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Levying evidence of the impact of Triphala in the mildly constipated human colon microbiota

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ABSTRACT

Triphala is a wildly used nutritional and phytotherapeutic formulation combining three dry fruits (*Terminalia bellirica*, *Terminalia chebula* and *Emblica officinalis*). Despite its long-standing use to treat gastrointestinal discomfort, there is limited understanding of Triphala's effects on gut microbiota, especially among individuals with mild constipation. Thus, this study aimed to investigate Triphala's impact on the constipated human colon microbiota. A short-term (72 h) static configuration of the *in vitro* Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) system was used to study the fermentation process of a standardized extract of Triphala in the gut microbiota. Chromatographic and enzymatic methods were used to analyze the microbe-derived metabolic production. Potential herb-human host interactions were assessed using *in vitro* cell-based methods. Triphala extract increased *Akkermansia muciniphila* but decreased *Bifidobacterium spp.* in the simulated colon microbiota. Metabolic profiling of Triphala treatment showed increased phenolic species and antioxidant potential and reduced ammonia, valeric, isovaleric, and isobutyric acids during fermentation, potentially benefiting intestinal health, especially in contexts of constipation. Fermentation metabolites enhanced transepithelial electrical resistance in a human epithelium model and inhibited aryl hydrocarbon receptor (AhR) transcriptional activity. Triphala's polyphenols likely cause this AhR antagonism. Overall, these findings state some potential explanations for the usefulness of Triphala as a natural remedy for gastrointestinal diseases. However, it also raises concerns about some harmful effects of Triphala in the gut microbial ecosystem of people suffering from mild constipation.

1. Introduction

Triphala is a traditional polyherbal remedy that has been used for centuries in Ayurvedic medicine, which is a traditional system of medicine in India. The word "Triphala" comes from Sanskrit and means "three fruits," as the formulation is made from a combination of three dried fruits. One of them is amalaki (*Emblica officinalis*) also known as Indian gooseberry. This fruit is rich in vitamin C and antioxidants (Scartezzini et al., 2006) and has shown immunomodulatory (Sai Ram et al., 2002) and gastroprotective effects (Al-Rehaily et al., 2002). The second one, bibhitaki (*Terminalia bellirica*) is a fruit known for having anti-inflammatory effects (Jayesh et al., 2020), radical scavenging activity and antibacterial activity against multidrug-resistant bacteria (Dharmaratne et al., 2018). Moreover, the calcium channel antagonism

Abbreviations: AhR, aryl hydrocarbon receptor; BA, biogenic amines; BCFA, branched chain fatty acids; CH223191, 1-Methyl-N-[2-methyl-4-[2-(2-methylphenyl) diazenyl]phen yl-1H-pyrazole-5-carboxamide; CYP, cytochrome P450; LDH, lactate dehydrogenase; GAE, gallic acid equivalents; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAOT, Power Antioxidant Total-Liquid® Technology; PS, Penicillin-Streptomycin; RPC, relative to positive control; SHIME®, Simulator of the Human Intestinal Microbial Ecosystem; TEER, transepithelial electrical resistance..

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and anticholinergic effects of *T. bellirica* fruits supported its traditional use in respiratory health as a bronchodilator and as an antispasmodic to treat digestive discomforts such as colic and diarrhea (Gilani et al., 2008). Finally, haritaki (*Terminalia chebula*) has shown antiulcerogenic activity (Sharma et al., 2011). Its cleansing effects on the gastrointestinal tract have long been investigated (Tamhane et al., 1997) and recently, multiple pharmacological studies have backed up its traditional use in other human diseases (Tiwari & Barooah, 2023). Thus, all three fruits making Triphala are historically used as herbal supplements and have demonstrated applicability to improve gastrointestinal health (Tarasiuk et al., 2018).

Intestinal constipation is a very common condition particularly affecting adult women (McCrea et al., 2009), but scarce therapeutic options are available other than the laxative drugs recommended for chronic states that in many cases have detrimental long-term colonic effects and potential carcinogenic risks (Noergaard et al., 2019). Commonly, phytomedicines are the therapy of choice in case of mild constipation (Iizuka & Hamamoto, 2015). Several studies have addressed the pharmacological applicability of Triphala formulations to improve gut motility and gastric emptying, being attractive alternatives to treat constipation (Munshi et al., 2011). Some modulatory effects of Triphala in the human gut microbiota of healthy subjects have been shown after dietary interventions with Triphala (Peterson et al., 2020). Moreover, a recent study pointed out some potential effects of a Triphala extract in a model of obese human adult microbiota (Kwandee et al., 2023). However, although Triphala's use in gastrointestinal health is one of the best known in Ayurvedic medicine (Peterson et al., 2017), its specific effects on constipation have not been reported.

Plant-based dietary supplements and phytomedicinal preparations that are not absorbed in the upper intestinal tract will impact the resident microbial communities in the colon (Dou et al., 2019). Though, as many traditionally used plants for gastrointestinal disorders, insufficient information is available on the impact of Triphala on the human colon microbiota (Thumann et al., 2019).

In this work, we aimed to provide evidence on the impact of a standardized dry extract of Triphala in the human colon microbiota of mildly constipated individuals by combining several *in vitro* methods to explore the complex interplay of the herb-gut microbiome-human host interactions. The production of several microbiota-derived metabolites such as short-chain fatty acids (*i.e.*, acetic acid, propionic acid, butyric acid, valeric acid), branched-chain fatty acids (*i.e.*, isopropionic acid, isobutyric acid), ammonia and biogenic amines was measured to assess the impact of the plant product on microbial metabolic activity. Furthermore, as oxidative stress impacts gut health by contributing to inflammation, barrier dysfunction, and microbial imbalance, the antioxidant capacity and the production of isoprostanes in the fermentation media were used to gain insight into how the plant product might influence the redox balance in the gut microbiota ecosystem. Changes in bacterial biomarkers (*e.g. Akkermansia muciniphila*, lactobacilli, bifidobacteria) contributed to developing a microbial profile of the constipated gut fermentation upon treatment with Triphala. Meanwhile, some potential interactions with the human host of the fermentation output from Triphala's treatment were analyzed in intestinal-derived cell lines.

2. Experimental section

2.1. Consumables, reagents, and human fecal samples

Consumable materials were obtained from ThermoFisher Scientific (Waltham, MA, USA), Greiner Bio-One (Vilvoorde, Belgium), and VWR International BV (Leuven, Belgium). Materials and reagents for fermentation experiments were all provided by Prodigest (Ghent, Belgium). Other chemical and biological reagents were supplied by Merck KGaA (Darmstadt, Germany) unless otherwise stated. Cell culture media and supplements were obtained from Greiner Bio-One or InvivoGen (Toulouse, France).

The committee of the University of Liège-CHU Hospital (File ID: 2020/293) approved the ethical dossier for the collection and use of human stool samples. Informed consent was signed by the three volunteer donors participating in this study. Bristol stool form scale was used to select these three mildly constipated (Type 2) individuals (Lewis & Heaton, 1997). The volunteer donors were female adults (33–44 years old) with no history of antibiotics, prebiotics, or probiotic consumption for at least 6 months before the study. A pool of fecal homogenate was prepared by mixing equal proportions from the three donors. Then, 30 mL of the pool were inoculated in each vessel and filled up to 600 mL using adult SHIME® fermentation media whose composition and preparation have been previously described (Goya-Jorge et al., 2023).

2.2. Plant material (Triphala dry extract)

Triphala dry extract was developed and provided by ORTIS SA. The extract was an equally proportioned mixture of three fruits (*Terminalia bellirica*, *Terminalia chebula* and *Emblica officinalis*) standardized to obtain a specific molecular profile including a tannin concentration higher than 10 %. Details on the quality control analyses of the dry extract of Triphala developed by Laboratories ORTIS SA. are provided in Table S1 (Supplementary Material).

2.3. Gut fermentation experimental design

The static-SHIME® system was set up for a 72 h- last fermentation in batches. Briefly, multiple parameters (*i.e.*, pH, temperature, anaerobiosis, constant agitation) were maintained in six vessels where the same fecal inoculum was added. In three of the fermenters, 0.5 g of a fine powder of Triphala dry extract was poured in a final volume of fermentation medium of 600 mL (approximately dose: 0.83 mg/mL). The remaining three vessels were kept as parallel controls. Sampling took place every 24 h and were stored (−20 °C) as pellets or supernatants until further analysis. A representation of the system is provided in Fig. S1 (Supplementary Material).

2.4. Analysis of microbe-derived metabolites

2.4.1. SCFA and BCFA by GC–MS

Short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) were directly determined in the supernatant of the fermentation samples without any previous centrifugation or filtration. Briefly, 25 µL of samples were mixed with the internal standard 2-methylvaleric acid (0.2 mg/mL), 15 µL of sulfuric acid (0.9 M) and water to complete 1 mL final volume. A validated method of solid-phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry (GC–MS) (Douny, Dufourny, et al., 2019) was used to determine SCFA and BCFA in the fermentation samples with the following quantification limits: acetic acid (2.44–121.95 mM), propionic acid (1.10–55.42 mM), butyric acid (0.80–40.18 mM), isobutyric acid (0.18–9.18 mM), isovaleric acid (0.14–6.92 mM), valeric acid (0.47–23.73 mM), and caproic acid (0.02–0.86 mM).

2.4.2. Biogenic amines and ammonia by UPLC-FLD

Samples were prepared as previously detailed (Goya-Jorge et al., 2022). Ultra-performance liquid chromatography was used with fluorescence detection (UPLC-FLD) following a previously validated method (Douny, Benmedjadi, et al., 2019) to determine biogenic amines (BA) with limits of quantification between 0.002 and 1.986 mM. From ten BA analyzed in control and treatment fermentation experiments every 24 h, six biogenic amines (*i.e.*, methylamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, and tyramine) were quantified. Ammonia production was analyzed only by the end of the fermentation period (72 h), in control and Triphala treatment experiments, following the same sample preparation procedure and chromatographic quantification method with limits of quantification between 0.235 and 32.88

mM.

2.4.3. Enzymatic determination of D- and L-lactic acids

Enzymatic UV tests were performed to detect D-lactate and L-lactate produced by the end of the fermentation (72 h) using an enzymatic kit from BioSenTec (Portet-sur-Garonne, France). A volume of 1 mL of fermentation supernatants was diluted in triethanolamine buffer (0.1 M) to reach a pH of 10 and protein precipitation was conducted using trichloroacetic acid (6.1 N) followed by centrifugation (3214 ×*g*, 20 min, 4 °C). Then, the manufacturer's instructions were followed for optical density (OD) measurements at 340 nm. Control assays were performed with standards to validate the method with detection limits between 0.33 and 2.22 mM.

2.4.4. Total phenols

Total polyphenols content was determined by the Folin-Ciocalteu method (Tabart et al., 2006). Briefly, 0.5 mL of filtered (0.45 μm) and centrifuged supernatant sample was mixed with 0.2 mL of Folin-Ciocalteu reagent. Three minutes later, 0.8 mL sodium carbonate 20 % (*w/v*) was added, and the mixture was heated at 100 °C for 1 min. After cooling, the absorbance was measured at 750 nm. Results were expressed as mg gallic acid equivalents per liter (GAE/L).

2.4.5. Isoprostanes

Fermentation samples were first mixed with 1 M potassium hydroxide and the internal standard (deuterated derivative ent-8-iso-15 (*S*)-Prostaglandin F2α-d9), then placed at 40 °C for 35 min in a thermostatic oven. To stop the hydrolysis, 11 % formic acid was added, and the resulting mixture was centrifuged at 30000 ×*g* for 5 min. The supernatant was then treated using a Novum MAX extraction plate from Phenomenex (Torrance, USA). The final extract was evaporated until dryness and reconstituted in a mixture of isopropanol, methanol, acetonitrile and water (25/25/25/25; *v*/v/*v*/v). Finally, 10 μL from each sample were injected.

15-F2t-isoprostane (Ent-8-iso-15(*S*)-Prostaglandin F2α) was determined following a previously validated protocol based on the coupling of ultra high-pressure liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) (Pincemail et al., 2017). The mass spectrometer was a Xevo™ TQ-S mass spectrometer from Waters (Milford, MA, USA) equipped with an electrospray ionization source (ESI) operating in negative ion mode and interfaced with a Waters Acquity UPLC I-Class inlet system. The chromatographic separation was achieved on Acquity UPLC® CSH™ C18 column (1.7 μm, 2.1 × 150 mm). The mobile phase contained a mixture of water and acetonitrile, both with acetic acid (0.1 %). The analytes were identified and quantified using multiple reactions monitoring mode. MassLynx v4.2 and TargetLynx v4.2 software were used for data acquisition and analyses. The limit of quantification of the method was 25 pg/mL.

2.4.6. Antioxidant capacity

The electrochemical Power Antioxidant Total-Liquid® Technology (PAOT) was used to determine the antioxidant capacity of the fermentation samples (20 μL) following a previously reported method (Pincemail et al., 2019). Results were expressed as PAOT scores using gallic acid (mg) equivalents per liter (GAE/L).

2.5. Gut microbial populations

DNA was extracted with the PSP® Spin Stool DNA Kit from Invitek Molecular GmbH (Berlin, Germany) from a 200 mg pellet of fermentation samples obtained by centrifugation (17,000 ×*g*, 5 min). DNA was diluted up to 4 ng/μL for qPCR reactions. Takyon™ No ROX SYBR master mix from Eurogentec (Seraing, Belgium) was used, and each reaction included: 10 μL of master mix, the oligonucleotide forward and reverse primers (see details in Table S2 of Supplementary Material), 2.5 μL of DNA or water for non-template control to reach 20 μL as final volume. Efficiency of the primers (90–110 %) was validated through calibration curves. Melt curves analysis was used for quality control. The amplification conditions were as follows: one cycle of denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 15 s, annealing temperature for 15 s, extension at 72 °C for 30 s and a final elongation step at 72 °C for 5 min. The optimal annealing temperatures and concentrations validated for the eleven primer pairs including ten target bacterial taxa and a total bacteria pair, are specified in Table S2. Results were processed as relative quantifications using the $2^{-\Delta\Delta Cq}$ method (Livak & Schmittgen, 2001) and relative to the inoculation moment (0 h) and the total bacteria sequence.

2.6. Intestinal cell-based experiments

AhR_HT29-Lucia™ cells were grown in DMEM supplemented with Fetal Bovine Serum (FBS, 10 %), PS (100 U/mL Penicillin G + 100 μg/mL de Streptomycin), Normocin™ (100 μg/mL) and Zeocin™ (100 μg/mL). Caco-2 cells and HT29-MTX cells were grown in DMEM supplemented with FBS (10 %) and PS. All cell lines were maintained in a humidified (5 %) incubator at 37 °C in flasks of 75 cm^2.

2.6.1. Cell culture inserts

For intestinal epithelial models, Caco-2 cells (1×10^5 cells/insert) and a 9:1 co-culture of Caco-2 (9×10^4 cells/insert) and HT29-MTX (1×10^4 cells/insert) were seeded in 12-well inserts, Ø pore = 1 μm (Thincerts®, Greiner Bio-One). Cells were allowed to grow for 21 days, during which the cell media in apical (500 μL) and basolateral (1 mL) compartments were replaced 3 times per week until obtaining a functional monolayer with a transepithelial electrical resistance (TEER) greater than 300 Ω.cm^2, as recommended (Hubatsch et al., 2007). The electrical resistance of the epithelial cell layers was checked regularly using the Millicell ERS-2 Voltohmmeter (Millipore, Merck KGaA). Apical compartments of the cell culture inserts were exposed for 24 h to filter-sterilized fermentation samples mixed with the culture medium in a 10 % dose of the final volume. Technical duplicates of the Caco-2/HT29-MTX cell inserts were exposed to the biological triplicated fermentation samples ($n = 6$). Measurements of the resistance were done before (0 h) and 24 h after adding the treatments. The net TEER (Ω·cm^2) was calculated by subtracting the measurement of a blank membrane and the resistance measured at 0 h time point, and this result was multiplied by the approximate growth area of the 12-well Thincerts® (1.13 cm^2).

2.6.2. AhR transcriptional activation

For measurements of AhR transcriptional activation, AhR_HT29-Lucia™ cells were seeded in 96wp at a density of 2×10^5 cells/mL, measured with the Scepter 3.0 cell counter from Merck KGaA. Supernatant from fermentation samples were filter sterilized (0.22 μm) and mixed with antibiotic-free cell medium. Two exposure doses were evaluated representing 10 % (20 μL of fermentation sample and 180 μL of cell medium) and 25 % (50 μL of fermentation sample and 150 μL of cell medium). Meanwhile, for dose-response curves of individual metabolites, at least five concentrations were tested. Results of AhR activation were expressed as fold induction for agonist tests. Antagonism of Triphala dry extract and the control antagonist compound 1-Methyl-N-[2-methyl-4-[2-(2-methylphenyl)diazenyl]phen yl-1H-pyrazole-5-carboxamide (CH223191) was measured by co-exposing cells to the EC_{50} of kynurenine determined through a dose-response curve (95 μM) and to the different dilutions of Triphala dry extract prepared in water. Results of this competitive antagonist test were expressed as percentages relative to the co-exposed positive control kynurenine (% RPC).

2.7. Statistical analysis

Statistical analysis was performed in GraphPad (San Antonio, CA, USA) (version 10.0.3). Normality of the data was assessed with Shapiro-Wilk tests and QQ-plots. When normality assumptions were met,

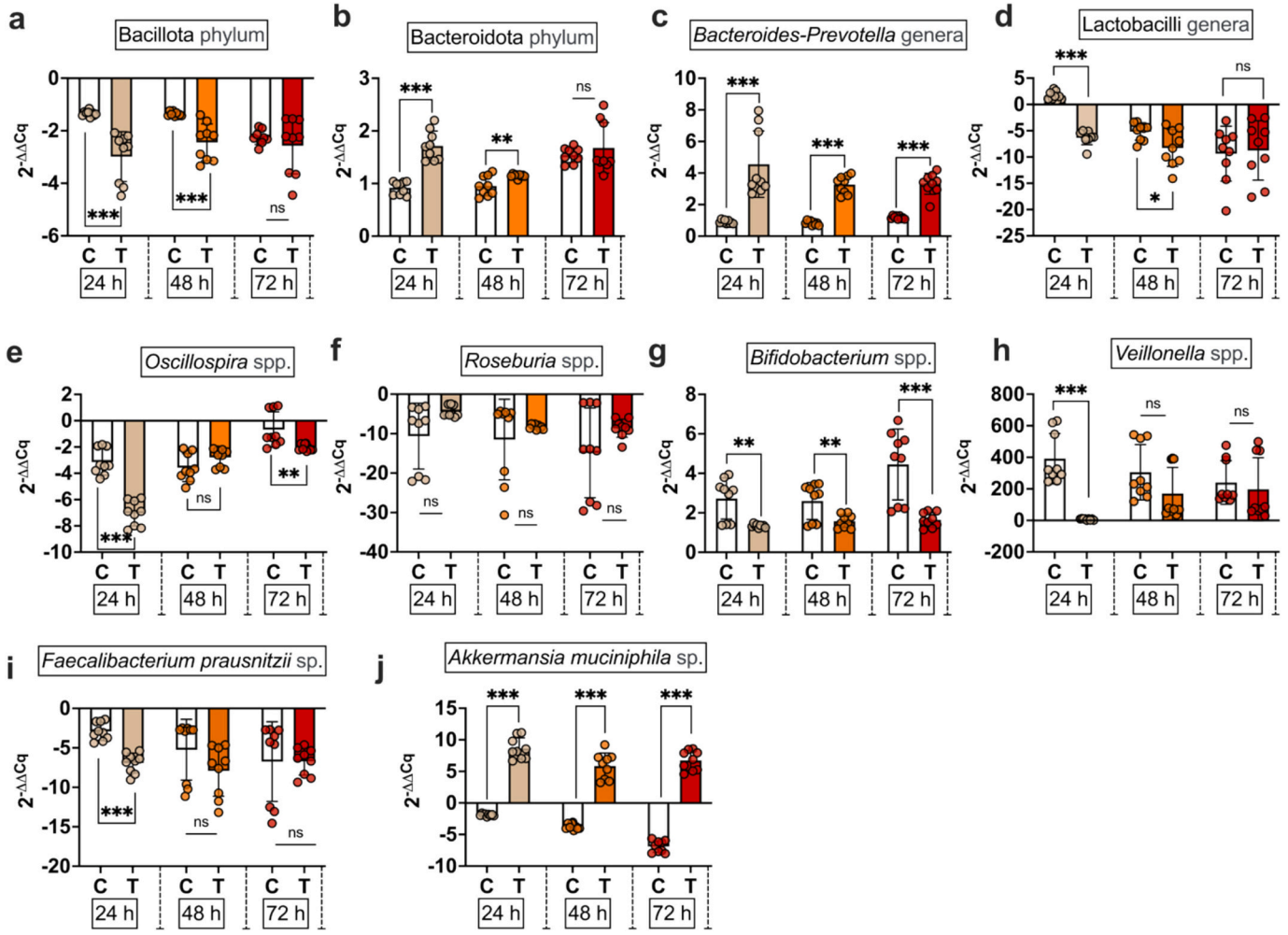

Fig. 1. Microbial profile in the static simulation of the human intestinal microbial ecosystem testing Triphala. **a-j**. Relative quantification ($2^{-\Delta\Delta Cq}$ method) of target bacterial taxa in the static-SHIME® by qPCR. Fold change values between zero and one are presented as their inverse (−1/X), indicating decreases compared to the inoculation sample (0 h).

comparisons were done using Unpaired Student *t*-tests. If assumptions were not met, non-parametric Mann-Whitney tests were performed. All experiments were performed at least in triplicate.

3. Results & discussion

Plant-based formulations can affect both the composition and functional dynamics of the gut microbial ecosystem, ultimately influencing digestive health (Ammar et al., 2023). The microbiota-host interactions may be particularly important when underlying pathological conditions affect both the host individual and its gut microbial populations (Gonza et al., 2024). Previous studies have approached the effects of Triphala extracts in the fecal microbiome in cases of obesity (Kwandee et al., 2023). Nevertheless, this is the first report on the impact of Triphala in the intestinal gut microbial ecosystem under mild constipation contexts, which was *in vitro* simulated using representative microbiota derived from three women suffering from this discomfort.

3.1. Herb-gut microbiome interactions

The microbial profile in the simulated human colon microbiota upon Triphala treatment was evaluated through several target bacterial taxa quantified daily on the fermentation samples (Fig. 1). Overall, Triphala did not seem to cause important disruptions in the target bacterial phyla by the end of the fermentation period (72 h). However, significant increases were observed in the Bacteroidota phylum 24 h after adding Triphala and, to a lesser extent, after 48 h of fermentation, when compared with the control experiment (Fig. 1b). On the contrary, Triphala caused a significant decrease in Bacillota phylum (Fig. 1a).

Akkermansia spp. is widely recognized as a key gut health biomarker associated with improved gut barrier integrity, reduced local and systemic inflammation, and important immunomodulatory effects (Panzetta & Valdivia, 2024). The significant increase of *Akkermansia muciniphila sp.* caused by Triphala (Fig. 1j) is consistent with recent studies (Upadhyay & Gupta, 2023) and supports the previously reported prebiotic action of this herbal product (Westfall et al., 2018). Enhancing the growth of *Akkermansia* in the mildly constipated gut microbiota could attribute to the plant extract a role in gut lining restoration (Reunanen et al., 2015) and in recovering intestinal health (Zhai et al., 2019).

Other health-promoting populations such as lactobacilli (Fig. 1d) and *Faecalibacterium prausnitzii* (Fig. 1i) seemed to be affected by the addition of Triphala, particularly at the beginning of the fermentation period. However, these populations and *Veillonella* genus (Fig. 1h) returned to similar levels to those of the control in the subsequent time points. Contrary, the reduction in *Bifidobacterium spp.* caused by Triphala was maintained during the 72-h fermentation, which could have a detrimental impact on constipation contexts (Mitelmão et al., 2021). The observed decrease in *Oscillospira* (Fig. 1e) caused by the treatment could have a positive health impact, as this genus has been related to the

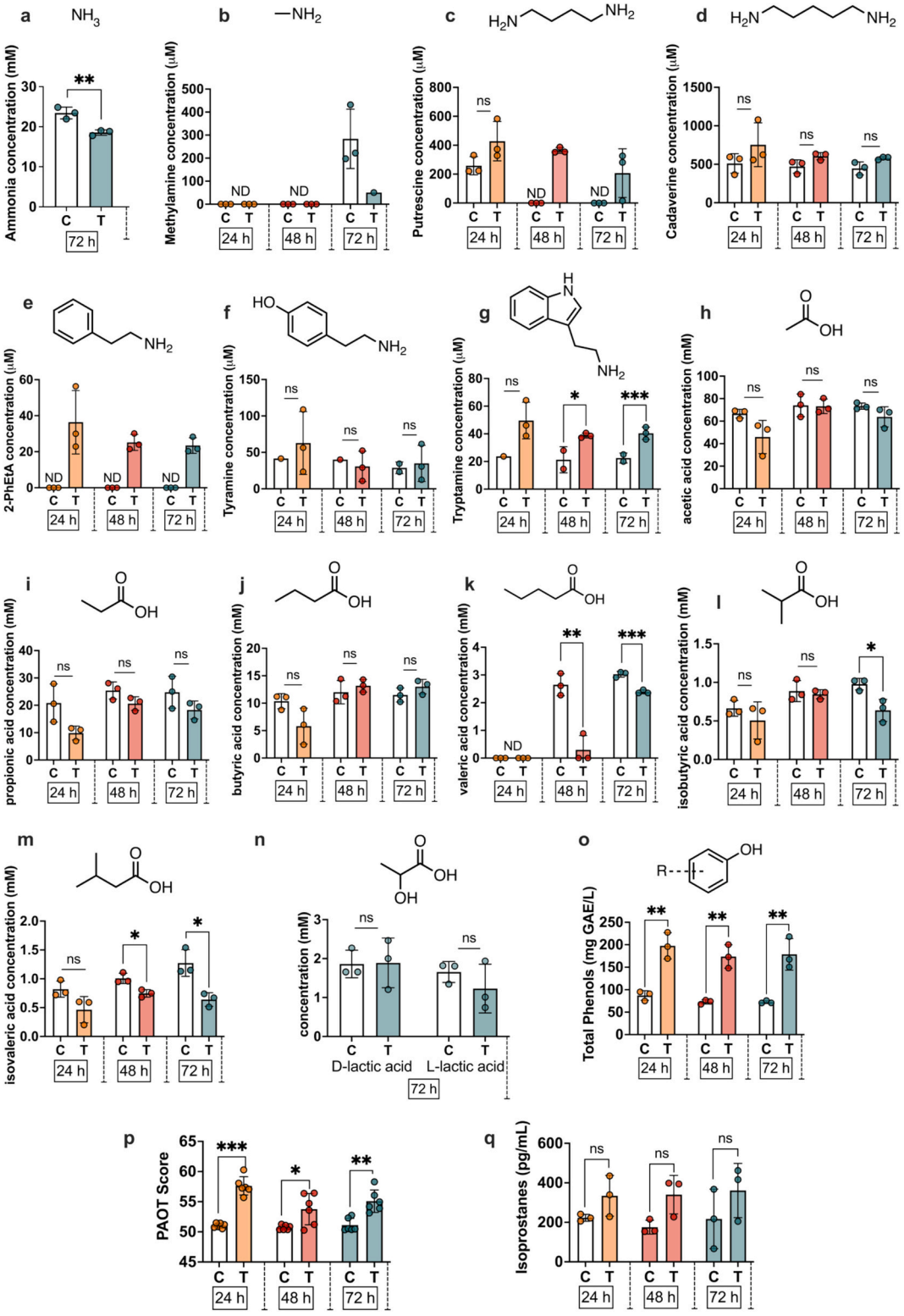

Fig. 2. Metabolic profile in the static simulation of the human intestinal microbial ecosystem testing Triphala dry extract. **a.** Ammonia quantification by UPLC-FLD (mM), **b-g.** Biogenic amine's quantification by UPLC-FLD (μM), **h-m.** SCFA quantification by SPME-GC/MS (mM), **n.** Enzymatic quantification of D- and L-lactic acids (mM), **o.** Total phenols quantification as gallic acid equivalent per liter (GAEL/L), **p.** Antioxidant capacity by power antioxidant total (PAOT)-liquid® technology expressed as PAOT score, **q.** Isoprostanes quantified by UHPLC/MS-MS (pg/mL). Statistical analyses were done using unpaired *t*-test or Mann-Whitney test depending on data normality to compare treatment *versus* control at each time point. Comparisons are presented as no significant (ns) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

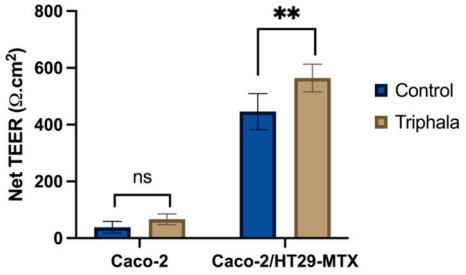

Fig. 3. Results of the net transepithelial electrical resistance assay (TEER). Statistical comparisons were done using unpaired *t*-test and are presented as no significant (ns) $p > 0.05$, ** $p \leq 0.01$.

aggravation of constipation states (Chen et al., 2020). Finally, the significant increase in *Bacteroides-Prevotella* caused by Triphala could impact both health-promoting and detrimental populations in the gut microbiota (Precup & Vodnar, 2019; Zafar & Saier, 2021).

3.2. Intestinal metabolic profile

Regarding the metabolic production during the 72-h simulated intestinal fermentation, Triphala was not affecting considerably most of the SCFA commonly impacted by herbal medicines (Feng et al., 2018), as shown in Fig. 2. Significant decreases were observed only in valeric acid (Fig. 2k), isovaleric acid (Fig. 2m) after 48 h and 72 h, and isobutyric acid (Fig. 2l) after 72 h, which could have positive implications for intestinal health (Rios-Covian et al., 2020). Another positive outcome identified for constipated subjects in the metabolic profile during fermentation was the decrease of ammonia caused by Triphala (Fig. 2a), as the ammonia accumulation in the human gut has been linked to toxic and pathogenic consequences (Walker, 2014). Moreover, Triphala caused a significant boost in the tryptophan-derived monoamine tryptamine (Fig. 2f). Thus, as tryptamine acts on 5-HT_4R, an important G-protein coupled receptor exclusively expressed in the colonic epithelium that contributes to the regulation of intestinal fluid (Bhattarai et al., 2018), an increase in this metabolite could have beneficial health consequences in constipation.

Triphala is a well-known source of polyphenols (Wang et al., 2023), which was demonstrated herein by the significant increase in the total

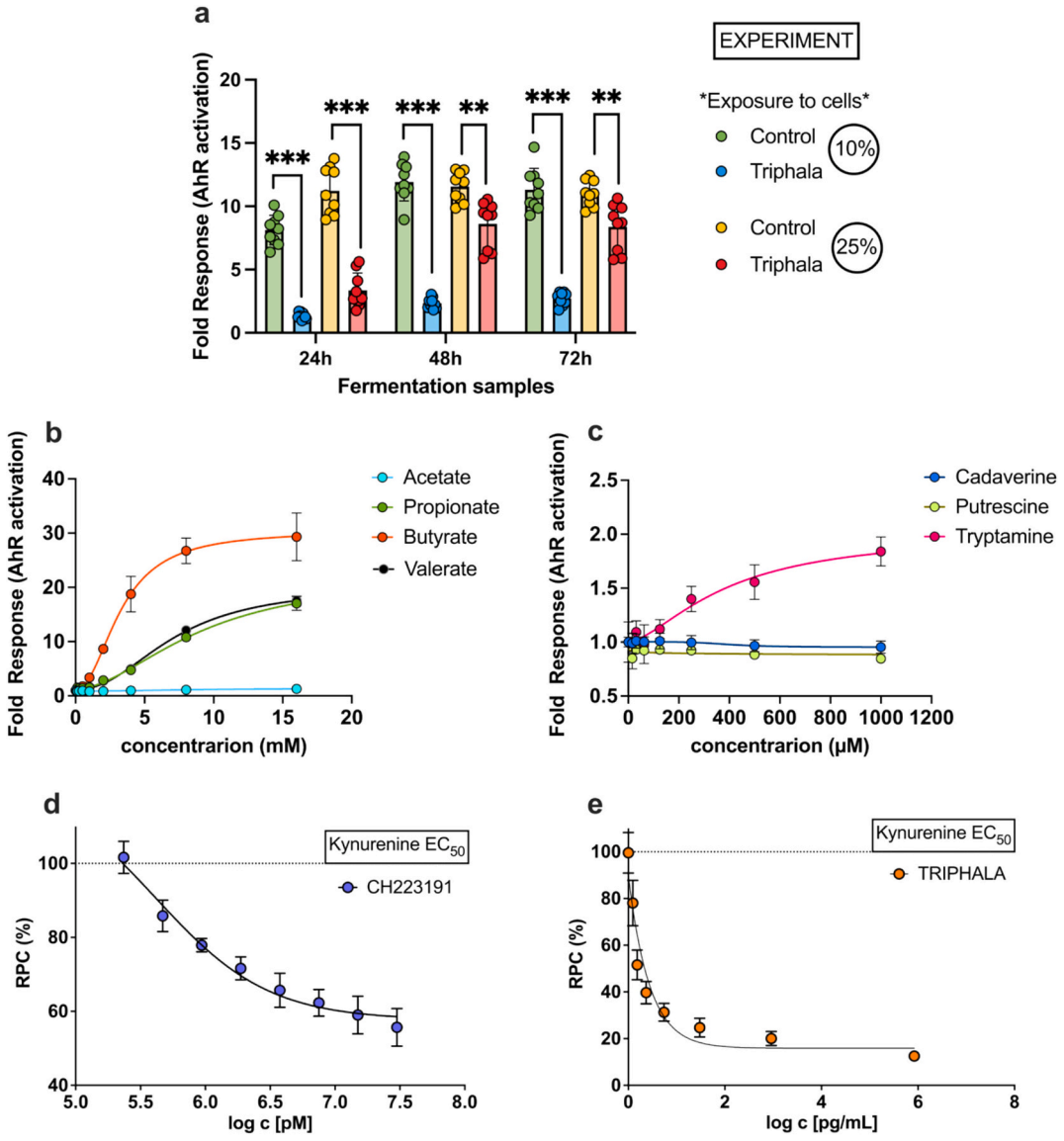

Fig. 4. Results of AhR reporter gene assays using AhR-HT29_Lucia™ cell line. unpaired t-test or Mann-Whitney test depending on data normality were used to compare treatment *versus* control at each time point and for the two exposure doses (10 % and 25 %). Comparisons are presented as ** $p \leq 0.01$, and *** $p \leq 0.001$.

phenolic content in the fermenters where Triphala was added (Fig. 2o). Furthermore, the antioxidant potential of Triphala's fermentation output was significantly higher than that of the control (Fig. 2p), probably related to such an increase in phenolic species. Since oxidative stress plays a role in intestinal diseases, particularly in the etiopathogenesis of constipation (Vermorken et al., 2016), this supports the existing evidence on Triphala to improve gut motility (Munshi et al., 2011). However, the quantification of isoprostanes, biomarkers of lipid peroxidation, revealed no significant differences in the presence of Triphala (Fig. 2q).

3.3. Triphala-host interactions

Metabolic output derived from Triphala's fermentation in the colonic microbiota increased the electrical resistance in the two intestinal epithelial models analyzed, as shown in Fig. 3. Yet, such a difference was found significant only in the co-culture of enterocyte-like Caco-2 cells with mucus-secreting HT29-MTX cell line Caco-2/HT29-MTX. These results revealed potential beneficial effects of the metabolic output from Triphala fermentation in the tightness and integrity of human intestinal epithelium, which is consistent with previous reports (Tarasiuk et al., 2018).

Studying the transcriptional activation of the immunological and bacterial sensor receptor AhR, it was noticed that the fermentation-derived metabolic pool from Triphala significantly reduced AhR activation (Fig. 4a). Subsequently, dose-dependent competitive antagonist effects of Triphala extract (Fig. 4e) were revealed, comparable to those of the known synthetic AhR antagonist CH223191 (Fig. 4d). Individual metabolites such as SCFA (*i.e.*, acetate, propionate, butyrate, valerate)-Figs. 4b and biogenic amines (*i.e.*, cadaverine, putrescine, tryptamine)-Fig. 4c tested in the same concentration range quantified during fermentation, caused agonist activation of AhR, as it has been described before (Modoux et al., 2022). From the SCFAs, the most potent one was butyrate and from the BA, it was tryptamine, both consistent with previous reports (Marinelli et al., 2019; Vikström Bergander et al., 2012).

AhR regulates the expression of cytochrome P450 enzymes (*e.g.*, CYP1A1, CYP1A2, CYP2B1), fundamental for xenobiotic metabolization but also associated with toxic and oncogenic pathways (Guengerich, 2013). Thus, although the activation of AhR is essential for various functions in the intestinal ecosystem, many toxicity mechanisms of environmental pollutants are based on AhR signal transduction (Avilla et al., 2020).

Polyphenols present in numerous plant products such as Triphala have shown dose- and structural-dependent antagonistic potential against AhR transactivity (Goya-Jorge et al., 2021; Van der Heiden et al., 2009). Moreover, phenolic metabolites produced in the gut are able to modulate AhR (Pinto et al., 2023). Therefore, the presence of a wide variety of phenolic ligands, many of them with the capacity to antagonize AhR, is a plausible explanation for the overall inhibition of AhR caused by Triphala's fermentation media. Consistently, the inhibitory potential of Cytochrome P450 enzymes exhibited by Triphala-based formulations has been previously reported (Harwansh et al., 2014; Ponnusankar et al., 2011).

4. Concluding remarks

The *in vitro* simulation of the intestinal microbial ecosystem revealed the potential impact of a standardized polyherbal formulation of Triphala in the mildly constipated human gut microbiota. Some health-promoting microbes were boosted, while others appeared to be affected by Triphala extract. In a cell-based method, Triphala showed efficacy in enhancing the barrier function of the intestinal epithelium. In AhR reporter gene assays, Triphala dry extract demonstrated strong antagonistic activity, which could be useful but could also affect the multiple functions of this transcription factor as a sensor of microbiota metabolites. This is the first study critically analyzing the positive and negative effects that Triphala could induce on the intestinal microbiota of mildly constipated people.

CRediT authorship contribution statement

Elizabeth Goya-Jorge: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Pauline Bondue:** Methodology, Investigation, Conceptualization. **Irma Gonza:** Validation, Resources. **Samiha Boutaleb:** Validation, Resources. **Caroline Douny:** Validation, Resources. **Marie-Louise Scippo:** Validation, Resources, Supervision. **Joël Pincemail:** Validation, Resources. **Patrice Chiap:** Validation, Resources. **Jeoffrey Christyn de Ribaucourt:** Resources, Funding acquisition, Project administration. **Fabienne Crahay:** Resources, Funding acquisition, Project administration. **Véronique Delcenserie:** Writing – review & editing, Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2025.106698.

Data availability

All data supporting the findings of this research work are available within the paper and its Supplementary Material.

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