 8.7%, respectively.

The drying process, particularly high temperatures and prolonged drying duration, may cause damage to certain phenolic compounds and result in a reduction in antioxidant capacity (Deng *et al*., 2019; Zielińska *et al*., 2018). This statement is consistent with the present findings, in which most antioxidant activity values of dried bay leaf samples were lower than those of fresh leaves. Previous findings by Prangdimurti *et al.* (2025) also reported a similar digestion dependent pattern in both fresh and dried *pulai* leaf preparations, where antioxidant activity and phenolic related parameters decreased during the gastric phase and subsequently increased during the intestinal phase. This trend was accompanied by higher bioaccessibility in fresh samples compared to dried ones, indicating enhanced release of bioactive compounds following intestinal digestion.

In addition to the effects of drying, digestive conditions should also be taken into account. The increase in TPC and antioxidant capacity during the intestinal phase is likely associated with the progressive disintegration of the food matrix and enzymatic hydrolysis occurring throughout gastrointestinal digestion. Phenolic compounds in plant tissues may

exist in free forms or be associated with various macromolecular components within the food matrix. During digestion, changes in pH conditions and digestive enzyme activity can gradually alter these interactions, thereby enhancing the release and accessibility of compounds previously trapped or bound within the plant matrix. In the intestinal phase, the presence of pancreatin and bile salts can further accelerate matrix disintegration and facilitate the release of compounds associated with proteins, carbohydrates, and other matrix components.

Thus, the increase in TPC following the intestinal phase does not necessarily indicate the formation of new phenolic compounds. Instead, this rise likely reflects an increased recovery of phenolic compounds or other compounds capable of reacting with the Folin reagent after their release from the food matrix and/or transformation during digestion, while still eliciting a response in the analytical method employed. Similarly, the increase in antioxidant capacity may stem from the release of antioxidant compounds previously bound within the matrix or from the formation of digestion products possessing antioxidant activity. Differing response patterns observed in DPPH, FRAP, and ABTS assays suggest that the digestion process likely influences not only the quantity of extractable or detectable compounds but also the chemical characteristics and reactivity of the antioxidant compounds generated or released during the process.

This explanation is further supported by Bouayed *et al.* (2011), who observed elevated phenolic levels in apples following the gastrointestinal phase as a result of pancreatin-driven mechanisms facilitating matrix-bound phenolic release. This study showed a pattern similar to that reported for bidara leaf tea, in which total phenolic and flavonoid contents decreased after the gastric phase and subsequently increased following the small intestinal phase (Rialdi *et al.,* 2024). The *wedang uwuh* beverage exhibited its highest phenolic content and antioxidant activity prior to digestion when subjected to a 15 min boiling process. However, simulated gastrointestinal digestion led to a reduction in these parameters as a result of pH changes and enzymatic reactions. In contrast, following digestion, samples prepared with a shorter boiling time of 5 min showed enhanced phenolic levels, antioxidant capacity, and antioxidant bioaccessibility (Fauziah *et al.,* 2023). These findings indicate that extraction methods, plant species used, and interactions among ingredients play important roles in determining the bioaccessibility of antioxidant compounds.

A significant decrease in all analytical parameters was observed for both fresh and dried bay leaf decoctions after the dialysis phase. This decrease may be attributed, at least partly, to the diffusion of low-molecular-weight compounds from the dialysis bag into the surrounding medium. The dialysis tubing used in this study had a nominal molecular weight cut-off (MWCO) of 12 kDa, which enables the separation of compounds primarily according to their molecular size and ability to pass through the membrane (de Morais *et al*., 2020).

Bioaccessibility index (BI)

Bioaccessibility refers to the fraction of phenolic compounds or antioxidant compounds released from the food matrix into the gastrointestinal tract, thereby becoming available for gastrointestinal absorption (Duque-Soto *et al.,* 2022). The BI PG and BI PI of total phenolic content, flavonoid content, and total antioxidant capacity in bay leaf decoction are presented in Figure 2. The BI of antioxidant compounds is expressed based on their antioxidant capacity. BI PI for antioxidant capacity measured by the DPPH and FRAP assays were below 100%, indicating a reduction in the antioxidant capacity potentially available for absorption compared with the corresponding undigested samples. However, an exception was observed for the ABTS assay in FBLD, for which the BI PI did not decrease but instead increased to nearly 2.5-fold the initial value. A similar pattern was observed for TPC and TFC, with BI PI values exceeding 100% for both FBLD and DBLD. These findings suggest that the measured levels of potentially bioaccessible compounds were not reduced after digestion and may reflect enhanced release or recovery of compounds, or the formation of digestion-derived products that remained detectable in the respective analytical assays.

Phenolic compounds are highly sensitive to pH and digestive enzymes. Qie *et al.* (2021) reported that catechin loss in tea was greater under gastrointestinal pH conditions than under gastric pH conditions. Changes in pH during digestion can affect the stability of phenolic compounds, partly by altering their chemical structure. Several studies have reported that most phenolic compounds are unstable at near neutral to alkaline pH, whereas others exhibit greater stability under acidic conditions (Pasquet *et al.,* 2024). In contrast to these findings, the increases in BI PG and BI PI observed in the present study exhibited patterns that differed from those reported previously. This discrepancy may be attributed to the nature of flavonoids, which are widely distributed in fruits and vegetables predominantly as glycosides bound to sugar moieties such as glucose. These glycosylated forms are known to exhibit greater stability during digestion, particularly under gastric conditions (Panche *et al*., 2016; Xie *et al.,* 2022). In this study, BI PG values in nearly all assays were lower than the corresponding BI PI values, indicating that most antioxidant compounds in bay leaf decoctions were released after the gastrointestinal phase.

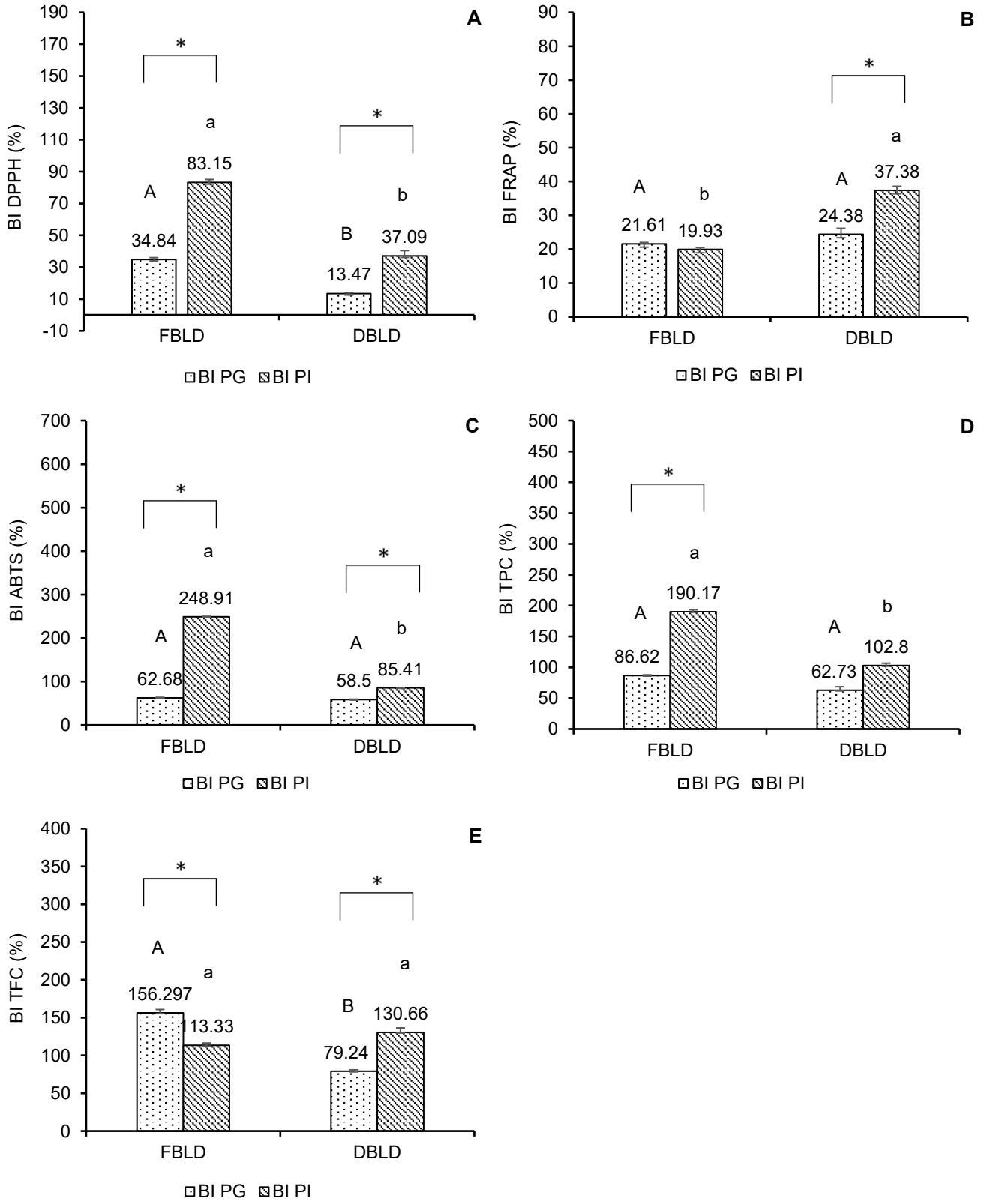


Figure 2. Bioaccessibility index of antioxidant activity, total phenolic content, and total flavonoid content of FBLD and DBLD. (A) DPPH, (B) FRAP, (C) ABTS, (D) TPC, and (E) TFC. Uppercase and lowercase letters indicate significant differences between FBLD and DBLD at the gastric (PG) and intestinal (PI) phases, respectively, while an asterisk indicates a significant difference between PG and PI within the same sample (p<0.05). Error bars represent ± standard deviations

The observed enhancement in total phenolic content following the gastrointestinal phase can be associated with the enzymatic conditions applied during the digestion simulation. Specifically, the presence of pancreatin and lipase, together with the catalytic actions of amylase and protease, promotes the liberation of phenolic compounds that were previously bound within the food matrix (Paz-Yépez *et al.,* 2019; Sęczyk *et al*., 2021). Extended exposure of food matrices to elevated temperatures may accelerate the degradation of phenolic compounds and antioxidant constituents, thereby increasing their vulnerability to further breakdown during digestion. Similarly, earlier research on roselle petals extraction has indicated that higher temperatures lead to a reduction in antioxidant activity (Suzery *et al.,* 2020). Comparable results have indicated that food product products subjected to thermal processing exhibit lower gastrointestinal-phase bioaccessibility of phenolic compounds than those processed using non-thermal methods (Pérez-Burillo *et al*., 2018). Heat processing can therefore affect the stability of phenolic compounds, leading to a reduction in antioxidant activity (Ashokkumar *et al.,* 2021). Moreover, thermal processing may modify the chemical structure of phenolic compounds, resulting in either enhanced or reduced bioaccessibility by facilitating their release from the food matrix, promoting chemical degradation, or inducing polymerization reactions (Ribas-Agustí *et al*., 2018; Sęczyk *et al*., 2021). Human intervention studies have identified numerous phenolic metabolites in plasma and urine following the consumption of fruit juices (Castello *et al.,* 2020). Phenolic compounds that are not absorbed in their native form in the small intestine can be transported to the colon, where they undergo further biotransformation by the gut microbiota into a range of catabolic metabolites.

The ABTS assay yielded the highest gastrointestinal-phase bioaccessibility index and antioxidant capacity compared with the DPPH and FRAP assays. Structural changes in phenolic and antioxidant compounds, together with digestive processes, may induce interactions among sample constituents, thereby influencing the detection of antioxidant activity when using similar or more sensitive analytical methods (Ma *et al.,* 2020). Apak *et al.* (2016) also reported that the ABTS assay exhibits greater sensitivity than DPPH for food samples with hydrophilic and lipophilic characteristics and high pigment content. Phenolic compounds that remain bound to the food matrix may become available for gastrointestinal absorption after undergoing extraction and metabolic transformation during digestion (Dall'Asta *et al*., 2012; Ribas-Agusti *et al.,* 2018). Furthermore, Wu *et al.* (2023), in their study on the bioaccessibility of fermented coffee beans antioxidants, demonstrated that digestion can significantly enhance antioxidant activity associated with phenolic bioaccessibility.

Phytochemical profile of Indonesian bay leaf extract

The profiles of compound groups in decoctions prepared from fresh and dried bay leaves at each analyzed phase, as determined by UHPLC–HRMS, are presented in Table 1. Based on their classification, the detected compounds were grouped into six categories: organic acids, simple phenolics, flavonoids, other bioactive compounds, carbohydrates, and unknown compounds. Identification of compounds in FBLD and DBLD before and after gastric phase using UHPLC-HRMS in Figure 3 revealed that most of the detected compound belonged to the antioxidant organic acids and simple phenolics groups. The analysis also showed most detected compounds belonged to unknown group after the gastrointestinal phase. The organic acid antioxidant group comprised fumaric acid, DL-malic acid, citric acid, and quinnic acid. The simple phenolic group included gentisic acid, gallic acid, and 5-(3′,4′,5′-trihydroxyphenyl)-*γ*-valerolactone-O-methyl-O-sulfate. Flavonoids such as cianidanol, gallocatechin, quercitrin, and isoquercetin were also detected. In the group of other bioactive compounds, 2-C-methylerythritol 4-phosphate and catalposide were identified. D-(+)-galactose was also detected and classified under the carbohydrate group.

In the post-gastrointestinal phase, only 3–6 identified compounds are visible on the chromatogram (Figure 4 E–F). Certain compounds, such as gallic acid and isoquercetin, are no longer detected in the post-gastrointestinal phase. The numerous peaks appearing within the 11–16 mins range of the post-intestinal phase correspond to bile salt derivatives, such as glycocholic acid, taurocholic acid, taurochenodeoxycholic acid, glycochenodeoxycholic acid (Lin *et al*., 2021). Several compounds detected in the samples could not be identified and were therefore labeled as unknown, totaling two compounds. These unknown compounds require further investigation to elucidate their chemical identities and potential biological activities. The presence of these unknown compounds is presumed to be associated with processing and digestive transformations, as they were predominantly detected in the gastrointestinal-phase bay leaf decoctions.

In addition, several detected signals could not be confidently assigned to specific compounds and were therefore classified as unidentified compounds. These signals were predominantly detected in the post-gastrointestinal phase, indicating changes in the chemical profile of bay leaf decoctions following simulated digestion.

Table 1. Identified compounds and relative intensity of fresh bay leaves decoction (FBLD) and dried bay leaves decoction (DBLD) before and after *in vitro* gastrointestinal digestion analyzed by UHPLC-HRMS in negative ionization mode

Compound	Molecular Formula	Molecular Mass	RT (min)	Relative Intensity (%)					
				FBLD			DBLD		
				PD	PG	PI	PD	PG	PI
Antioxidant organic acids									
Fumaric acid	$C_4H_4O_4$	116.00980	1.60	0	1.49	0	1.33	0.34	0
DL-Malic acid	$C_4H_6O_5$	134.02055	1.21	27.80	19.99	7.46	18.82	13.76	8.41
Citric acid	$C_6H_8O_7$	192.02628	1.23; 1.45	7.33	2.48	4.69	11.30	14.51	7.92
Quinnic acid (1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid)	$C_7H_{12}O_6$	192.06243	1.14	4.95	2.66	0	3.09	2.44	1.46
Subtotal antioxidant organic acids				40.08	26.63	12.15	34.54	31.05	17.79
Simple phenolics									
Gentisic acid	$C_7H_6O_4$	154.02549	1.13	0	0	0	0.94	0	0
Gallic acid	$C_7H_6O_5$	170.02070	2.00	11.14	12.69	0	15.41	18.75	0
5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone-O-methyl-O-sulphate	$C_{12}H_{14}O_9S$	334.03574	10.48; 8.99	31.80	34.06	0	29.52	25.54	23.05
Subtotal simple phenolicss				42.94	46.75	0	45.87	44.29	23.05
Flavonoids									
Cianidanol	$C_{15}H_{14}O_6$	290.07843	6.26; 7.26	0	2.27	0	1.48	1.23	0
(+)-Gallocatechin	$C_{15}H_{14}O_7$	306.07343	3.75	0	1.92	0	1.80	0.76	0
Quercitrin	$C_{21}H_{20}O_{11}$	448.09954	9.47	1.17	0.87	0	1.02	0.62	0.02
Isoquercetin	$C_{21}H_{20}O_{12}$	464.09482	8.63	15.81	15.95	0	9.92	12.20	0
Subtotal flavonoids				16.98	21.00	0	14.22	14.81	0.02
Other bioactive compounds									
2-C-methylerythritol 4-phosphate	$C_5H_{13}O_7P$	216.03944	1.10	0	3.18	0	1.63	7.63	4.51
Catalposide	$C_{22}H_{26}O_{12}$	482.14140	10.03	0	0	0	0	0.16	0
Subtotal other bioactive compounds				0	3.18	0	1.63	7.78	4.51
Carbohydrates									
D-(+)-Galactose	$C_6H_{12}O_6$	180.06236	1.15	0	0.80	0	1.65	0.63	0
Subtotal carbohydrates				0	0.80	0	1.65	0.63	0
Unknown compounds									
Unknown 1	$C_{12}H_{18}O_7S$	306.07737	6.65	0	1.64	0	2.09	1.43	1.13
Unknown 2	$C_{25}H_{42}O_7$	454.29244	14.57	0	0	87.85	0	0	53.50
Subtotal unknown compounds				0	1.64	87.85	2.09	1.43	54.63

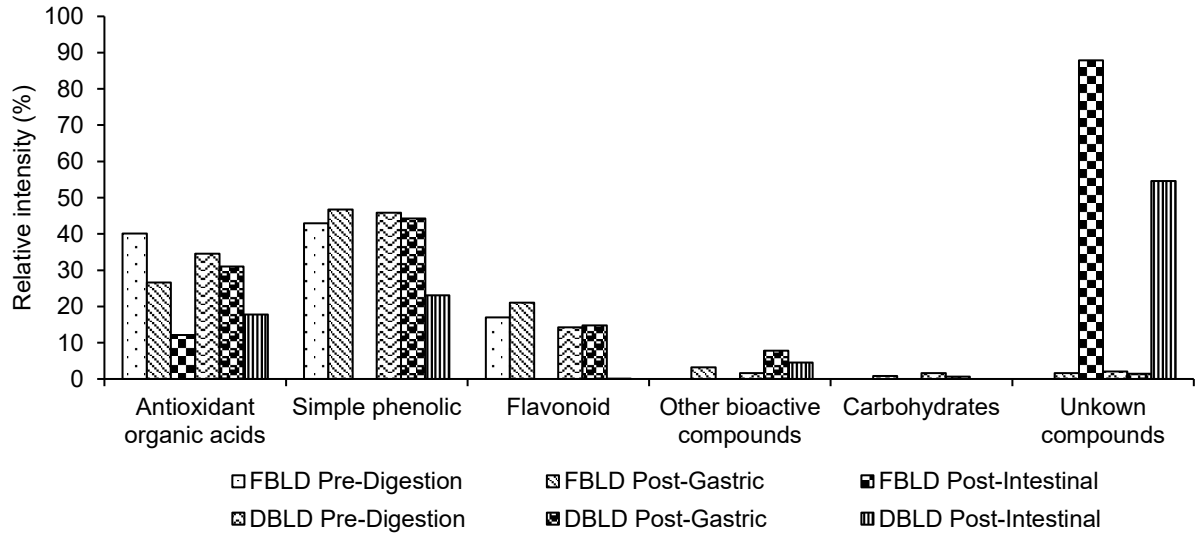
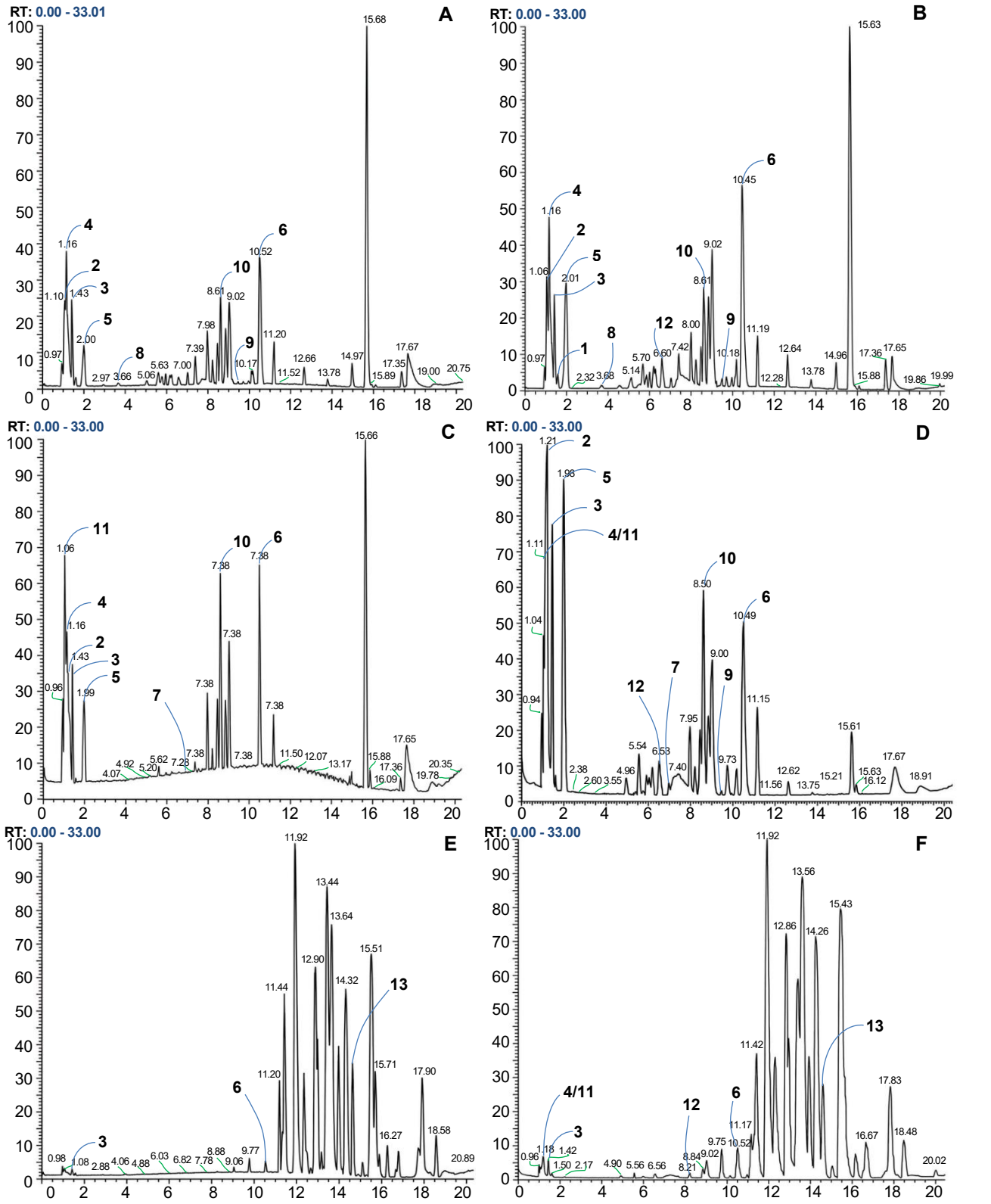


Figure 3. Relative intensities (%) of compound groups identified in fresh bay leaves decoction (FBLD) and dried bay leaves decoction (DBLD) before and after simulated gastrointestinal digestion using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry in negative ionization mode



Note: 1= Fumaric acid; 2= DL-Malic acid; 3= Citric acid; 4= Quinnic acid (1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid); 5= Gallic acid; 6= 5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone-O-methyl-O-sulphate; 7= Cianidanol; 8= (+)-Gallocatechin; 9= Quercitrin; 10= Isoquercetin; 11= 2-C-methylerythritol 4-phosphate; 12= Unknown 1; 13= Unknown 2; FBLD= fresh bay leaf decoctions; DBLD= dried bay leaf decoctions; retention time (RT): 0.00–33.00 min (x-axis)

Figure 4. Chromatograms in negative ionization mode for (A) FBLD pre-digestion, (B) DBLD pre-digestion, (C) FBLD post-gastric, (D) DBLD post-gastric, (E) FBLD post-gastrointestinal, and (F) DBLD post-gastrointestinal

However, because their chemical identities could not be definitively established based on the available UHPLC-HRMS data and database comparison, no specific structural transformation or formation mechanism can be concluded. Further targeted analyses and structural confirmation would be required to elucidate the identity and origin of these compounds.

CONCLUSION

This study presents information on the quantity of bioactive compounds released during digestion and available for absorption from bay leaf infusions following simulated gastrointestinal digestion. Fresh bay leaf decoction (FBLD) generally exhibited higher initial antioxidant capacity and phytochemical content compared to dried bay leaf decoction (DBLD). Bioaccessibility values exceeding 100% in the small intestinal phase were observed for ABTS antioxidant capacity (specifically for FBLD), total phenolic content (TPC) and total flavonoid content (TFC). Further UHPLC-HRMS analysis revealed extensive transformation of phenolic and flavonoid compounds during gastrointestinal digestion. These findings suggest that the choice between fresh and dried bay leaves in developing functional beverages. Further studies for *in vivo* validation are required to determine whether the compounds are truly absorbed and exert biological effects *in vivo*.

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