



## Phytochemical Characterization and Bioactivity of Compounds Present in the Infusion of *Artemisia Herba-Alba*

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### KEYWORDS

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### ABSTRACT:

Medicinal plants, known for their chemical compounds with pharmacological effects, have been a global and ancient method of treatment. Among them, the genus *Artemisia* (Asteraceae), with over 300 species, stands out for its therapeutic use throughout history. *Artemisia herba-alba*, in particular, has long been valued for its effectiveness in treating digestive issues and other ailments, gaining increasing attention for its biological and chemical properties. However, despite their proven effectiveness, probiotics often face challenges in surviving the harsh conditions of the digestive system, highlighting the need for new strategies to enhance their viability and beneficial effects. One promising approach is the integration of probiotics into plant matrices, such as *Artemisia herba-alba* infusions, which can help preserve the microorganisms and improve the bioavailability of bioactive compounds. This study aims to evaluate the combined effects of an infusion containing ultrabiotic probiotics and *Artemisia herba-alba* on microbial survival, the bioavailability of active substances, and their post-digestion antioxidant and anti-inflammatory properties. The research is based on a comprehensive review of the literature on plant matrices, probiotics, and *Artemisia*, followed by experimental validation of these findings. The results offer promising opportunities for enhancing medicinal applications and provide deeper insights into the interactions between probiotics and plant matrices.

### Introduction

Medicinal plants have long been essential to human therapeutic practices due to their ability to produce chemicals with a wide range of pharmacological effects. Among these, the genus *Artemisia* from the Asteraceae family stands out for its abundance of secondary metabolites and its numerous medicinal, culinary, and pharmacological applications. This genus, which encompasses over 400 species classified into four main sections, is highly valued for its

biological properties, including antibacterial, antimalarial, anti-inflammatory, and antidiabetic activities. Notably, *Artemisia herba-alba*, commonly known as "mountain thyme" or "desert wormwood," has been widely used in various traditional healing practices, particularly in North Africa and the Middle East. Its bioactive potential has also been the subject of extensive scientific investigation in multiple contexts.



The bioactive compounds found in *A. herba-alba*, including flavonoids, polyphenols, essential oils, and tannins, contribute significantly to its medicinal properties. This species is known for its ability to suppress inflammatory responses, reduce lipid peroxidation, and inhibit free radical formation, making it a promising candidate for the development of new therapeutic and commercial strategies. However, while extracts and infusions of this plant have demonstrated promising biological activities, innovative methods for enhancing its effectiveness could unlock further potential (El-Lakany et al., 2019; Varela et al., 2020).

Probiotics—live microorganisms that confer health benefits when consumed in adequate amounts—are also critical for regulating immune responses and maintaining the balance of the gut microbiota (Zhao et al., 2021; Liu et al., 2022). Despite their potential, their survival and efficacy in the gastrointestinal tract are hindered by harsh environmental factors such as bile salts and stomach acid (Sun et al., 2018). One promising approach to overcome these limitations is the integration of probiotics into modified food matrices, particularly those derived from plants. These bioactive-rich matrices not only protect the probiotics but also synergize with them, enhancing their beneficial effects (Luo et al., 2021; Singh et al., 2023).

## Experimental section:

### 1. Apparatus

All experiments were conducted from March to May 2024 in the biology laboratory at the Faculty of Natural and Life Sciences, Amar Thlidji University, Laghouat. The probiotic complex *Ultrabiotique Immune Defence System*, purchased from a pharmacy, which contains four bacterial strains (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus plantarum*, and *Bifidobacterium breve*), was one of the biological components used in the study. *Artemisia herba-alba*, collected from the Laghouat region, was used as the plant matrix. In vitro adhesion assays were carried out using laboratory-prepared chicken epithelial cells. Enzymes such as pepsin (2000 U/ml),  $\alpha$ -amylase (75 U/ml), and pancreatin (100 U/ml) were also used. Various chemicals and reagents were required, including salts such as potassium chloride (KCl at 0.5 M), sodium chloride

(NaCl at 2 M), ammonium bicarbonate ( $\text{NH}_4(\text{CO}_3)_2$  at 0.5 M), sodium carbonate ( $\text{Na}_2\text{CO}_3$  at 7.5%), and magnesium chloride hexahydrate ( $\text{MgCl}_2(\text{H}_2\text{O})_6$  at 0.15 M). Phosphate-buffered saline (PBS) and phosphate buffers were utilized, along with acids like trichloroacetic acid (TCA), ascorbic acid, gallic acid, HCl (6 M), and NaOH (1 M). Additional reagents included DPPH, hydrogen peroxide, bile salts, and 10% Folin-Ciocalteu.

A variety of instruments were used, including a heated magnetic stirrer, autoclave, Pasteur oven, drying oven, electronic, analytical, and precision balances, Sigma centrifuge, UV-VIS UV-1900i spectrophotometer, vortex, pH meter, refrigerator, optical microscope, rotary evaporator, and heating mantle. This equipment enabled the successful completion of all the experimental procedures.

## 2. Methods

### 2.1 Preparation of the mugwort infusion and phytochemical analyses

The preparation of the infusion followed the method adapted from Jilani et al. (2015). One gram of *Artemisia herba-alba*, collected from the Laghouat region, was immersed in 200 ml of boiling distilled water (100°C) for ten minutes without further heating. The resulting infusion was allowed to cool to room temperature before being used for testing. The total polyphenol and flavonoid content of the infusion were determined through phytochemical analysis. Polyphenols were quantified using the Folin-Ciocalteu method, with the absorbance measured at 765 nm after incubation with 7% sodium carbonate. Flavonoids were quantified using the method described by Jilani et al. (2015), by reacting with 2% aluminium chloride and measuring the absorbance at 430 nm, using quercetin as the reference standard.

### 2.2 Antioxidant activity

The infusion's antioxidant activity was evaluated using three methods: iron reducing power (FRAP), DPPH radical scavenging assay, and total antioxidant capacity (TAC). For the TAC test, the infusion was incubated with a reagent mixture containing sulphuric acid, sodium phosphate, and ammonium molybdate, and absorbance was measured at 695 nm. The DPPH test, used to assess the capacity of the infusion to



neutralize DPPH free radicals, was performed by measuring absorbance at 517 nm. The FRAP test evaluated the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> at 700 nm. The human red blood cell membrane stabilization (HRBC) assay was used to assess the anti-inflammatory properties of the infusion. A cell suspension was created by centrifuging fresh blood mixed with Alsever's solution. Membrane stability was measured using spectrophotometry at 560 nm after incubation with the infusion at 56°C.

### 2.3 Evaluation of probiotic properties in vitro

The probiotic strains (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus plantarum*, and *Bifidobacterium breve*) were assessed for their resistance to acidic pH, tolerance to bile salts, hydrophobicity of cell surfaces, self-aggregation, co-aggregation, and adhesion to epithelial cells. Optical density measurements at 620 nm were taken after incubating the strains in media mimicking intestinal and stomach conditions for the pH and bile salt resistance tests. Self-aggregation and co-aggregation assays were performed to examine the ability of the strains to combine with each other or with pathogens like *Escherichia coli*. Hydrophobicity was evaluated using organic solvents such as xylene, toluene, and chloroform.

### 2.4 Survival and bioavailability under conditions simulated to that of the digestive tract

The viability of probiotic strains and *Artemisia herba-alba* infusions was evaluated in an in vitro digestive system that simulated the oral, gastric, and intestinal stages. The simulated solutions were prepared according to the method described by Valerio et al. (2020). Following each stage, MRS agar count and spectrophotometric readings were used to assess the probiotic strains' viability. The bioavailability of flavonoids and phenolic compounds was also evaluated at each stage using the previously mentioned techniques. The anti-inflammatory and antioxidant properties of the infusions, both individually and in combination with the probiotic strains, were assessed following each stage of digestion using the TAC, DPPH, FRAP, and HRBC tests.

## 3. Findings and discussion

### 3.1 Mugwort infusion's quality and biological activity

#### 3.1.1. Composition of phytochemicals

The total polyphenol content of the mugwort infusion was calculated using the gallic acid calibration curve ( $y = 0.0264x - 0.0025$ ;  $R^2 = 0.99$ ) and was found to be  $50.525 \pm 0.48$  mg EAG/g. These results are in agreement with previous studies, such as those by Brown et al. (2019), which found that the polyphenol content varies depending on plant species, solvents, and environmental factors (Lupsor et al., 2019; Milutinovi et al., 2021). Ethanol extracts typically contain higher levels of polyphenols, suggesting that the extraction method significantly affects the bioactive compounds. The flavonoid concentration, calculated to be  $13.08 \pm 0.005$  mg EQ/g, was lower than values reported in other studies (Bouyahya et al., 2017).

#### 3.1.2. Antioxidant activity

Using the ascorbic acid calibration curve ( $y = 0.0029x + 0.0986$ ;  $R^2 = 0.96$ ), the total antioxidant capacity of the infusion was found to be  $105.265 \pm 0.46$  µg EAA/ml. The phenolic compounds and flavonoids in the infusion likely contribute to this activity by acting as hydrogen or electron donors, preventing peroxide formation and reducing oxidative damage (Hejina et al., 2021). The correlation between polyphenol concentration and antioxidant activity supports the use of these compounds as rapid markers for evaluating antioxidant effectiveness. The DPPH radical scavenging assay demonstrated that the infusion could neutralize  $94 \pm 0.01\%$  of DPPH radicals. This result reflects the ability of flavonoids and polyphenols to donate hydrogen atoms, neutralizing free radicals (Bougandoura et al., 2013). The FRAP assay measured the reduction of ferricyanide (Fe<sup>3+</sup>) to its ferrous form (Fe<sup>2+</sup>), yielding a result of  $115.965 \pm 1.09$  mg/g, indicating strong reducing power and confirming the antioxidant potential of the infusion.

#### 3.1.3. Anti-inflammatory activity of the infusion

The anti-inflammatory activity of the infusion was evaluated using the HRBC assay, which measures the ability of the infusion to stabilize red blood cell

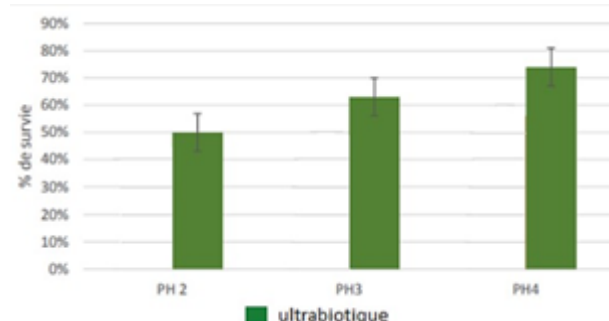


membranes and reduce hemolysis. The infusion demonstrated a  $50 \pm 0.001\%$  inhibition of hemolysis, indicating strong anti-inflammatory properties. This activity is likely due to the polyphenolic compounds in *A. herba-alba*, which are known to reduce the synthesis and release of pro-inflammatory cytokines (Hejina et al., 2021).

### 3.2 Evaluation of the probiotic properties of Ultrabiotique in vitro

#### 3.2.1. Resistance to gastric pH

For probiotic strains to be effective in the gastrointestinal tract, they must be able to endure in acidic environments (Rajam et al., 2022). According to our findings, the survival rate gradually drops as the pH drops: it was  $74 \pm 0.007\%$  at pH 4,  $63 \pm 0.006\%$  at pH 3, and  $50 \pm 0.001\%$  at pH 2. These findings are similar to those of Joghataei et al. (2019), who found that *Lactobacillus* strains exposed to acidic stomach conditions had survival rates ranging from 21% to 96% (Rajam et al., 2022).



**Figure 9: Survival rate of ultrabiotics at different pH levels**

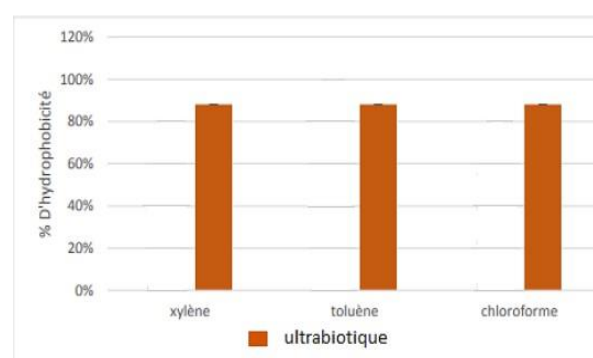
#### 3.2.2. Resistance to bile salts

Bile salts, which are found in the human gut at quantities of 0.3% to 0.5%, provide an additional threat to the survival of probiotic strains (Chen et al., 2020). In a medium that contained 0.3% bile salts, ultrabiotic bacteria had a high survival rate, calculated at  $82 \pm 0.001\%$ , replicating duodenal conditions (Chen et al., 2020).

#### 3.2.3. Hydrophobicity of cell surfaces

The strains' adherence to polar and apolar solvents—specifically, xylene, toluene, and chloroform—was measured in order to determine how hydrophobic they

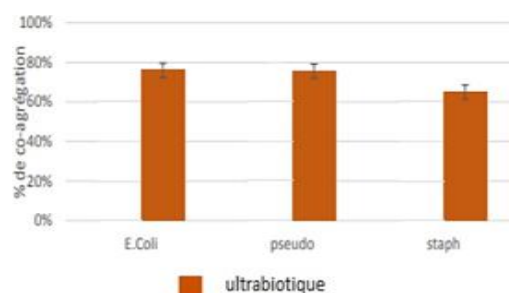
were. All three solvents had a consistent hydrophobicity rate of  $88 \pm 0.01\%$ , according to the data. The proteins and polysaccharides on the cell surface of Ultrabiotic strains contribute to their high hydrophobicity, as evidenced by their strong affinity for both polar and apolar solvents (Gohil et al., 2023). Chloroform, an acidic polar solvent, indicates the capacity of cells to donate electrons, whereas xylene, an apolar solvent, is used to evaluate general hydrophobicity (Ren et al., 2012).



**Figure 10: Hydrophobicity (%) of the ultrabiotic strain**

#### 3.2.4. Self-aggregation and co-aggregation

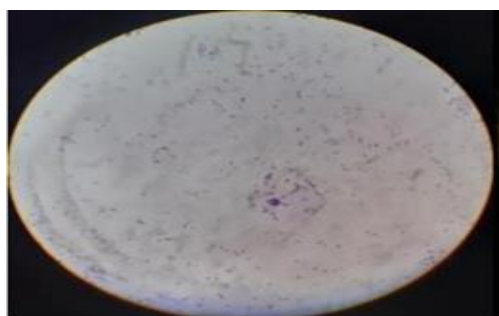
While co-aggregation restricts colonisation by pathogenic bacteria, self-aggregation of probiotic strains promotes intestine colonisation (Isenring et al., 2021; Singhal et al., 2021). The self-aggregation rate of ultrabiotic strains was  $66 \pm 0.001\%$ , which was greater than the 13% to 63% range reported by Sohn et al. (2020). These findings point to a strong colonisation ability, which is most likely related to the strains' surface characteristics, such as hydrophobic and electrostatic interactions (Bah et al., 2019). Ultrabiotique demonstrated high rates of co-aggregation with *Pseudomonas aeruginosa* ( $76 \pm 0.02\%$ ), *Staphylococcus aureus* ( $65 \pm 0.02\%$ ), and *Escherichia coli* ( $76 \pm 0.07\%$ ). These findings are consistent with those of Anandharaj et al. (2015), who showed that *Lactobacillus* strains and different pathogens co-aggregated significantly at rates ranging from 19% to 68%. This characteristic helps to modify the gut flora and improves pathogen inhibition.



**Figure 11: Co-aggregation (%) of ultrabiotics with test samples.**

### 3.2.5. Ability to adhere to epithelial cells

Probiotic strains' capacity to stick to epithelial cells is essential for intestinal colonisation and pathogen competitive exclusion, both of which enhance host health (Ren et al., 2012; Guo et al., 2023; Lebeer et al., 2008; Wells et al., 2010; Sánchez et al., 2017). Microscopic analysis (figure 5) demonstrated that Ultrabiotic cells adhered significantly; if there were more than 15 adhesions, the test was deemed successful. The bacterial cell surface and the epithelial membranes interact chemically and physicochemically during this intricate adhesion process, which is impacted by Van der Waals forces and electrostatic interactions. Research has also demonstrated that the surrounding environment and the extracellular makeup of bacteria might influence this adhesion process (De Melo Pereira et al., 2018; Bermudez-Brito et al., 2012; Zárate & Nader-Macías, 2006; Kainulainen & Korhonen, 2014).



**Figure 12: Photomicrograph of ultrabiotic adhesion to chicken epithelial cells (Gx100).**

### 3.3 Ultrabiotique's antioxidant action

Two methods were used to evaluate the antioxidant activity of Ultrabiotique strains in vitro: iron reduction capacity (FRAP) and DPPH free radical scavenging. The findings indicate a notable resistance to oxidative stress, with a DPPH free radical scavenging percentage of  $84 \pm 0.008\%$  (Wang et al., 2022; Gill & Prasad, 2008; Pieniz et al., 2014). Teichoic acid and peptidoglycan, two bioactive substances found in probiotic cell walls that can lower oxidative stress, are responsible for this effect (Won et al., 2021; Amaretti et al., 2013; Wu et al., 2014; Sah et al., 2014).

### 3.4 Impact of oral-gastrointestinal digestion on mugwort infusion

#### 3.4.1. Impact on polyphenol and flavonoid content

The bioaccessibility of the phenolic and flavonoid components in the mugwort infusion was significantly impacted by oral-gastrointestinal digestion. In comparison to the control ( $50.525 \pm 0.48$  mg EAG/g), the polyphenol content dropped to  $14.75 \pm 0.48$  mg EAG/g after the oral phase, and to  $13.955 \pm 0.31$  mg EAG/g following the stomach phase, and to  $8.425 \pm 0.10$  mg EAG/g after the intestinal phase. Following intestinal digestion, flavonoids also decreased, going from  $2.65 \pm 0.05$  mg EQ/g to  $1.285 \pm 0.04$  mg EQ/g. The reason for these losses is because polyphenols and flavonoids are sensitive to digestive circumstances such as pH changes, reactive oxygen species, and interactions with digestive enzymes like pepsin, proteases, and  $\alpha$ -amylase. Furthermore, structural alterations that impact their bioavailability and bioactive qualities may result from the shift from the acidic gastric environment to the alkaline intestine environment (Catalkaya et al., 2020; Tagliazucchi et al., 2010; Bouayed et al., 2011; D'Archivio et al., 2010; Scalbert & Williamson, 2000; Pérez-Jiménez et al., 2013).

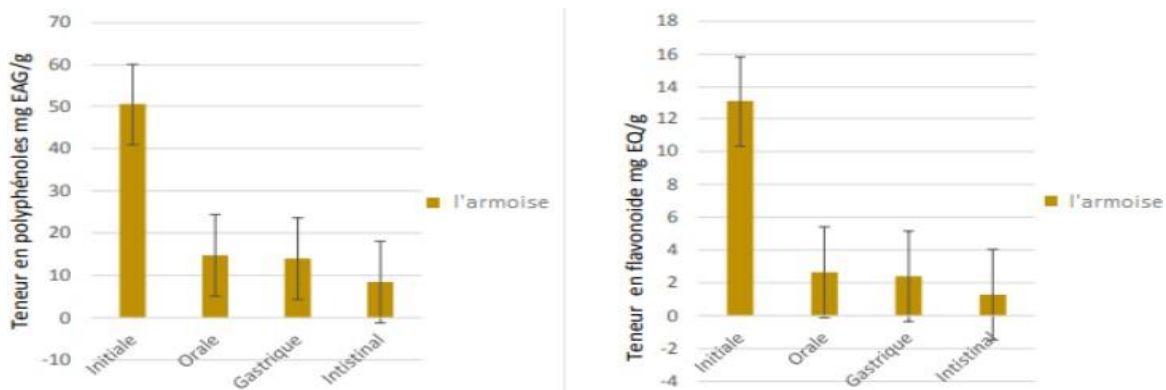


Figure 13: Effect of digestion on polyphenol and flavonoid content

### 3.4.2. Impact on antioxidant activity

Throughout the various stages of digestion, the mugwort infusion's antioxidant activity dramatically dropped. From 105.265 mg EAA/g before digestion, the total antioxidant capacity decreased to  $48.025 \pm 3.4$  mg EAA/g following the oral phase, and to  $37.33 \pm 0.9$  mg EAA/g after the gastric phase, and to  $2.505 \pm 1.4$  mg EAA/g after the intestinal phase. Iron reducing capacity (115.965 mg EAA/g before digestion, decreasing to  $98.145 \pm 4.17$  mg EAA/g,  $88.89 \pm 3.66$  mg EAA/g, and  $20.74 \pm 0.5$  mg EAA/g)

and DPPH free radical scavenging activity ( $94\%$  before digestion, decreasing to  $67 \pm 0.01\%$ ,  $34 \pm 0.04\%$ , and  $14 \pm 0.02\%$  respectively) also showed a similar decline. These declines are most likely caused by polyphenols' instability in digestive environments, namely pH changes and sub-alkaline oxidation processes, which change their molecular makeup and, in turn, their antioxidant capacity (Laib et al., 2021; Tagliazucchi et al., 2010; Bermudez-Brito et al., 2012; D'Archivio et al., 2010; Bouayed & Bohn, 2010; Pérez-Jiménez et al., 2013).

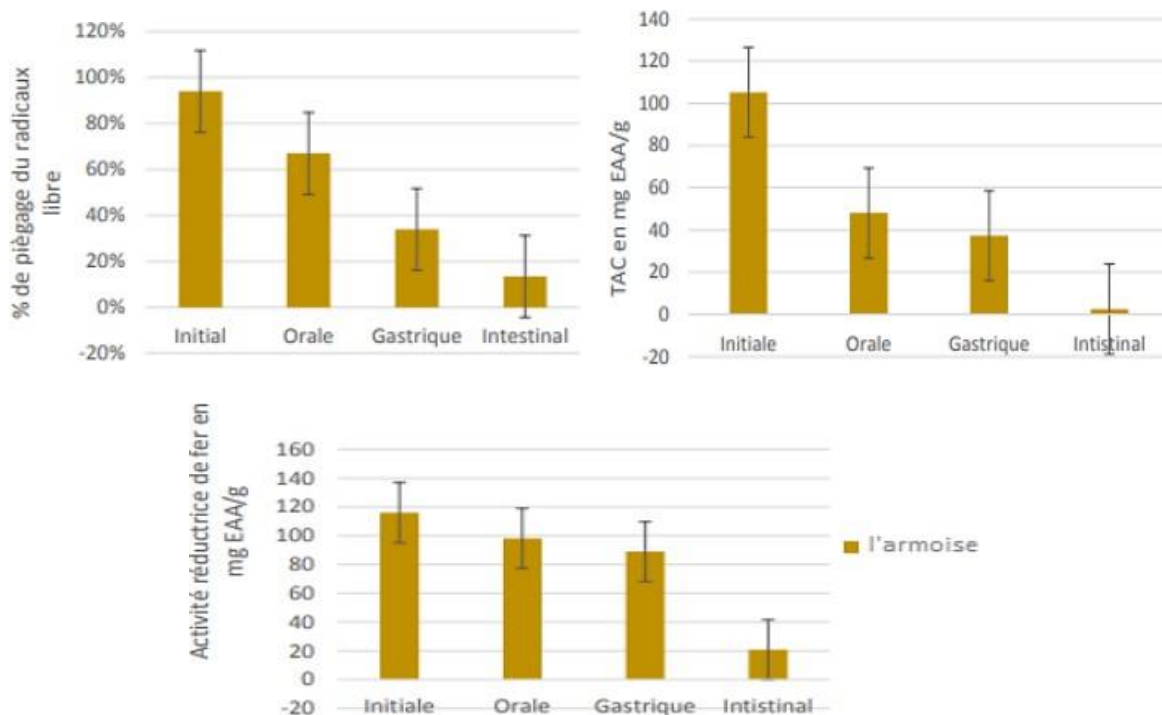


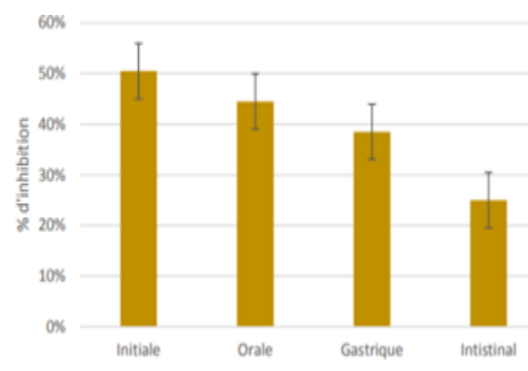
Figure 14: Effect of digestion on TAC, PDPH radical inhibition percentage and iron reducing power



These findings emphasise the significance of phenolic compounds' bioaccessibility and bioavailability, which are impacted by digestive circumstances and enzymatic interactions. Mugwort infusion has substantial residual action, indicating possible utility in functional applications, even though its antioxidant qualities decrease with digestion.

### 3.4.3. Anti-inflammatory activity

Following the various stages of oral-gastrointestinal digestion, the anti-inflammatory efficacy of the mugwort infusion was assessed. This activity gradually decreased from  $51 \pm 0.001\%$  prior to digestion to  $45 \pm 0.001\%$  following oral digestion, and to  $39 \pm 0.001\%$  following gastric digestion, and to  $25 \pm 0.001\%$  following intestinal digestion, according to the results. These findings demonstrate that digestion has a detrimental impact on the infusion's anti-inflammatory qualities, most likely as a result of the active ingredients' breakdown by digestive enzymes and fluctuating pH levels (D'Archivio et al., 2010; Bouayed & Bohn, 2010; Cătălina et al., 2019; Pérez-Jiménez et al., 2013; Henning et al., 2011).

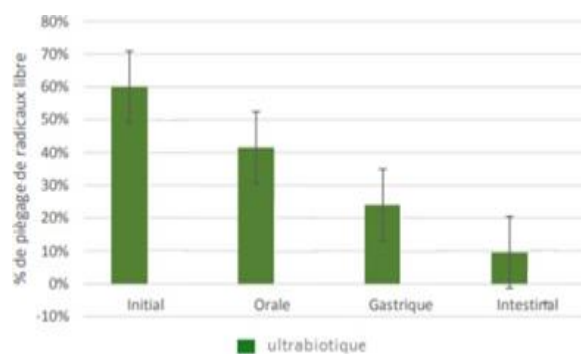


**Figure 15: Effect of digestion on anti-inflammatory activity**

### 3.5 Impact of oral-gastrointestinal digestion on Ultrabiotique

After two minutes of incubation, the initial vitality of the ultrabiotic bacteria was  $6.94 \times 10^9$  CFU/mL, demonstrating good resistance to oral digesting conditions. However, following the intestinal ( $0.1785 \times 10^9$  CFU/mL) and stomach ( $0.7675 \times 10^9$  CFU/mL) stages, a notable decline in the quantity of viable cells was noted, suggesting that the hostile environment had an impact on their survival. These findings

demonstrate how sensitive the strains are to bile salts and acidic environments as compared to the control ( $45.5 \times 10^9$  CFU/mL) (Mandal et al., 2016; Rajam et al., 2022; Ouwehand et al., 2016; Marteau et al., 1997; do Carmo et al., 2018).



**Figure 16: Effect of treatment on the percentage of inhibition of the DPPH radical**

The DPPH radical scavenging test was used to evaluate how digestion affected Ultrabiotique's antioxidant activity. The free radical inhibition rate gradually decreased from  $60 \pm 0.001\%$  before digestion to  $42 \pm 0.007\%$  following oral digestion, and to  $24 \pm 0.014\%$  following gastric digestion, and  $10 \pm 0.007\%$  following intestinal digestion, according to the data. This decrease indicates a notable decline in antioxidant capacity, most likely brought on by the active components' instability during digestion.

### 3.6 Impact of digestion on the 'mugwort infusion - Ultrabiotic' combination

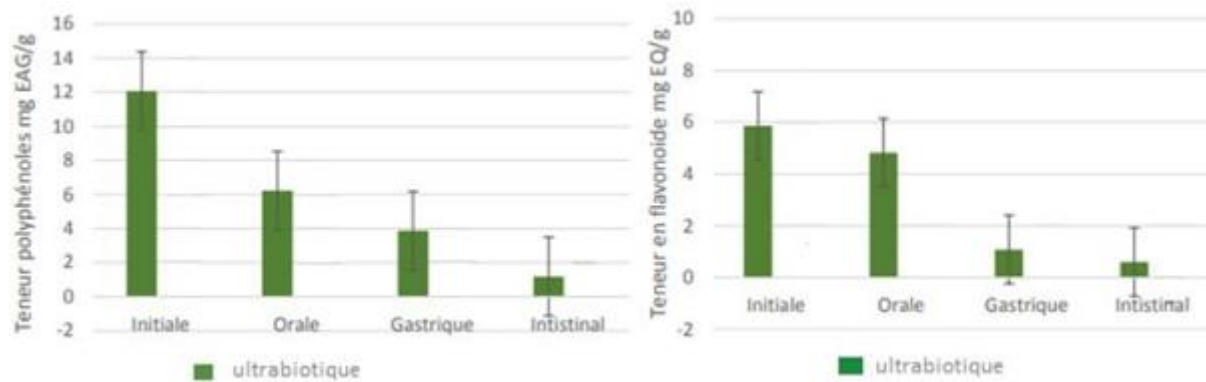
#### 3.6.1. Bioavailability of polyphenols and flavonoids

Following each digestion phase, the infusion-steep combination's polyphenol and flavonoid content decreased, according to the results. Following the oral phase, the levels of polyphenols decreased from  $12.06 \pm 0.11$  mg EAG/g (control) to  $6.22 \pm 0.31$  mg EAG/g,  $3.87 \pm 0.10$  mg EAG/g, and  $1.19 \pm 0.05$  mg EAG/g following the stomach phase. With levels of  $5.865 \pm 0.4$  mg EQ/g (control),  $4.83 \pm 0.02$  mg EQ/g (oral phase),  $1.085 \pm 0.007$  mg EQ/g (gastric phase), and  $0.6 \pm 0.05$  mg EQ/g (intestinal phase), a similar pattern was noted for flavonoids. These findings imply that the bioavailability of phenolic compounds is impacted by the combination of probiotic strains and infusion, most likely as a result of their enzymatic breakdown or transformation during digestion



(Tagliacruzchi et al., 2010; Cătălina et al., 2019; Henning et al., 2021; González-Barrio et al., 2023;

Pérez-Jiménez et al., 2013).

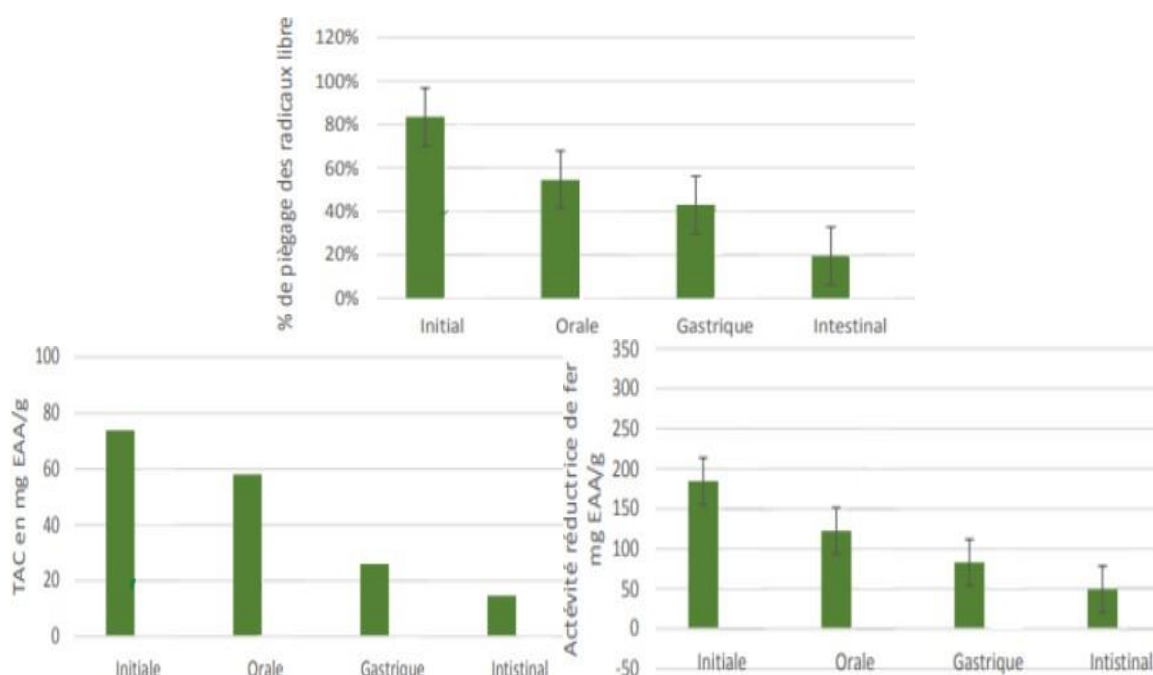


**Figure 17: Effect of digestion on polyphenol and flavonoid content**

### 3.6.2. Antioxidant activity

The TAC, FRAP, and DPPH tests were used to assess the combination's antioxidant activity. The findings indicate that following the several stages of digestion, the infusion-strain combination maintains a greater level of antioxidant activity than the infusion alone. TAC, for instance, rose from  $70.851 \pm 0.22$  mg EAA/g before digestion to  $54.92 \pm 2.90$  mg EAA/g following the oral phase, and to  $24.718 \pm 0.52$  mg

EAA/g following the gastric phase, and to  $13.995 \pm 3.5$  mg EAA/g following the intestinal phase. These numbers continue to be greater than those derived by infusion alone. The synergistic effect of probiotic strains on the conservation of antioxidant activity was confirmed by the same patterns seen for free radical scavenging activity (DPPH) and iron reduction capacity (FRAP) (Zhang et al., 2022; Wang et al., 2021; Ouwehand et al., 2016; González-Barrio et al., 2023; Henning et al., 2021).



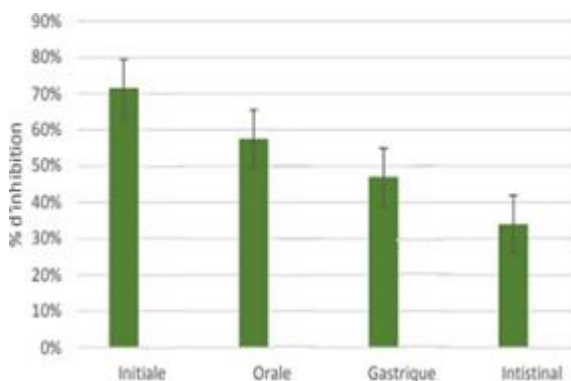
**Figure 18: Effect of digestion on PDPH percentage, iron-reducing power and total antioxidant activity**



### 3.6.3. Anti-inflammatory activity

Following the digestion phases, the infusion-strain combination's anti-inflammatory effectiveness also improved as compared to infusion alone. Before digestion, the values were  $55 \pm 0.006\%$ ; and  $47 \pm 0.007\%$  after the oral phase, and were  $41 \pm 0.005\%$  after the gastric phase, and  $36 \pm 0.0001\%$  after the intestinal phase.

These findings support earlier findings that probiotics and medicinal plants can enhance the anti-inflammatory effect (Hevia et al., 2021; Lee et al., 2024; Markowiak et al., 2023; Wang et al., 2023; García-Cordero et al., 2022).

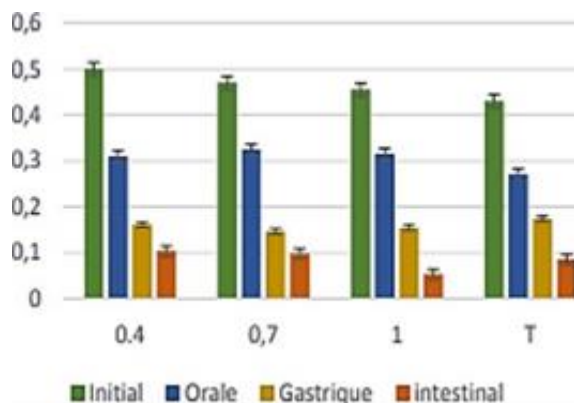


**Figure 19: Impact of digestion on the anti-inflammatory activity of the combination**

### 3.7 H<sub>2</sub>O<sub>2</sub> scavenging activity

The H<sub>2</sub>O<sub>2</sub> scavenging activity of the infusion-strain combination gradually decreased throughout the different digestion phases. For instance, the activity decreased from  $0.31 \pm 0.001$  (control) to  $0.1515 \pm 0.001$  after the oral phase,  $0.1035 \pm 0.004$  after the gastric phase, and  $0.091 \pm 0.005$  after the intestinal phase in the presence of 0.4% H<sub>2</sub>O<sub>2</sub>. Similar patterns were observed for H<sub>2</sub>O<sub>2</sub> concentrations of 0.68% and 1%, indicating a reduction in effectiveness under digestive conditions. This reduction in scavenging activity during digestion has also been observed in other studies. For example, research on cocoa shell flour and extract has demonstrated that gastrointestinal digestion can influence antioxidant capacity and radical scavenging activities, including H<sub>2</sub>O<sub>2</sub> scavenging, due to the transformation and degradation of phenolic compounds during digestion (González-Barrio et al., 2023). Similarly, studies on

synthesized coumarin compounds have shown varying degrees of H<sub>2</sub>O<sub>2</sub> scavenging activity, highlighting the importance of chemical structure in antioxidant potential (Al-Amiery et al., 2015).



**Figure 20: Impact of digestion on H<sub>2</sub>O<sub>2</sub> scavenging activity of the combination**

### Conclusion

The combination of probiotic preparations and mugwort has been shown to be effective in improving their properties as they pass through the intestinal tract. The main objective of this study was to evaluate the synergistic effect of this combination under simulated conditions, and the results obtained confirm its potential.

Analyses have shown that mugwort is rich in bioactive compounds, including polyphenols and flavonoids, which explains its excellent biological activities.

On the other hand, the Ultrabiotic strain has shown remarkable probiotic properties, such as significant tolerance to gastric pH, resistance to bile salts (0.3%), high hydrophobicity, aggregation and co-aggregation capacities, as well as adhesion to epithelial cells. Antioxidant activity, as measured by the free radical scavenging capacity of DPPH, was confirmed both before and after digestion.

By integrating probiotics with an infusion of mugwort into a system simulating gastrointestinal digestion, the results showed effective survival of Ultrabiotic strains, although some losses of bioactive compounds were noted. The combination resulted in increased antioxidant and anti-inflammatory activities, confirming that this combination can be a versatile



solution to protect probiotic strains against hostile conditions in the digestive tract while enhancing their health benefits.

## Perspectives

In light of the results obtained, several perspectives open up to expand this work:

- Developing preservation methods: Develop encapsulation techniques to protect probiotic strains and bioactive compounds in mugwort in the digestive system.
- Evaluating in vivo effects: Conduct in vivo studies to confirm the survival, viability, and antioxidant and anti-inflammatory profiles of this combination under real-world physiological conditions.
- Creating functional foods: Harness this combination to develop new functional food products, such as probiotic-enriched infusions, to combat inflammatory and oxidative diseases.

These perspectives offer promising opportunities to enhance this combinatorial approach and develop innovative solutions in the field of health and nutrition.

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