

RESEARCH ARTICLE OPEN ACCESS

A Digestive Herbal Mixture Preparation Stimulates Proton Secretion in Human Parietal Cells through Phenolic Compounds Targeting Bitter Taste Receptors

Phil Richter¹  | Maria-Riera Piqué-Borràs²  | Gerald Künstle²  | Veronika Somoza^{1,3} ¹Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany | ²Preclinical Research and Development Pharma, Weleda AG, Arlesheim, Switzerland | ³Institute of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria**Correspondence:** Phil Richter (p.richter@leibniz-lsb@tum.de)**Received:** 9 January 2026 | **Revised:** 20 February 2026 | **Accepted:** 5 March 2026**Keywords:** digestive aid | plant extracts | polyphenols | proton secretion | TAS2Rs

ABSTRACT

The effects of herbal remedies aiding digestion are well known, but the underlying mechanisms remain poorly understood. Notably, many of these preparations are bitter, and bitter-tasting phenolic food constituents have been shown to induce digestive processes by activating extra-oral bitter taste receptors (TAS2Rs). This study examined the effects of a commercially available herbal mixture preparation used to aid digestive discomfort and nine solvent extracts thereof on proton secretion of immortalized human parietal cells (HGT-1). The bitter taste of the preparation was sensorially evaluated, and its impact on the gene expression of the polyphenol-related receptors *TAS2R4*, *TAS2R5*, and *TAS2R39* was analyzed. Functional TAS2R involvement was validated using CRISPR-Cas9 knock-out and siRNA knock-down approaches. Total polyphenol content was quantified using Folin-Ciocalteu reagent. The bitter-tasting herbal mixture preparation and several of its solvent extracts stimulated proton secretion by HGT-1 cells concentration-dependently. Four extracts exhibited high total polyphenol content and induced strong secretory responses, whereas five extracts showed lower content and weaker effects ($p < 0.01$). Reduced responses were observed in *TAS2R4ko*, *TAS2R5kd*, and *TAS2R39ko* cells. In conclusion, bitter-tasting plant extracts can stimulate TAS2R-mediated digestive processes depending on their total polyphenol content, thereby supporting their use as promising remedies for alleviating digestive discomfort.

1 | Introduction

Phenolic compounds, also known as polyphenols, are among the main representatives of secondary plant metabolites alongside terpenoids and alkaloids [1]. A number of health-promoting effects have been reported for polyphenolic compounds in the past, e.g., immunomodulatory [2–4] and anti-inflammatory activities. The latter of which were in most cases being attributed to the inhibition of enzymes involved

in inflammatory reactions [5–7] and antioxidant properties [8, 9].

Polyphenols have also been demonstrated to target bitter taste receptors (TAS2Rs), either in the oral cavity, resulting in the perception of bitterness, or extra-orally, leading to, for example, bronchodilation [10, 11]. This shows that TAS2Rs, whose expression is not limited to the oral cavity, where they are an evolutionary trait intended to prevent the ingestion of bitter and

Abbreviations: ddH₂O, double-distilled water; DMEM, Dulbecco's modified Eagle's medium; GA, gallic acid; gLMS, general labeled magnitude scale; HGT-1, immortalized human parietal cells; IPX, intracellular proton index; kd, knock-down; ko, knock-out; KRHB, Krebs-Ringer-HEPES buffer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT-qPCR, real-time quantitative polymerase chain reaction; SEM, standard error of the mean; SNARF-1-AM, 1,5-carboxy-seminaphthorhodafluor acetoxymethylester; TAS2R, bitter taste receptor.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH.

therefore potentially toxic compounds, may also play a functional role in extra-oral tissues and organs [12]. The presence and functionality of TAS2Rs have also been demonstrated in the brain, the intestines, and the stomach [13]. The stomach is one of the key TAS2R-expressing [14] organs that come into direct contact with food constituents and bitter-tasting compounds immediately after ingestion. Previous studies have shown that phenolic compounds in wine stimulate the proton secretion of gastric HGT-1 cells, with catechin, syringic acid, and procyanidin B2 found in red wine playing a decisive role in this process [15].

Studies on other phenolic wine compounds showed that gallic acid stimulated proton secretion in HGT-1 cells via functional involvement of TAS2R4, as validated by means of CRISPR-Cas9 knock-out [16]. Moreover, *trans*-resveratrol contained in red wine, cranberries, and currants [17] also led to a stimulation of the proton secretory activity of HGT-1 cells involving TAS2R50, and reduced the lipopolysaccharide-induced release of the pro-inflammatory IL-6 in human gingival fibroblasts via the same receptor [18].

Aside from the in vitro studies that demonstrated a TAS2R-dependent polyphenol-induced stimulation of proton secretion by immortalized parietal cells, the link between bitter-tasting compounds and gastric acid production was also confirmed in healthy subjects. After oral administration of caffeine, the resulting gastric acid secretion [14] was attenuated by homoeriodictyol, a bitter taste masking compound of flavonoid structure. Homoeriodictyol targets TAS2R14 and -39 as agonist [19], and TAS2R20, -31, -43, and -50 as antagonist [14]. Besides homoeriodictyol, there are also some other representatives of bitter masking, TAS2R antagonizing polyphenols, such as lariciresinol, matairesinol and dihydroeucumin derivatives [19–21] which may help to reduce gastric acid secretion induced by bitter-tasting plant extracts. These findings indicate that polyphenolic compounds may activate or inhibit TAS2Rs [20, 21], suggesting potential dietary or therapeutic strategies for modulating gastric acid secretion.

Given that gastric acid production is a key mechanism of balanced digestion, we investigated whether the total polyphenol content of a commercially available herbal mixture preparation intended to support digestion relates to the extent of proton secretion in immortalized parietal HGT-1 cells, a well-established surrogate model of gastric acid secretion [22]. The herbal mixture preparation studied contains nine selected standardized plant extracts of *Achillea millefolium*, *Artemisia absinthium*, *Centaureum erythraea*, *Cichorium intybus*, *Gentiana lutea*, *Juniperus communis*, *Peucedanum ostruthium*, *Salvia officinalis*, and *Taraxacum officinale* [23].

For two of these plant extracts, key flavor active constituents have already been identified to interact with TAS2Rs. First, the bicyclic monoterpenoid ketone camphor, present in the essential oil of *Achillea millefolium* [24], was shown to interact with TAS2R4, -10, -14, and -47 [25]. Second, the sesquiterpene lactone absinthin present in *Artemisia absinthium* has been identified as a ligand of TAS2R10, -14, -46, and -47 [24, 25]. In addition to these specific constituents, which have been well studied with regard to their TAS2R interactions, plant extracts also contain many

polyphenolic compounds [26], whose potential effects on TAS2Rs remain to be elucidated. Notably, TAS2R4, -5, and -39 have been reported as primary targets of plant-derived polyphenols [27, 28].

Given the complexity of TAS2R activation and inhibition by individual phenolic compounds as well as by their mixtures, it should be emphasized that pure, isolated compounds are rarely ingested. Instead, complex plant extracts or mixtures containing diverse constituents are typically consumed, potentially eliciting distinct physiological responses through modulation of specific receptor profiles. Such mixtures may also produce synergistic or antagonistic interactions, particularly with respect to the previously described agonistic and antagonistic effects of polyphenols on TAS2Rs chiefly involved in cellular proton secretion as a key mechanism of gastric acid-aided digestion. Based on the current state of the literature, we hypothesized that the total polyphenolic content of a bitter-tasting herbal preparation comprising nine different plant extracts functionally modulates TAS2R-dependent proton secretion in immortalized human parietal cells.

2 | Experimental Section

2.1 | Chemicals

The standardized ethanolic plant extracts of *Artemisia absinthium*, *Achillea millefolium*, *Centaureum erythraea*, *Cichorium intybus*, *Gentiana lutea*, *Juniperus communis*, *Peucedanum ostruthium*, *Salvia officinalis*, and *Taraxacum officinale*, as well as the commercially available specific mixture of these extracts, Amara Drops, (hereafter referred to as “herbal mixture preparation”) were provided by Weleda AG (Schwäbisch Gmünd, Germany). The manufacturing protocols and composition analysis have been previously published [23]. In brief, 10 g of the Amara Drops are composed of the following amounts of ethanolic extracts, 0.15 g *Artemisia absinthium*, 2.0 g *Achillea millefolium*, 0.075 g *Centaureum erythraea*, 0.6 g *Cichorium intybus*, 0.36 g *Gentiana lutea*, 0.05 g *Juniperus communis*, 0.15 g *Peucedanum ostruthium*, 1.0 g *Salvia officinalis*, and 0.32 g *Taraxacum officinale*. 1,5-Carboxy-seminaphthorhodafluor acetoxymethylester (SNARF-1-AM) and Dulbecco's modified Eagle's medium GlutaMAX (DMEM) were obtained from Thermo Fisher Scientific. Fetal bovine serum (FBS Supreme), trypsin/ethylenediaminetetraacetic acid, and penicillin-streptomycin were purchased from PANBiotech GmbH (Aidenbach, Germany). Phosphate-buffered saline was bought from Biozym Scientific GmbH (Hessisch Oldendorf, Germany). Dimethyl sulfoxide (DMSO) was purchased from Carl Roth (Karlsruhe, Germany). PeqGOLD RNA Kit was obtained from VWR Peqlab (USA). All items for performing real-time qPCR (RT-qPCR) were purchased from BioRad (Feldkirchen, Germany). All other compounds were ordered from Merck KGaA (Darmstadt, Germany). Double-distilled water (ddH₂O) from Elga Purelab Classic (Veolia Water Solutions & Technologies, France) was used for all experiments. Krebs-Ringer-HEPES buffer (KRHB) is composed of 130 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 11.7 mM D-glucose, and 10 mM HEPES; pH was adjusted to 7.4 with KOH.

2.2 | Sensory Experiments

For the sensory analysis of the bitterness intensity of the commercially available herbal mixture preparation, a representative recombinant of the nine ethanol plant extracts thereof was prepared and diluted to a concentration of 300 µg/mL. The evaluation referred to the corresponding ethanol concentration (solvent control). This study was conducted in accordance with the principles of the Declaration of Helsinki. The 19 trained panelists were informed before the tests were carried out and provided written informed consent prior to enrollment. To further minimize the risk, the panelists were instructed not to swallow the sample solutions but to spit them out. Bitter intensity was rated according to a general labeled magnitude scale (gLMS), with 0 representing “no sensation”, 1.4 “barely detectable”, 6 “weak”, 17 “moderate”, 35 “strong”, 51 “very strong” and 100 “strongest sensation imaginable” [29].

2.3 | Cell Culture

Immortalized human parietal cells (HGT-1) were obtained from Merck KGaA (Darmstadt, Germany) and cultured under previously published standard conditions at 37 °C in a humidified atmosphere at 5% CO₂ in DMEM containing 10% FBS and 1% penicillin and streptomycin [14, 30]. Cells up to passage 20 were used for all experiments.

2.4 | Cell Viability

To exclude cytotoxic effects of the extracts, HGT-1 cells were spread at a density of 100,000 cells/well in transparent 96-well plates. On the following day, the cells were washed with KRHB and then incubated for 30 min (37 °C, 5% CO₂) with the respective extracts at the highest concentrations used. After another washing step, the cells were incubated for 15 min with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (37 °C, 5% CO₂). The water-soluble tetrazolium salt is reduced to the water-insoluble formazan in the presence of NADH and NADPH. Since only metabolically active cells can form NADH and NADPH, this assay is commonly used to determine cellular viability in response to compounds under investigation [31]. After treatment, the formazan is dissolved in DMSO, and the absorbance at 570 nm (reference 650 nm) is determined using an Infinite M200 plate reader (Tecan, Switzerland). A higher absorbance indicates a large amount of reduction equivalents and, therefore, higher cell viability than low absorbance values. The cells were treated with KRHB as a positive control (= 100%), whereas quinine has a cytotoxic effect at higher concentrations (5 mM) and acted as a negative control [32]. No cytotoxic effect (viability ≥ 96%) was found for any of the extracts or for their solvent (compared to KRHB-treated control; $p > 0.05$; Figure S1).

2.5 | Cellular Proton Secretion Assay

To determine the effects of the extracts on the proton secretory activity of HGT-1 cells as outcome measure of a cellular bitter response [14], the cells were spread at a density of 100,000

cells/well in black 96-well plates. On the following day, the cells were washed with KRHB and incubated with SNARF-1-AM at 37 °C for 30 min. The pH-sensitive fluorescent dye enables intracellular measurement of the pH value. Increased secretory activity, that is, stimulation of proton release, reduces the concentration of intracellular protons. This results in a negative intracellular proton index (IPX). In contrast, positive IPX values indicate an inhibition of proton secretion. After incubation, the cells are washed again and incubated for 15 min with the respective extracts. To exclude any interference of the results by the known autofluorescence of aromatic plant constituents such as quercetin and kaempferol [33], which remain in or on the cells in relevant quantities, the cells were washed again in order to read the fluorescence directly afterwards, from which the IPX can then be calculated in comparison to cells treated with solvent alone. For better comparability of the measured values, the effect sizes were normalized to the effects induced by the established positive control 1 mM histamine (set to 1), which consistently leads to a stimulation of the proton secretion of HGT-1 cells [22]. Values > 1 indicate a stronger stimulation of secretory activity than histamine, while values < 1 indicate a weaker stimulation.

2.6 | Quantitation of mRNA Expression

To determine the effects of the extracts on *TAS2R4*, -5, and -39 gene expression of HGT-1 cells, these were spread at a density of 500,000 cells/well in 24-well plates. On the following day, the cells were washed with KRHB and incubated for 15 min with the herbal mixture preparation (300 µg/mL) or the corresponding solvent control. After incubation, the cells were washed with PBS and lysed directly in the plate. RNA isolation followed by concentration and integrity determination, gDNA removal, synthesis of cDNA, and real-time quantitative polymerase chain reaction (RT-qPCR) were performed using the peqGOLD RNA Kit (VWR Peqlab, USA), the iScript gDNA Clear cDNA Synthesis Kit (BioRad, Feldkirchen, Germany), and the SsoAdvanced Universal SYBR Green Supermix (BioRad, Feldkirchen, Germany) as previously described [30]. The primer sequences for the *TAS2Rs* were taken from Liszt et al. [14]. *PPIA* [34] and *GAPDH* [18] were used as reference genes. The effects of the compounds on gene expression were analyzed in comparison with control cells treated with the corresponding solvent.

2.7 | Stable *TAS2R4* and *TAS2R39* Knock-Out Using CRISPR-Cas9

For experiments with *TAS2R4* [16, 35] and *TAS2R39* [36] homozygote KO HGT-1 cells, the same clones were used as previously published.

2.8 | Transient *TAS2R5* Knock-Down Using siRNA

Before performing the functional cellular proton secretion assays, the transfection efficiency was determined using two different siRNA sequences targeting *TAS2R5* (HSS122866 and HSS182480). For this, the cells were spread at a density of 100,000 cells/well in 24-well plates. On the following day, the cells were transfected for 72 h with Lipofectamine RNAiMAX (Thermo Fisher Scientific,

USA) in Opti-MEM according to the manufacturer's protocol. Mock and positive controls (MAPK1; VHS40312) were conducted as previously published [37].

2.9 | Quantification of Total Polyphenol Content by Means of Folin-Ciocalteu Reagent

To determine the total polyphenol content, the standardized ethanolic plant extracts were first diluted tenfold in water. In a 96-well format, five parts of a tenfold diluted Folin-Ciocalteu reagent (Merck KGaA Darmstadt, Germany) were added to one part of the plant extract. After incubation for 5 min at room temperature in the dark, six parts of 10.75% an aqueous Na_2CO_3 solution were added and incubated again at RT for 30 min. The absorbance at 760 nm was then determined using an Infinite M200 plate reader (Tecan, Switzerland). Gallic acid (GA; 3.125 – 200 $\mu\text{g/mL}$) was used as a standard [38].

2.10 | Statistics

All data were expressed as mean $\pm$ standard error of the mean (SEM) unless otherwise indicated. At least three biological replicates were prepared from each experiment. Statistical analyses of the different treatments with the untreated control were performed after a Nalimov outlier test with the *t*-test Holm–Šidák method or one-way ANOVA followed by a Holm–Šidák *post hoc* test. Different *p*-values are indicated with asterisks according to the following scheme: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

3 | Results

3.1 | Evaluation of the Bitterness of the Commercially Available Herbal Mixture Preparation and by Means of Sensory Trials and Investigation of the Influence of These and Their Solvent Fractions on Cellular Proton Secretion

Previous studies have shown that bitter-tasting phenolic compounds stimulate and aid mechanisms of gastric digestion by inducing gastric acid secretion. Herbal remedies to treat gastric discomfort contain polyphenols, whose bitterness has been validated in the past, although they are able to both stimulate and reduce proton secretion in the parietal cells. Therefore, it was investigated whether the total amounts of polyphenolic compounds play a major role in the ability of plant extracts used as digestive remedies to stimulate proton secretion as the key mechanism of gastric acid secretion. To investigate the link between the effect on proton secretion, the taste quality, and TAS2R activation, the bitterness of a recombination of the extracts representing the herbal mixture preparation was evaluated by sensory trials (300 $\mu\text{g/mL}$). The results showed that the recombine causes a bitter taste between *moderate* and *strong* ($p \leq 0.0001$) compared to the solvent control (0.9% EtOH) with the corresponding EtOH concentration, whose bitterness was rated as weak (Figure S2).

According to the manufacturer, one dose (15 drops) of the herbal mixture preparation, a specific mixture of nine standardized

plant extracts (Figure 1A) from *A. absinthium*, *A. millefolium*, *C. erythraea*, *C. intybus*, *G. lutea*, *J. communis*, *P. ostruthium*, *S. officinalis*, and *T. officinale*, contains approximately 4 mg of dissolved herbal ingredients. Taking into account possible dilutions in the stomach after ingestion of the drops, concentrations between 5 and 500 $\mu\text{g/mL}$ were tested for their effects on the proton secretion by HGT-1 cells. As a result, a concentration-dependent stimulation of cellular proton secretion was shown when the cells were incubated with the herbal mixture preparation (orange bars in Figure 1B). As an established positive control, the cells were treated with histamine (1 mM), which elicited a TAS2R-independent stimulation. This effect ($\text{IPX}_{\text{histamine}} = -0.27 \pm 0.10$) was set to 1 for normalization of intra-assay variations [14, 39]. The tested concentrations between 100 and 500 $\mu\text{g/mL}$ of the herbal mixture preparation increased the cellular proton secretion in a concentration-dependent manner ($p \leq 0.05$) without affecting cell viability ($p > 0.05$).

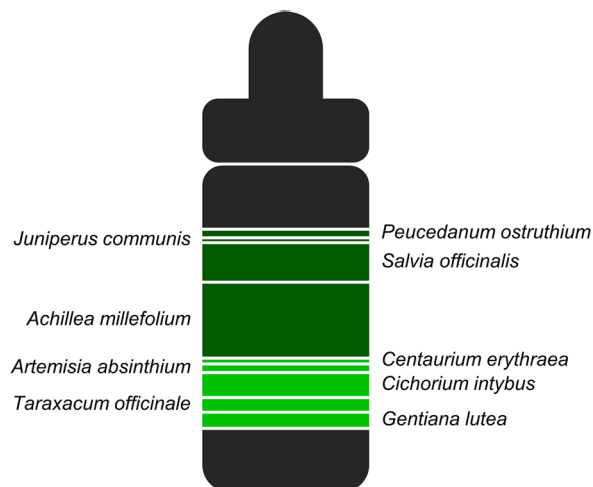
The herbal mixture preparation is composed of varying amounts of the nine ethanolic plant extracts [23] (Figure 1A). The impact of the individual extracts that compose the mixture in certain proportions, as described in the method section, on cellular proton secretion was investigated by also subjecting them to the bitter response assay (1–300 $\mu\text{g/mL}$). It was found that seven of the nine extracts had a stimulating effect on proton secretion (green bars in Figure 1B), which is also concentration-dependent (Figure 2), as demonstrated for the herbal mixture preparation. The extracts from *C. erythraea*, *A. absinthium*, and *C. intybus* led to an increased cellular secretion of protons, with *P. ostruthium*, *J. communis*, *S. officinalis*, and *A. millefolium* inducing the strongest effects ($p \leq 0.0001$). The tinctures from *T. officinale* and *G. lutea* showed no significant effects in the same tested concentration range between 1 and 300 $\mu\text{g/mL}$. No difference in the effect on proton secretion was found between a recombine prepared from the individual extracts and the herbal mixture preparation ($p = 0.58$; hatched orange bar in Figure 1B).

Notably, the mixture representing the combination of the four extracts with higher effects on proton secretion had a stronger effect than the reference group of five extracts with smaller effect sizes ($p \leq 0.05$; hatched green bars in Figure 1B). At the same time, however, the effects of both recombinants were substantially weaker than the effect of the herbal mixture preparation, which contains all nine extracts ($p \leq 0.05$).

3.2 | Effects of the Extracts on TAS2R Gene Expression

To gain basic insights into whether TAS2Rs are involved in polyphenol-induced cellular responses, the influence on TAS2R gene expression was determined. Since it is already known from literature that bitter-tasting polyphenols, like (–)-epicatechin, proanthocyanidins, and gallic acid, mainly interact with the bitter taste receptors TAS2R4, -R5, and -R39 [16, 27, 28], the influence of the herbal mixture preparation on the regulation of the genes of these three receptors was investigated. Incubation of HGT-1 cells with the herbal mixture preparation (300 $\mu\text{g/mL}$) resulted in a downregulation of TAS2R4 by $-40.5 \pm 5.5\%$ ($p \leq 0.01$), TAS2R5 by $-23.3 \pm 4.0\%$ ($p \leq 0.01$), and TAS2R39 by $-45.1 \pm 9.5\%$ ($p \leq 0.05$) compared to solvent-treated control cells (Figure S3).

A



B

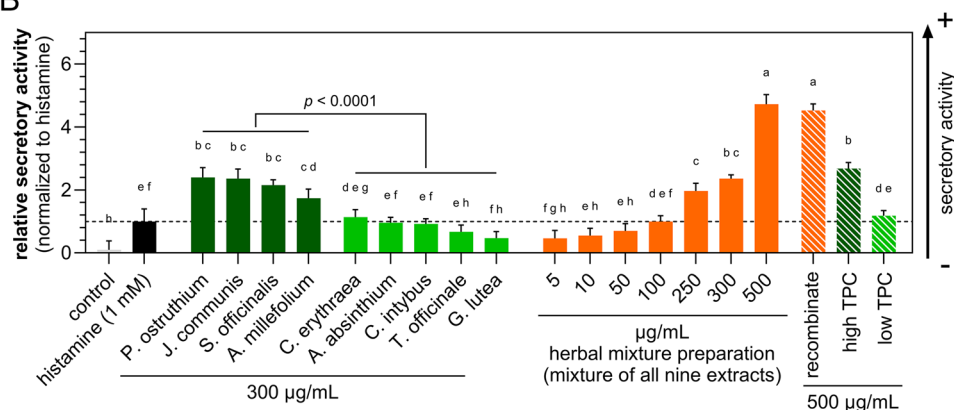


FIGURE 1 | (A) Representation of the respective proportions of the nine plant extracts used in herbal mixture preparation. (B) Effects of the standardized ethanolic extracts (300 µg/mL) from *P. ostruthium*, *J. communis*, *S. officinalis*, and *A. millefolium* with high polyphenol content (dark green bars) and *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea* and with low polyphenol content (light green bars) on proton secretion of HGT-1 cells normalized to the positive control 1 mM histamine (relative secretory activity). Concentration-dependent impact of the commercially available herbal mixture preparation (5–500 µg/mL, orange bars), a complete recombine (500 µg/mL, hatched orange bar), a combination of extracts with high (500 µg/mL, hatched dark green bar) and low (500 µg/mL, hatched light green bar) polyphenol content on cellular proton secretion. Data shown as mean ± SEM after incubation for 15 min, $n = 5$, t. r. = 4–6, control: KRHB containing the respective EtOH concentration, pos. control: histamine (1 mM, black bar). Statistics: one-way ANOVA Holm-Šidák *post hoc* test; significant differences are expressed with different letters ($p \leq 0.05$).

The found modulation of the mRNA transcript levels of *TAS2R4*, *TAS2R5*, and *TAS2R39* allowed a first validation of the possible involvement of these three receptors in the polyphenol-induced stimulation of cellular secretory activity.

3.3 | Functional Role of Bitter Taste Receptors *TAS2R4*, *TAS2R5*, and *TAS2R39* in Extract-Mediated Stimulation of Proton Secretion

Since (i) the gene expression of the receptors *TAS2R4*, *TAS2R5*, and *TAS2R39* was regulated by the herbal mixture preparation and (ii) these three receptors have already been associated with polyphenolic compounds in the past, they were selected as potential targets. To investigate the functional involvement of specific TAS2Rs, the cellular proton secretion assay was repeated with knock-down (kd) and knock-out (ko) HGT-1 cells (Figure 3). While existing clones were used for the experiments with *TAS2R4*

[16] and *TAS2R39* [36] knock-out cells, the generation of HGT-1 cells with reduced *TAS2R5* expression was first established and verified. In direct comparison, the siRNA HSS122866 (Thermo Fisher Scientific, USA) targeting *TAS2R5* showed better efficiency ($-64.2 \pm 1.7\%$; $p \leq 0.05$) and was therefore used for the functional assays (Figure S4).

In *TAS2R4*ko HGT-1 cells, a reduced release of protons was observed compared to wild-type cells after treatment with the extracts from *A. millefolium*, *C. erythraea*, *J. communis*, *P. ostruthium*, and *S. officinalis* ($p \leq 0.05$). Treatment of HGT-1 cells with *TAS2R5* expression reduced by siRNA showed lower stimulation of proton secretion after exposure to the four extracts of *A. absinthium*, *A. millefolium*, *C. erythraea*, and *P. ostruthium* compared to mock-transfected cells ($p \leq 0.05$). Treatment of *TAS2R39*ko cells also led to a reduction in the stimulating effect of the extracts from *A. millefolium*, *C. erythraea*, *P. ostruthium*, *S. officinalis*, and *T. officinale* ($p \leq 0.05$). The recombine of

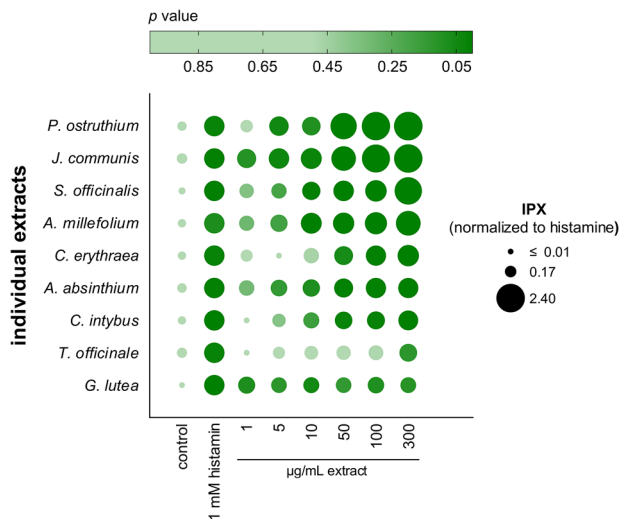


FIGURE 2 | Visualization of the concentration-dependent effects of the individual extracts (1 – 300 µg/mL) from *P. ostruthium*, *J. communis*, *S. officinalis*, *A. millefolium*, *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea* on proton secretion of HGT-1 cells normalized to the positive control histamine (1 mM). Darker shades of green represent higher significance levels compared to the solvent-treated control. The dot sizes correlate with the measured effect size normalized to histamine. Data shown as mean after incubation for 15 min, $n = 5$, t. r. = 4 – 6, control: KRHB containing the respective EtOH concentration, pos. control: histamine (1 mM). Statistics: one-way ANOVA Holm-Šidák *post hoc* test.

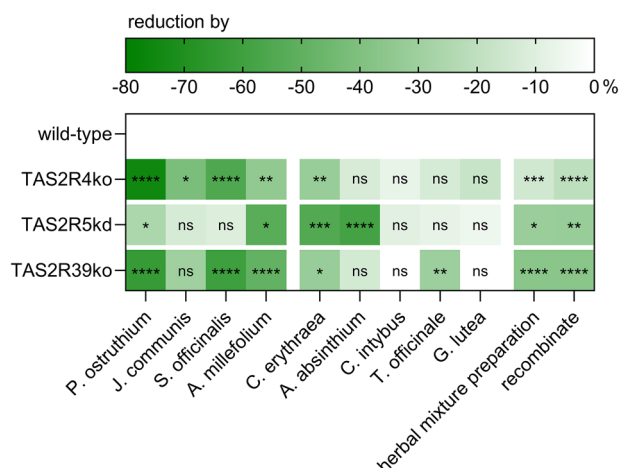


FIGURE 3 | Heatmap showing the reduction of stimulatory effects on proton secretion of HGT-1 cells obtained by knock-out (TAS2R4ko and TAS2R39ko) or knock-down (TAS2R5kd) approaches compared to wild-type/mock-transfected cells (= 0%, white) incubated with the ethanolic extracts (300 µg/mL) from *P. ostruthium*, *J. communis*, *S. officinalis*, *A. millefolium*, *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea*, the herbal mixture preparation and the recombine normalized to the positive control histamine (1 mM). Data shown as mean ± SEM, $n = 5$, t. r. = 6, intense green shades represent strong reductions caused by the knock-out or knock-down of the respective receptor, while white stands for no changes, control: KRHB containing the respective EtOH concentration, statistics: *t*-test Holm-Šidák method, significant differences within the groups (related to the effects of the respective wild-type/mock transfected cells) are expressed with * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

TABLE 1 | Total polyphenol content in gallic acid equivalents (µg GA/mL) of the nine ethanolic plant extracts, the herbal mixture preparation, a recombine thereof, and the theoretical content based on the values of the individual extracts. Data shown as mean ± SD, $n = 6$.

Extract	Absolute quantification total polyphenol content (µg GA/mL)	Representative proportion of all constituents (%)
<i>P. ostruthium</i>	2063.7 ± 31.6	8.9 ± 0.1
<i>J. communis</i>	2859.5 ± 13.6	13.6 ± 0.1
<i>S. officinalis</i>	3702.0 ± 81.7	15.5 ± 0.3
<i>A. millefolium</i>	2644.0 ± 16.5	13.2 ± 0.1
<i>C. erythraea</i>	708.0 ± 10.5	2.9 ± 0.0
<i>A. absinthium</i>	572.1 ± 4.4	2.0 ± 0.0
<i>C. intybus</i>	217.8 ± 3.1	1.3 ± 0.0
<i>T. officinale</i>	490.7 ± 4.4	1.7 ± 0.0
<i>G. lutea</i>	316.2 ± 3.9	0.7 ± 0.0
Herbal mixture preparation	1087.7 ± 15.3	9.9 ± 0.1
Recombine	1120.7 ± 7.6	10.2 ± 0.1
Theoretical amount	998.3 ± 8.3	9.1 ± 0.1

all nine solvent extracts reduced the effects by $20.6 \pm 3.3\%$ ($p \leq 0.0001$), $32.5 \pm 6.7\%$ ($p \leq 0.01$), and $37.0 \pm 4.0\%$ ($p \leq 0.0001$) in TAS2R4ko, TAS2R5kd, and TAS2R39ko HGT-1 cells, respectively. Compared to HGT-1 wild-type cells, also the proton secretion-stimulating effect of the herbal mixture preparation was reduced by $15.0 \pm 2.6\%$ ($p \leq 0.001$), $32.0 \pm 7.1\%$ ($p \leq 0.05$), and $36.7 \pm 2.2\%$ ($p \leq 0.0001$) in TAS2R4ko, TAS2R5kd, and TAS2R39ko HGT-1 cells, demonstrating a functional involvement of these three bitter taste receptors in plant extract-induced increased proton secretion as a mechanism of gastric acid secretion (Table S1).

3.4 | Determination of Total Polyphenol Content in the Plant Extracts

As the effects of herbal mixture preparation and its solvent extracts on cellular proton secretion were substantially modulated by polyphenol-related TAS2Rs, total polyphenol content was determined using Folin-Ciocalteu reagent, with gallic acid (GA) used as a standard (Table 1). It was found that the four extracts of *A. millefolium*, *J. communis*, *P. ostruthium*, and *S. officinalis* had higher total polyphenol content ($p \leq 0.0001$) of over 2000 µg GA/mL (corresponding to a content of between 8.9 and 15.5% of the total ingredients), while analysis of the other extracts of *A. absinthium*, *C. erythraea*, *C. intybus*, *G. lutea*, and *T. officinale* revealed a total polyphenol content of less than 800 µg GA/mL (corresponding to a content $\leq 2.9\%$). The herbal mixture preparation showed a total polyphenol content of $1,087.7 \pm 15.3$ µg GA/mL (9.9%). A recombine based on the specific amounts of the nine individual extracts, as well as their

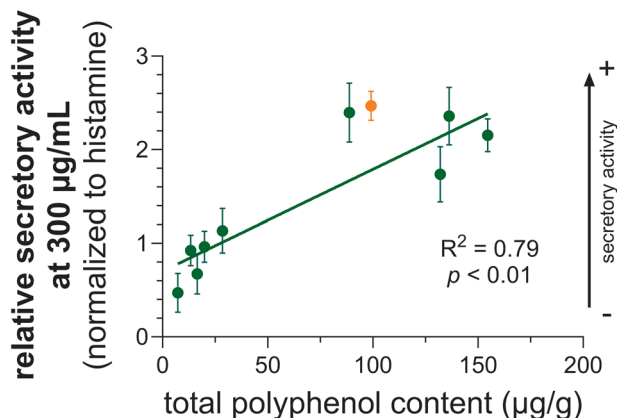


FIGURE 4 | Pearson correlation of the content of polyphenols in the nine plant extracts (300 µg/mL) vs. their histamine-normalized effects on proton secretion of HGT-1 cells (relative secretory activity). Positive correlation was found for the total polyphenol content ($R^2 = 0.79$; $p \leq 0.01$) and the IPX values. Each green dot represents one of the nine standardized ethanolic extracts from *A. absinthium*, *A. millefolium*, *C. erythraea*, *C. intybus*, *G. lutea*, *J. communis*, *P. ostruthium*, *S. officinalis*, and *T. officinale*. The orange data point indicates the combined herbal mixture preparation of the extracts. Data shown as mean $\pm$ SEM, $n = 5$, t. r. = 6.

theoretical summation, resulted in contents with only very small deviations ($< 10\%$; Table 1).

A Pearson correlation was found between the stimulating effects on proton secretion and the total polyphenol content (Figure 4). Extracts with high total polyphenol content, such as the standardized tinctures of *A. millefolium*, *J. communis*, *P. ostruthium*, and *S. officinalis*, led to greater stimulation ($R^2 = 0.79$; $p \leq 0.01$) of cellular proton secretion than extracts with low total polyphenol content, such as *A. absinthium*, *C. erythraea*, *C. intybus*, *G. lutea*, and *T. officinale*.

4 | Discussion

It was hypothesized that the herbal mixture preparation and its solvent extracts stimulate proton secretion by immortalized human parietal cells through TAS2R activation, with the magnitude of the response determined by their total polyphenol content. The modulating properties of individual polyphenols, such as catechin, syringic acid, and procyanidin B2 [15], on proton secretion by immortalized human parietal cells have been well documented, and the involvement of bitter taste receptors (TAS2Rs) in these effects has been described in the past [16, 18]. Building on this foundation, the present study examined nine standardized extracts of *Artemisia absinthium*, *Achillea millefolium*, *Centaurea erythraea*, *Cichorium intybus*, *Gentiana lutea*, *Juniperus communis*, *Peucedanum ostruthium*, *Salvia officinalis*, and *Taraxacum officinale* and their combined herbal mixture preparation to determine whether bitter-tasting, polyphenol-containing plant extracts influence gastric proton secretion via TAS2R involvement. Based on this, it was investigated whether (i) the herbal mixture preparation exhibits a perceptible bitter taste, (ii) the individual extracts and their combination stimulate

proton secretion by HGT-1 cells, and whether (iii) these effects are mediated by specific TAS2Rs and correlate with their total polyphenol content.

This work, therefore, aims to investigate how complex mixtures of polyphenols in plant extracts influence proton secretion as a key mechanism of digestion, to assess the potential of herbal remedies to aid gastric discomfort. In addition, the findings may provide insights for future optimization of the formulation to enhance its efficacy.

Activation of TAS2Rs in the oral cavity by the recombined mixture of all nine plant extracts examined, as is the case when plant-based remedies are ingested, was validated in a sensory trial. Here, the panelists reported a stronger bitterness for the recombinant of all nine extracts compared to the solvent control. This already indicates that the mixture of polyphenols from various plant sources leads to a predominantly agonistic effect on TAS2Rs.

The suitability of immortalized parietal cells (HGT-1) as a surrogate model of TAS2R-mediated bitter taste perception has been shown in earlier works [14, 32]. In contrast to taste cells in the oral cavity, the interaction of bitter-tasting food constituents with TAS2Rs expressed in the stomach does not lead to the perception of taste quality in the brain, but to a stimulation of parietal proton secretion [14]. Proton secretion is the basis for the formation of gastric acid (HCl) in the stomach and, therefore, a key mechanism of gastric protein digestion, which can be modulated by food-derived bitter compounds [20]. In addition to plant-derived bitter peptides [37], it was also found that bitter-tasting polyphenols such as gallic acid, catechin, syringic acid, and procyanidin B2 have a modulating effect on the proton secretion of HGT-1 cells [15, 16]. In the present work, a concentration-dependent stimulation of proton secretion was found for the mixture of the nine standardized plant extracts of *P. ostruthium*, *J. communis*, *S. officinalis*, *A. millefolium*, *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea*, with higher extract concentrations inducing stronger secretory activities. This is consistent with the findings of the sensory trial, in which the recombined mixture was also perceived as bitter.

Since the herbal mixture preparation consists mainly of the extracts of *A. millefolium* and *S. officinalis*, it can be assumed that these two extracts contribute substantially to its effect. The effects of the nine plant extracts on the secretory activity of HGT-1 cells were analyzed to determine their individual contributions to the overall response induced by the herbal mixture preparation. A stimulating effect could be attributed to the seven extracts of *P. ostruthium*, *J. communis*, *S. officinalis*, *A. millefolium*, *C. erythraea*, *A. absinthium*, and *C. intybus* which also showed a concentration-dependent increase in proton secretion. The remaining two extracts of *T. officinale* and *G. lutea* showed no effect on the cellular secretory activity. Examination of all extracts revealed a clear distinction between two groups, with (i) the extracts obtained from *P. ostruthium*, *J. communis*, *S. officinalis*, and *A. millefolium* exhibiting a stronger stimulatory effect on proton secretion, while (ii) the remaining five extracts from *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea* had a weak or no effect.

The results from both the sensory evaluation and the proton secretion assay using HGT-1 cells indicate the bitterness of the extracts and, consequently, the activation of TAS2Rs. In order to investigate the involvement of distinct TAS2Rs, the impact of the extract mixture on TAS2R4, -5 and -39 which have been reported to be activated by polyphenolic agonists [27, 28], was first examined. This resulted in a downregulation of the gene expression of these three TAS2Rs by the herbal mixture preparation. This modulation of TAS2R gene expression after 15 min of incubation suggests a possible targeting of the extract constituents to these receptors. Feedback regulation of this type has previously been described for GPCRs, whereby receptor activation by specific ligands leads to modulation of mRNA transcript levels. These short-term changes in mRNA do not necessarily reflect an immediate change in receptor protein abundance or signaling capacity [40]. The observed synergism might be explained by multiple TAS2Rs being targeted by the extract mixture polyphenols in parallel.

While it has already been shown that TAS2R4 can be activated by polyphenolic compounds such as gallic acid and (-)-epicatechin [16, 27], agonists of plant origin are also known for TAS2R5, -39, and -43. Known agonists of TAS2R5 include punicalagin and various representatives of the procyanidins [28]. In addition to (-)-epicatechin, pentagalloylglucose also binds to TAS2R39 [27]. However, functional involvement of specific TAS2Rs can only be verified by knock-out experiments using CRISPR-Cas9 or knock-down approaches by means of siRNA.

The work presented here used CRISPR-Cas9-edited knock-out HGT-1 cells with nonfunctional variants of TAS2R4 or TAS2R39, as well as HGT-1 cells with reduced *TAS2R5* expression using specific siRNA, to confirm the functional involvement of these selected TAS2Rs. Particularly strong reductions in the effect size, namely, cellular proton secretion, and the associated involvement of TAS2R4 were observed for the *P. ostruthium* and *S. officinalis* extracts. TAS2R5 played an essential role for the *A. absinthium*, *A. millefolium*, *C. erythraea*-induced stimulation of secretory activity and a relevant TAS2R39 involvement was shown for the extracts from *A. millefolium*, *P. ostruthium*, and *S. officinalis*. *J. communis* also had a strong effect on proton secretion, although the involvement of TAS2R5 and -39 could be excluded and the absence of TAS2R4 only minimally reduced the effect, suggesting that other TAS2Rs, possibly also activated by polyphenols or other constituents, are also involved. Consequently, at least one relevant TAS2R could be functionally assigned to the four extracts of the first group, which induced strong effects on proton secretion. Remarkably, the stronger influences on the secretory activity of HGT-1 cells of the solvent extracts from *P. ostruthium*, *J. communis*, *S. officinalis*, and *A. millefolium* assigned to group one could, in most cases, be reduced by knock-out and knock-down approaches more effectively than those assigned to group two. This suggests that these four extracts play a major role in TAS2R-induced stimulation of proton secretion by the herbal mixture preparation.

The combination of multiple plant extracts broadens the spectrum of targeted TAS2Rs, resulting in a potentially synergistic enhancement of the effects. In the case of the herbal mixture preparation, for example, the interactions of the constituents from *A. absinthium* and *C. erythraea* with TAS2R5 and those

from *P. ostruthium* and *S. officinalis* with TAS2R4 and TAS2R39 demonstrate their involvement, which ultimately induce the beneficial digestive effects of the commercially available herbal mixture preparation or its recombinant with identical composition. Confirming the hypothesis, the stimulatory effect of the herbal mixture preparation or a correspondingly prepared recombinant was moderately reduced by all three knock-out or knock-down approaches, demonstrating that the herbal mixture preparation activates a broad spectrum of TAS2Rs, resulting in a strong effect even at lower concentrations of the individual extracts.

To validate a correlation between the total polyphenol content of the extracts and their influence on stimulating proton secretion in immortalized human parietal cells, which consequently affects gastric acid production, the total polyphenol content of the ethanolic plant extracts was determined. The four extracts from *P. ostruthium*, *J. communis*, *S. officinalis*, and *A. millefolium*, which already had a stronger influence on the proton secretion of HGT-1 cells, showed higher total content of polyphenols in the quantitation experiments than the extracts from *C. erythraea*, *A. absinthium*, *C. intybus*, *T. officinale*, and *G. lutea*. This confirms the classification of the extracts into the two groups, (i) those with high total polyphenol content, which strongly stimulate proton secretion and may therefore benefit digestion, and (ii) those with lower total polyphenol content, which have no or only weak effects on the secretory activity of parietal cells.

To address the question of whether the four extracts with the higher levels of polyphenols chiefly account for the strong effect of the herbal mixture preparation, recombinates of both groups were examined for their effects on the proton secretion of HGT-1 cells, based on their respective proportions of the mixture. It was found that the mixture of the four plant extracts with high total polyphenol content stimulated proton secretion more strongly than the mixture of the remaining five extracts, as hypothesized. However, the two recombinations containing high and low amounts of total polyphenols did not reach the potential induced by the herbal mixture preparation, neither individually nor cumulatively. This suggests that synergistic interactions contribute to the overall effect, potentially resulting from the simultaneous activation of multiple TAS2Rs by individual polyphenols, but possibly also by other constituents.

In summary, polyphenol-rich herbal extracts were shown to stimulate proton secretion by immortalized human parietal cells as the fundamental mechanism of gastric acid production involving the bitter taste receptors TAS2R4, TAS2R5 and TAS2R39. The combination of these herbal extracts leads to an activation of several candidates of the TAS2R family. Herbal preparations with complex compositions can thereby have beneficial effects on digestion by stimulating the secretion of gastric acid to aid mechanisms of digestion via TAS2Rs. Overall, the stimulatory activity of the herbal mixture preparation studied can be attributed to its complex composition of nine different plant extracts, which is, however, largely determined by the extracts *S. officinalis* and *A. millefolium*. These two extracts are among the four key extracts with high total polyphenol content that induce strong stimulation of cellular proton secretory activity. *P. ostruthium* and *J. communis* also exhibit pronounced stimulatory properties, but their low proportions in the commercially available herbal preparation suggest

only a minimal influence on proton secretion by immortalized parietal cells. Overall, these findings may form the basis for future improvements in modulatory remedies that target TAS2Rs to stimulate digestive processes. The results also suggest that the herbal mixture examined here could aid digestion due to its ability to promote gastric secretory activity. However, clinical studies are required to substantiate the efficacy of polyphenol-rich plant extracts in supporting digestive function.

Acknowledgments

The authors thank Kristin Kahlenberg for excellent technical assistance. Open access funding enabled and organized by Projekt DEAL.

Funding

The authors declare that this study received funding from Weleda AG. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Conflicts of Interest

The authors declare the following competing financial interest(s): M.-R. Piqué-Borràs and G. Küntle are employed by Weleda AG, Arlesheim/Switzerland.

Data Availability Statement

The datasets generated and analyzed during this study are available in the Zenodo repository under the DOI: 10.5281/zenodo.17699271

References

1. R. M. H. Shoker, "Revisiting the Medicinal Value of Terpenes," *IJRASB* 7 (2020): 354–358, <https://doi.org/10.31033/ijrasb.7.5.47>.
2. G. Andersen, K. Kahlenberg, D. Krautwurst, and V. Somoza, "[6]-Gingerol Facilitates CXCL8 Secretion and ROS Production in Primary Human Neutrophils by Targeting the TRPV1 Channel," *Molecular Nutrition & Food Research* 67 (2023): 2200434, <https://doi.org/10.1002/mnfr.202200434>.
3. S. Crouvezier, B. Powell, D. Keir, and P. Yaqoob, "The Effects of n-3 Polyunsaturated Fatty Acids on Peptide Cytokine Production by Human Mononuclear Cells," *Cytokine* 13 (2001): 337–341, <https://doi.org/10.1006/cyto.2000.0837>.
4. C. M. John, P. Sandrasaigaran, C. K. Tong, A. Adam, and R. Ramasamy, "Immunomodulatory Activity of *Orthosiphon stamineus* Benth. Extract in Hepatic Inflammation," *Cellular Immunology* 271 (2011): 431–440, <https://doi.org/10.1016/j.cellimm.2011.08.017>.
5. Y.-D. Min, C.-H. Choi, H. Bark, H.-Y. Son, H.-H. Park, S. Lee, J.-W. Park, E.-K. Park, H.-I. Shin, and S.-H. Kim, "Quercetin Inhibits Expression of Inflammatory Cytokines Through Attenuation of NF- κ B and p38 MAPK in HMC-1 Human Mast Cell Line," *Inflammation Research* 56 (2007): 210–215, <https://doi.org/10.1007/s00011-007-6172-9>.
6. N.-H. Nam, "Naturally Occurring NF-KB Inhibitors," *Mini - Reviews in Medicinal Chemistry* 6 (2006): 945–951, <https://doi.org/10.2174/138955706777934937>.
7. A. Olivera, T. W. Moore, F. Hu, A. P. Brown, A. Sun, D. C. Liotta, J. P. Snyder, Y. Yoon, H. Shim, A. I. Marcus, A. H. Miller, and T. W. Pace, "Inhibition of the NF-KB Signaling Pathway by the Curcumin Analog, 3,5-Bis(2-pyridinylmethylidene)-4-piperidone (EF24)," *International Immunopharmacology* 12 (2012): 368–377, <https://doi.org/10.1016/j.intimp.2011.12.009>.

8. M. A. Carluccio, L. Siculella, M. A. Ancora, M. Massaro, E. Scoditti, C. Storelli, F. Visioli, A. Distanti, and R. de Caterina, "Olive Oil and Red Wine Antioxidant Phenolics Inhibit Endothelial Activation: Antiatherogenic Properties of Ischemic Conditioning," *Arteriosclerosis, Thrombosis, and Vascular Biology* 23 (2003): 622–629, <https://doi.org/10.1161/01.ATV.0000062884.69432.AO>.
9. K. E. Heim, A. R. Tagliaferro, and D. J. Bobilya, "Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationships," *Journal of Nutritional Biochemistry* 13 (2002): 572–584, [https://doi.org/10.1016/S0955-2863\(02\)00208-5](https://doi.org/10.1016/S0955-2863(02)00208-5).
10. D. R. Reed and A. Knaapila, "Genetics of Taste and Smell: Lessons From Animal Models and Human Studies," *Progress in Molecular Biology and Translational Science* 94 (2010): 213–240, <https://doi.org/10.1016/B978-0-12-375003-7.00008-X>.
11. D. A. Deshpande, W. C. H. Wang, E. L. McIlmoyle, K. S. Robinett, R. M. Schillinger, S. S. An, J. S. K. Sham, and S. B. Liggett, "Bitter Taste Receptors on Airway Smooth Muscle Bronchodilate by Localized Calcium Signaling and Reverse Obstruction," *Nature Medicine* 16 (2010): 1299–1304, <https://doi.org/10.1038/nm.2237>.
12. J. Chandrashekar, K. L. Mueller, M. A. Hoon, E. Adler, L. Feng, W. Guo, C. S. Zuker, and N. J. Ryba, "T2Rs Assemble Into a Family of G Protein-Coupled Receptors That Respond to Bitter Compounds," *Cell* 100 (2000): 703–711, [https://doi.org/10.1016/S0092-8674\(00\)80706-0](https://doi.org/10.1016/S0092-8674(00)80706-0).
13. F. Jalševac, M. Descamps-Solà, C. Grau-Bové, H. Segú, T. Auguet, F. X. Avilés-Jurado, F. Balaguer, R. Jorba, R. Beltrán-Debón, M. T. Blay, X. Terra Barbadora, M. Pinent, and A. Ardévol, "Characterization of the Expression of Taste Receptors and Their Signaling Molecules in the Human Gastrointestinal Tract and Their Associations With Obesity," *Front Endocrinol (Lausanne)* 15 (2024): 1436580, <https://doi.org/10.3389/fendo.2024.1436580>.
14. K. I. Liszt, J. P. Ley, B. Lieder, M. Behrens, V. Stöger, A. Reiner, C. M. Hochkogler, E. Köck, A. Marchiori, J. Hans, S. Widder, G. Krammer, G. J. Sanger, M. M. Somoza, W. Meyerhof, and V. Somoza, "Caffeine Induces Gastric Acid Secretion via Activation of Bitter Taste Receptors TAS2R43 and TAS2R7," *PNAS* 114 (2017): E6260–E6269, <https://doi.org/10.1073/pnas.1703728114>.
15. K. I. Liszt, R. Eder, S. Wendelin, and V. Somoza, "Identification of Bitter-Tasting Compounds in Red Wine That Influence Gastric Acid Secretion," *Journal of Agricultural and Food Chemistry* 63 (2015): 7033–7041, <https://doi.org/10.1021/acs.jafc.5b02879>.
16. S. Sterneder, V. Stoeger, C. A. Dugulin, K. I. Liszt, A. Di Pizio, K. Kornthuer, A. Dunkel, R. Eder, J. P. Ley, and V. Somoza, "Identification of the Red Wine Peptide GR-9 and the Color Compound Malvidin-3-glucoside as Stimulators of Gastric Acid Secretion," *Journal of Agricultural and Food Chemistry* 69 (2021): 10510–10521, <https://doi.org/10.1021/acs.jafc.1c03061>.
17. J. A. Rothwell, J. Perez-Jimenez, V. Neveu, A. Medina-Remón, N. M'hiri, P. García-Lobato, C. Manach, C. Knox, R. Eisner, D. S. Wishart, and A. Scalbert, "Phenol-Explorer 3.0: A Major Update of the Phenol-Explorer Database to Include Compounds Generated by the Human Metabolism," *Database (Oxford)* 2013 (2013): bat070, <https://doi.org/10.1093/database/bat070>.
18. J. Tiroch, S. Sterneder, A. Di Pizio, B. Lieder, K. Hoelz, A.-K. Holik, M. Pignitter, M. Behrens, M. Somoza, J. P. Ley, and V. Somoza, "Bitter-Tasting Hops Compounds Alpha-Acids, Beta-Acids, and Iso-alpha-Acids Induce Gastric Acid Secretion via Activation of TAS2R4 and TAS2R14," *Journal of Agricultural and Food Chemistry* 69 (2021): 2802–2813, <https://doi.org/10.1021/acs.jafc.0c07058>.
19. W. S. U. Roland, L. van Buren, H. Gruppen, M. Driesse, R. J. Gouka, G. Smit, and J.-P. Vincken, "Bitter Compounds as Molecular Determinants for Dietary Choices: Identification of TAS2R Receptors Yielding Successive Profiles of Food Compounds," *Journal of Agricultural and Food Chemistry* 61 (2013): 10106–10119, <https://doi.org/10.1021/jf403387p>.
20. K. I. Liszt, J. Hans, J. P. Ley, E. Köck, and V. Somoza, "Theobromine Induces Gastric Acid Secretion by Activation of the Bitter Taste Receptor

- tor TAS2R43,” *Journal of Agricultural and Food Chemistry* 65 (2017): 3833–3841, <https://doi.org/10.1021/acs.jafc.7b01051>.
21. S. Sterneder, J. Seitz, J. Kiefl, E. Rottmann, M. Liebig, M. Blings, S. Seilwind, Y. Zhou, J. Wei, H. Guan, Q. Zhu, J. Kreißl, K. Lamottke, J. P. Ley, and V. Somoza, “Identification of Bitter-Tasting Coffee Compounds Stimulating Gastric Acid Secretion via Bitter Taste Receptors,” *Journal of Agricultural and Food Chemistry* 72 (2024): 19688–19703, <https://doi.org/10.1021/acs.jafc.4c04583>.
22. C. Weiss, M. Rubach, R. Lang, E. Seebach, S. Blumberg, O. Frank, T. Hofmann, and V. Somoza, “Identification of a Coffee Compound That Effectively Inhibits Mechanisms of Gastric Acid Secretion in Human Gastric Parietal Cells (HGT-1),” *Journal of Agricultural and Food Chemistry* 58 (2010): 2720–2726, <https://doi.org/10.1021/jf903614d>.
23. M.-R. Piqué-Borràs, J. Röhrli, and G. Küntle, “Herbal Amara Extract Induces Gastric Fundus Relaxation via Inhibition of the M₂ Muscarinic Receptor,” *Neurogastroenterology and Motility* 37 (2025): 14924, <https://doi.org/10.1111/nmo.14924>.
24. S. Czigle, S. Bittner Fialová, J. Tóth, P. Mučaji, M. Nagy, and B. O. T. O. On, “Bitter Plants—Amara: From Traditional Use to Food Supplement,” *Molecules* 27 (2022): 2881, <https://doi.org/10.3390/molecules27092881>.
25. W. Meyerhof, C. Batram, C. Kuhn, A. Brockhoff, E. Chudoba, B. Bufo, G. Appendino, and M. Behrens, “The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors,” *Chemical Senses* 35 (2010): 157–170, <https://doi.org/10.1093/chemse/bjp092>.
26. S. Quideau, D. Deffieux, C. Douat-Casassus, and L. Pouységu, “Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis,” *Angewandte Chemie (International ed in English)* 50 (2011): 586–621, <https://doi.org/10.1002/anie.201000044>.
27. S. Soares, S. Kohl, S. Thalmann, N. Mateus, W. Meyerhof, and V. de Freitas, “Different Phases of Dietary Phenols and Salivary Proteins Interaction: The Influence of Salivary Proteins on Tannin Bitterness and Astringency,” *Journal of Agricultural and Food Chemistry* 61 (2013): 931–939, <https://doi.org/10.1021/jf304198k>.
28. S. Soares, M. S. Silva, I. García-Estevéz, P. Großmann, N. Brás, E. Brandão, N. Mateus, V. de Freitas, M. Behrens, and W. Meyerhof, “Human Bitter Taste Receptors Are Activated by Different Classes of Polyphenols,” *Journal of Agricultural and Food Chemistry* 66 (2018): 8814–8823, <https://doi.org/10.1021/acs.jafc.8b03569>.
29. D. Snyder, K. Fast, and L. Bartoshuk, “Beauty and the Blech: Using Cross-Modality Matching to Identify Differences in Sensory Experience,” *J Conscious Stud* 11 (2004): 96–107.
30. P. Richter, K. Sebal, K. Fischer, M. Behrens, A. Schnieke, and V. Somoza, “Development of a Human Gastric Parietal Cell Model (HGT-1) Stably Expressing the Bitter Taste Receptor TAS2R43,” *Journal of Agricultural and Food Chemistry* 70 (2022): 14757–14768, <https://doi.org/10.1021/acs.jafc.2c05228>.
31. T. L. Riss, R. A. Moravec, A. L. Niles, S. Duellman, H. A. Benink, T. J. Worzella, and L. Minor, *Assay Guidance Manual: Cell Viability Assays* (Bethesda (MD), 2004).
32. P. Richter, G. Andersen, K. Kahlenberg, A. U. Mueller, P. Pirkwieser, V. Boger, and V. Somoza, “Identification of the Casein-Derived Peptides YPFPG and YPF as Stimulators of Gastric Acid Secretion via the Bitter Taste Receptor TAS2R43,” *Journal of Agricultural and Food Chemistry* 72 (2024): 7076–7088, <https://doi.org/10.1021/acs.jafc.3c09085>.
33. O. Monago-Maraña, I. Durán-Merás, T. Galeano-Díaz, and A. La Muñoz de Peña, “Determination of Theobromine, Theophylline, Caffeine and Saccharin in Food Samples by a High-Order Calibration Strategy and Liquid Chromatography With a Diode Array Detector,” *Food Chemistry* 196 (2016): 849–856, <https://doi.org/10.1016/j.foodchem.2015.10.041>.
34. J. Walker, J. Hell, K. I. Liszt, M. Dresel, M. Pignitter, T. Hofmann, and V. Somoza, “Identification of Coffee Components That Stimulate Gastric Acid Secretion in Human Gastric Parietal Cells (HGT-1),” *Journal of Agricultural and Food Chemistry* 60 (2012): 1405–1412, <https://doi.org/10.1021/jf204306z>.
35. K. Gradl, P. Richter, and V. Somoza, “Casein and Its Digestion Products Attenuate Caffeine-Induced Gastric Acid Secretion in HGT-1 Cells,” *Food Chemistry* 465 (2025): 144174, <https://doi.org/10.1016/j.foodchem.2025.144174>.
36. H. N. Orth, P. Pirkwieser, J. Benthin, M. Koehler, S. Sterneder, E. Parlar, E. Schaudy, J. Lietard, T. Michel, V. Boger, A. Dunkel, M. M. Somoza, and V. Somoza, “Deciphering Gastric Bitter Taste Receptor Activation by Diet-Derived Compounds Using High-Density Peptide Microarrays,” *International Journal of Molecular Sciences* 26 (2025): 6017, <https://doi.org/10.3390/ijms26136017>.
37. P. Richter, K. Sebal, K. Fischer, A. Schnieke, M. Jilati, V. Mittermeier-Klessinger, and V. Somoza, “Gastric Digestion of the Sweet-Tasting Plant Protein Thaumatin Releases Bitter Peptides That Reduce *H. pylori* Induced Pro-Inflammatory IL-17a Release via The TAS2R16 Bitter Taste Receptor,” *Food Chemistry* 448 (2024): 139157, <https://doi.org/10.1016/j.foodchem.2024.139157>.
38. A. Blainski, G. C. Lopes, and J. C. P. de Mello, “Application and Analysis of the Folin-Ciocalteu Method for the Determination of the Total Phenolic Content From *Limonium Brasiliense* L.,” *Molecules* 18 (2013): 6852–6865, <https://doi.org/10.3390/molecules18066852>.
39. C. L. Labois, C. Augeron, M.-H. Couturier-Turpin, C. Gespach, A.-M. Cheret, and F. Potet, “Characterization of a Newly Established Human Gastric Cancer Cell Line HGT-1,” *Cancer Research* 42 (1982): 1541–1548.
40. K. L. Pierce, R. T. Premont, and R. J. Lefkowitz, “Seven-Transmembrane Receptors,” *Nature Reviews Molecular Cell Biology* 3 (2002): 639–650, <https://doi.org/10.1038/nrm908>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting information to this manuscript include data on the reductions by the knock-out and knock-down approaches (Table S1), cell viability (Figure S1), sensory results (Figure S2), impact on TAS2R gene expression (Figure S3), and transfection efficiency (Figure S4).

Supporting File: mnfr70443-sup-0001-SupMat.pdf.