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Original Article

Effect of Different Samples of *Viruddha Āhāra* on Lactobacillus Inhibition-An Exploratory *in vitro* Study

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ABSTRACT

Background: *Viruddha Āhāra* (incompatible food combinations) are described in Ayurveda as dietary practices that disturb digestion and metabolism, leading to *doshic* imbalance and disease. Though classically established, their biological basis remains poorly explored in modern terms. Since gut microbiota, especially *Lactobacillus* species, play a crucial role in digestion and host health, this study aimed to assess the effect of selected *Viruddha Āhāra* combinations on *Lactobacillus plantarum* growth *in vitro*. **Objectives:** To evaluate and compare the influence of classical *Viruddha Āhāra* combinations and their individual components on the growth of *Lactobacillus plantarum* using standard microbiological techniques. **Materials and Methods:** Four *Viruddha Āhāra* categories were selected: (1) honey-ghee (*Matra Viruddha*), (2) unripe, ripe, and overripe mango (*Sampat Viruddha*), (3) tea-ice cream (*Vīdhi Viruddha*), and (4) milk-orange (*Samyoga Viruddha*). Sterile samples were inoculated with *L. plantarum* (0.5 McFarland standard) in MRS broth and incubated under CO_2 conditions for 48 hr. Serial dilutions were plated using the quadrant streak method, and colony-forming units (CFU) were calculated. **Results:** The honey sample showed no growth, while ghee and honey-ghee (1:1) showed reduced CFU (10×10^5 and 15×10^5) compared to control (29×10^5). Unripe mango supported highest growth (70×10^5) followed by ripe (44×10^5) and overripe (29×10^5). Tea and ice cream combination (25×10^5) showed slightly higher growth than control (23×10^5). The milk-orange mixture exhibited complete inhibition (0 CFU), confirming classical *āmla-kṣīra viruddha* (milk-sour incompatibility). **Conclusion:** The study provides preliminary experimental evidence that certain *Viruddha Āhāra* combinations can inhibit *Lactobacillus* growth, suggesting potential disruption of gut microbial balance. These findings support Ayurvedic dietary cautions and encourage further exploration using multi-strain and *in vivo* models.

Keywords: *Āhāra*, *ama*, Gut Microbiome, Incompatible Diet, Serial Dilution, *Viruddha*.

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INTRODUCTION

Āhāra is an integral factor for providing a healthy life. Its prime importance is signified by its placement as one among the *trayopastambhas* (three-pillars to support a healthy living) (Vagbhata, Annaraksha Adyaya, 2017). More than the appropriate selection of *Āhāra* (food) specific to the individual, time, place, doshic predominance, etc., it is crucial for anyone to know the right combinations of food so as to maximize its health benefits. On the contrary improper combinations of *Āhāra* is understood as *Viruddha* (incompatible). Unlike the regular *Āhāra* which bestows good health and growth, *Viruddha Āhāra* (incompatible food) hampers entirely the digestion, metabolism, growth and other important factors needed for a healthy and long life. The innate nature of such *Āhāra* causes abnormal aggravation of doshas without expelling them out of the body (Agnivesa, Atreya Bhadrakapeeya Adhyaya, 2018). As a result, it produces symptoms like *Garavisha* (poison) and diseases like impotency, blindness, skin disorders, fistula, flatulence, and even death (Vagbhata, Annaraksha Adyaya, 2017) (Agnivesa, Atreya Bhadrakapeeya Adhyaya, 2018). Some of such combinations that are prone to cause the above said complications, as mentioned in the classics include - *Kala Viruddha* (incompatible by time), *Matra Viruddha* (incompatible by proportion), *Satmya Viruddha* (incompatible by habituation), *Samskara Viruddha* (incompatible by processing), *Veerya Viruddha* (incompatible by potency), *Samyoga Viruddha* (incompatible by combination), *Krama Viruddha* (incompatible by order) etc., (Agnivesa, Atreya Bhadrakapeeya Adhyaya, 2018).

Lactobacillus species are the commonest occurring micro-organisms accounting to 6% of the duodenal bacteria and 0.3% of colonic bacteria in humans. Although the abundance of their presence in the human gut varies due to diverse factors, their presence is identified as functional probiotics in regulating biological processes like - immunoregulation, anti-cancer

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activity, metabolic regulation of the host, improvement in intestinal barrier functions etc., Specific *Lactobacilli* strains were shown to inhibit abnormal bacterial presence in the gut and restore its normal flora. They also reduce the intestinal inflammation by downregulating the inflammatory response particularly in diseases like intestinal cancer and in other bowel inflammatory conditions (Tobias, 2025). The activity of *Lactobacillus* in regulating digestion and metabolism is one such among many parallels to the concept of *Agni* (digestive fire) and *Āhāra*paka (digestion and metabolism) in Ayurveda. While we know that *Viruddha Āhāra* is a major contributor to impaired *agni*, the disabled metabolism due to a weak *agni* further tends to create ama (metabolic toxins) after fermentation of the residual undigested food matter inside gut (Hridya, 2025). While the gut microbiome has a tendency get altered under the influence of diet and nutrition, *Viruddha Āhāra* may act as a potent factor to cause dysbiosis eventually leading to a disturbed gut microbiota balance (Shen, 2025). Hence it is plausible that *Viruddha Āhāra* can potentially affect the growth and survival of beneficial gut flora, particularly *Lactobacillus* species. Unfortunately, there is a lack of experimental evidence to support the above mentioned corelation. Establishing this link could provide a modern biological basis for understanding *Viruddha Āhāra* at the level of digestive and metabolic impairment. Based on these grounds, the present study was attempted to explore the effect of different *Viruddha Āhāra* combinations in the growth of *Lactobacillus* species through in-vitro techniques.

MATERIALS AND METHODS

Preparation of *Viruddha Āhāra* samples was done in the practical laboratory of Rasashastra and Bhaishajya Kalpana of KAHER's Shri BMK Ayurveda Mahavidyalaya, Belagavi, Karnataka.

The study was conducted at the micro-biology laboratory of Dr. Prabhakar Kore Basic Science Research Centre, Belagavi, Karnataka.

4 samples of *Viruddha Āhāra* were selected for the present study as follows

Matra Viruddha - equal proportions of honey and ghee.

Sampat Viruddha - Unripe mango, ripened mango and over-ripened mango.

Vidhi Viruddha - Tea with ice cream.

Samyoga Viruddha - Orange and milk.

Ingredients like - Honey and Ghee were procured from GMP certified Ayurvedic Pharmacy in Belagavi. Unripe mango, ripened mango, over-ripened mango, orange, milk, tea and ice cream (vanilla flavour) were purchased from the local markets of Belagavi.

Viruddha Āhāra Sample 1 (Matra Viruddha)

Only honey (MV 1): Only honey was stored in a sterile container.

Only ghee (MV 2): Only ghee was stored in a sterile container.

Honey and ghee (MV 3): Equal proportions of honey and ghee was made into a homogenous mixture and stored in a sterile air tight container.

Viruddha Āhāra Sample 2 (Sampat Viruddha)

Unripe mango (SV 1): Unripe mango was blended into a paste and the juice was extracted by manual pressing. The same was stored in a sterile container.

Ripened mango (SV 2): Ripened mango pulp was obtained by blending in a blender without adding water after peeling the skin. The pulp was then expressed into concentrated juice and stored in sterile container.

Over-ripened mango (SV 3): Over-ripened mango's juice was extracted the same way as described above and stored in a sterile container.

Viruddha Āhāra Sample 3 (Vidhi Viruddha)

Only tea (VV 1): Milk tea procured from market was stored in a sterile container and labelled as VV 1.

Only ice-cream (VV 2): Required amount of ice-cream from the market was stored in a sterile container and labelled as VV 2.

Tea and ice-cream (VV 3): Hot milk and cold ice-cream were mixed together in equal proportions and stored in the sterile container.

Viruddha Āhāra Sample 4 (Samyoga Viruddha)

Only milk (SY 1): Milk from the market was stored directly in a sterile container.

Only orange (SY 2): Orange skin was peeled and the blended in a mixer. The juice was filtered and stored in a sterile container.

Milk and orange (SY 3): Equal quantities of milk and orange juice extracted was mixed together. After curdling it was stored in a sterile container.

All the above samples were subjected to *in vitro Lactobacillus* inhibition test at the micro-biology laboratory of Dr. Prabhakar Kore Basic Science Research Centre, Belagavi, Karnataka.

Lactobacillus inhibition test

Materials

Sterile 0.85% NaCl/PBS, sterile micropipettes (P10, P200, P1000) + tips, sterile serological pipettes (1, 5, 10 mL), vortex mixer, sterile petri dishes (MRS agar plates), sterile spreaders / glass beads, CO_2 incubator (37°C), sterile dilution tubes (50 × 1.5 mL microtubes + 10 × 15 mL centrifuge tubes), sterile filtration

units (0.22 µm) for sample extracts, McFarland standard, colony counter, biosafety cabinet, PPE, disinfectants, autoclave access, marker pens, plate labels, timer. Culture media and reagents were: De Man, Rogosa and Sharpe (MRS) broth and MRS agar (Manufacturer: HIMEDIA, catalogue no. MV369 and MV 641 respectively), analytical grade Sodium Chloride (NaCl) and sterile distilled water. All glassware and media were sterilized by autoclaving at 121°C for 15-20 min prior to use.

Preparation of media

MRS Broth and MRS Agar were prepared according to the manufacturer's instructions. For the batches used in this study the following were prepared (batch volumes adjusted as required).

MRS Broth: 55.15 g of MRS powder per L (prepared here as 1.65 g in 30 mL for a small batch), dissolved in distilled water and autoclaved at 121°C for 15-20 min.

MRS Agar: 67.15 g of MRS agar powder per L (prepared here as 5.372 g in 80 mL for a small batch), dissolved in distilled water, autoclaved at 121°C for 15-20 min, poured (≈20 mL per plate) into sterile Petri dishes and allowed to solidify and dry in a sterile hood. Plates were stored inverted at 4°C for short term and equilibrated to room temperature before use.

Preparation and standardization of bacterial inoculum

A fresh overnight culture of *L. plantarum* was prepared in MRS broth at 37°C. The culture was adjusted to 0.5 McFarland standard (≈1.0-2.0 × 10^8 CFU/mL) by dilution with sterile 0.85% NaCl (w/v) or MRS broth. Turbidity was matched to the McFarland standard visually. This standardized suspension served as the inoculum.

Preparation and sterilization of *Viruddha Āhāra* sample extracts

Viruddha Āhāra sample were sterilized by passage through a sterile 0.22 µm syringe filter immediately prior to use and stored on ice until added to reaction mixtures. A sterile vehicle control was included.

Reaction setup (bacteria + sample exposure)

Reaction mixtures were prepared in sterile microcentrifuge tubes in triplicate for each sample and control. Each tube contained a final volume of 1.0 mL composed as follows:

800 µL sterile MRS broth

100 µL sterile-filtered sample extract (test) or 100 µL vehicle (vehicle control) or 100 µL sterile broth (positive growth control).

100 µL *L. plantarum* suspension (0.5 McFarland)

Thus, bacterial inoculum comprised 10% (v/v) of the reaction. A sterility control (MRS broth + sample extract, no bacteria) was included to confirm sample sterility. Reaction tubes were vortexed briefly to mix and incubated at 37°C in a CO_2 incubator (5% CO_2) for 24 hours. All incubations and handling were performed aseptically.

Serial dilution procedure

After incubation, each reaction tube was vortexed and processed for serial ten-fold dilutions to obtain a dilution series up to 10^{-5}. Sterile 0.85% NaCl (w/v) was used as diluent (prepare fresh; typical 0.85 g NaCl per 100 mL sterile distilled water). The dilution scheme was as follows (all steps in sterile 1.5 mL microtubes):

Label dilution tubes 10^{-1} through 10^{-5} and add 900 µL sterile 0.85% NaCl to each.

Add 100 µL of the incubated reaction mixture to tube 10^{-1} → vortex 5-10 s.

Transfer 100 µL from 10^{-1} to 10^{-2} → vortex. Repeat sequentially to obtain 10^{-3}, 10^{-4} and 10^{-5} dilutions. (Five 10-fold dilutions yields a final plated dilution factor of 10^{-5}.)

Plating (quadrant method) and incubation

Quantitative plating was performed using the quadrant layout described in the study protocol (four plates used; each plate subdivided into four quadrants to allow simultaneous plating of three test samples and one control per plate). To improve quantitative reliability, 100 µL of each dilution to be plated was spread within the assigned quadrant (see below). The plating scheme:

Plate A: Honey | Ghee | Honey + Ghee | Control (inoculum only).

Plate B: Unripe mango | Ripe mango | Overripe mango | Control.

Plate C: Tea | Ice-cream | Tea + Ice-cream | Control.

Plate D: Orange | Milk | Orange + Milk | Control.

Implementation details for quadrant plating

Each quadrant received 100 µL of the same dilution (recommended: plate at least from two dilutions per sample, e.g., 10^{-3} and 10^{-5}, to ensure countable colonies). In practice plate 100 µL of the 10^{-4} and 10^{-5} dilutions on separate replicate plates (or duplicate quadrant plates) if feasible.

Using a sterile glass spreader or sterile glass beads, the inoculum was spread evenly within the quadrant area, taking care to avoid cross-contamination between quadrants. Allow the spot to absorb/dry with lid closed in the biosafety cabinet.

Plates were incubated inverted at 37°C in a CO_2 incubator (5% CO_2) for 24-48 hr and inspected at 24 hr and 48 hr.

After incubation, colonies in each quadrant were counted. For quantitative comparison the plate/quadrant with colony counts in the readable range (30-300 colonies) was selected. CFU per

mL of the original reaction mixture was calculated as: Number of colonies X dilution factor (10^5).

RESULTS

The growth pattern of *Lactobacillus plantarum* varied markedly across different samples of *Viruddha Āhāra* combinations. For Sample 1 (Honey-Ghee group), *Lactobacillus* growth was completely inhibited in honey (0 CFU), whereas ghee and the honey-ghee combination showed moderate growth (10×10^5 and 15×10^5 CFU, respectively) compared to the control (29×10^5 CFU) (Figure 1). In Sample 2 (Mango group), the highest colony count was observed with unripe mango (70×10^5 CFU), followed by ripe mango (44×10^5 CFU) and overripe mango (29×10^5 CFU), all showing greater growth than the control (13×10^5 CFU) (Figure 2). For Sample 3 (Tea-Ice cream group), minimal growth was seen with tea (4×10^5 CFU) and ice cream (9×10^5 CFU), while the combination of tea and ice cream showed comparable growth to the control (25×10^5 and 23×10^5 CFU, respectively) (Figure 3). In Sample 4 (Milk-Orange group), milk (13×10^5 CFU) and orange (23×10^5 CFU) individually supported bacterial growth; however, their combination (milk with orange) completely inhibited *Lactobacillus* growth (0 CFU), similar to the control plate (0 CFU) (Figure 4). Table 1 shows the complete tabulated results for comparison with different samples of *Viruddha Āhāra*.

DISCUSSION

The present study aimed at bridging and drawing a plausible link between *Viruddha Āhāra* and *Lactobacillus* growth. The results obtained here seem to give varied results with each of the *Viruddha Āhāra* sample used. Classical texts suggest many such food substances to cause incompatibility. Out of them, the present study selected the following incompatibles from Charaka Samhita - honey and ghee mixed in equal proportions, any fruit that is consumed at its improperly ripened stage, mixing hot substances together with cold ones and mixing of milk with sour fruit (orange) (Agnivesa, Atreya Bhadrakapeeya Adhyaya, 2018).

With the first sample, we see that there is a complete inhibition of growth of *Lactobacilli* with MV1 (only honey). Honey exhibits strong antimicrobial activity attributable to multiple physicochemical factors. Its high osmolarity and low water activity create an unfavourable environment for microbial proliferation, as the supersaturated sugar content exerts an osmotic effect that draws water out of microbial cells, thereby inhibiting their growth in vitro. In addition, honey contains several antimicrobial constituents that contribute to its inhibitory action. Upon dilution, glucose oxidase present in honey generates hydrogen peroxide, which serves as a potent antimicrobial agent. Certain varieties, such as Manuka honey, possess additional non-peroxide antibacterial components, including methylglyoxal and various phenolic compounds, which exhibit bacteriostatic or bactericidal effects against a broad spectrum of microorganisms. Furthermore, the acidic nature of honey (typically with a pH between 3.2 and 4.5) enhances its antimicrobial efficacy by creating an environment unsuitable for most pathogenic bacteria (Almasaudi, 2020). Sample 1 has shown increased colony count when used with ghee alone. Ghee, being a clarified butter composed largely of triglycerides, offers a lipid-rich matrix that may provide protective microenvironments for *Lactobacillus plantarum*. Analogous studies show that low-water activity matrices, such as certain oils or low-moisture food carriers, help maintain viability of lactobacilli under osmotic or heat stress. For example, *L. plantarum* cultures survive better under moderate osmotic stress in media adjusted for water activity (Champagne, 2012). Oil-based matrices have been documented to preserve *L. rhamnosus* viability during storage and stress exposure substantially better than non-lipid matrices (Endo, 2014). Likewise, structured lipid or gel systems provide protection in simulated digestive or environmental insults (Melchior, 2021). However, because ghee is essentially free of free water and readily metabolizable carbohydrates, it does not provide optimal growth conditions. Thus, any increase in colony counts in serial dilution assays using ghee rather than a full nutrient medium likely reflects survival or limited proliferation of the initial inoculum, perhaps via use of residual nutrients or lipid metabolism rather than robust growth. Furthermore, potential antimicrobial trace components in ghee (from processing or natural milk contents) could diminish growth relative to control media.

When comparing growth in ghee alone vs the 1:1 honey + ghee mixture, and then vs control medium, the following factors likely contribute the following - Mixing honey with ghee creates a composite environment where the lipid phase can partially shield

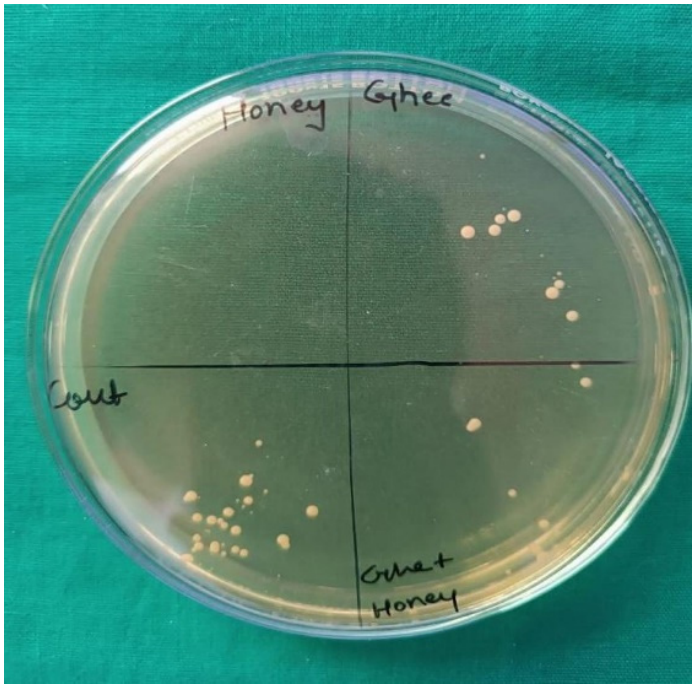

Figure 1: Result of *Lactobacillus* growth seen in plate 01 using samples MV1, MV2 and MV3.

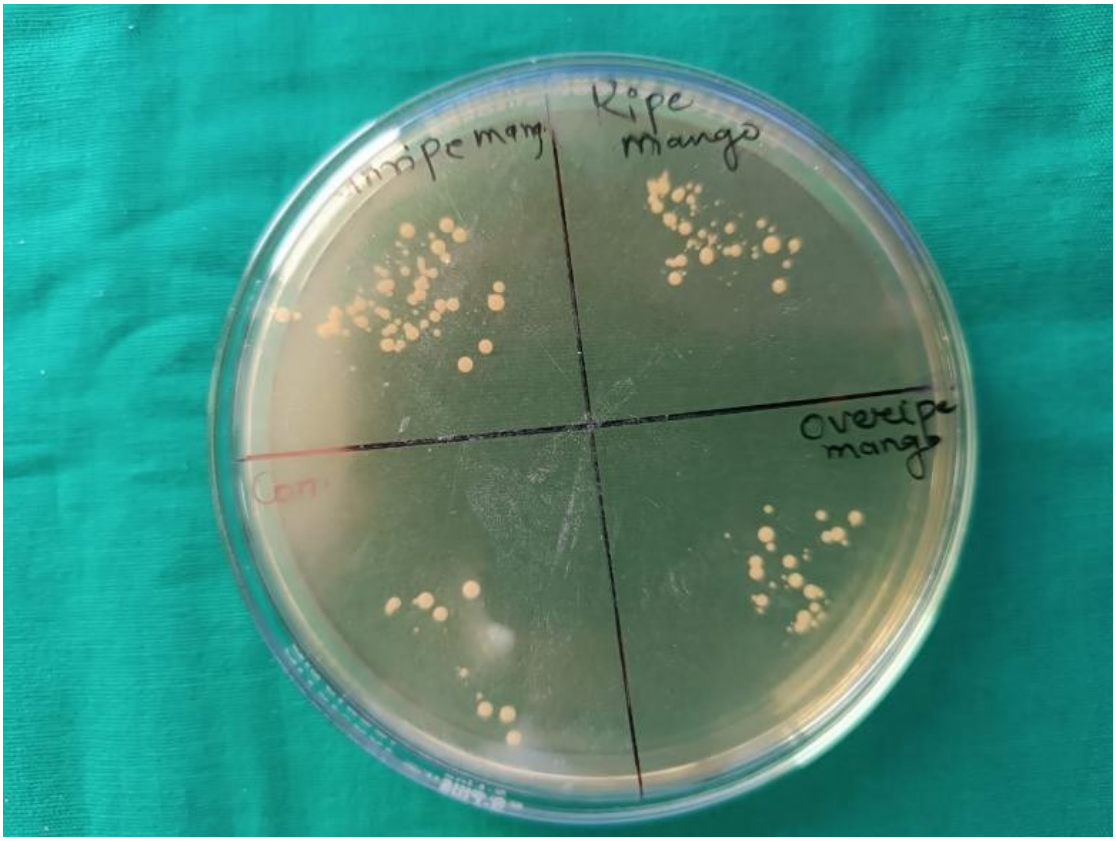

Figure 2: Result of *Lactobacillus* growth seen in plate 02 using samples SV1, SV2 and SV3.

bacterial cells from the full strength of honey's aqueous phase. This reduces osmotic or chemical stress (e.g. high sugar concentration, phenolics or peroxide) relative to pure honey, but still more stressful than the control medium. Lipids may sequester or limit the mobility/exposure of hydrogen peroxide and phenolics, reducing their bacteriostatic or bactericidal action. Also, phase separation / emulsion effects may reduce effective osmotic stress on bacteria. The physicochemical study of honey + ghee mixtures shows changes in pH, HMF, antioxidant and phenolic content when mixed, which supports this attenuation of honey's effect (Annapoorani, 2010) (Sowa, 2017). The result is that growth in the mixture is better than in ghee alone (which may lack water, carbs, or other growth-promoting factors), because the honey adds some residual nutrients and perhaps some water. But it is lower than in the full nutrient control, because the mixture is still imposing some inhibitory effects from honey, and lacks the full spectrum of nutrients present in control. Supporting evidence from animal studies shows that equal mixtures of honey + ghee generate increased formation of Amadori products, Advanced Glycation End products (AGEs), increased oxidative stress, altered incretins, liver mild inflammation, etc (Aditi, 2020). These effects imply that honey + ghee mixtures are biologically active / chemically different from pure honey or ghee, which parallels how the mixture in vitro might behave differently from either component alone.

All mango extract samples (unripe, ripe, over-ripe) enhanced *Lactobacillus* growth relative to the sterile control, with unripe mango yielding the highest CFU counts, followed by ripe and over-ripe samples. This pattern likely reflects ripening-associated changes in substrate composition and the microenvironment. Unripe mangoes contain more complex carbohydrates, oligosaccharides, and dietary fibre that may act as prebiotic substrates for *Lactobacillus* sp., supporting robust growth (e.g., mango polyphenols & fibre studies showing increased *L. plantarum* with mango supplementation) (Kim, 2021). During ripening, starch is hydrolysed into simple sugars, which are more readily fermented but may also alter osmotic pressure or pH favourably compared to over-ripe fruit. In over-ripening, biochemical decomposition, the activity of native microbes, and accumulation of organic acids or secondary metabolites may create less favourable conditions, diminishing net cultivable growth. Studies of mango juice fermentation demonstrate shifts in phenolic profiles (bound vs free), organic acid accumulation, and altered antioxidant capacities in later fermentation stages, which may parallel changes in overly ripe fruit environments (Liu, 2025; Tran, 2024). Finally, some phenolic compounds present in mango fruit can stimulate *Lactobacillus* growth, but breakdown products or oxidation derivatives formed during over-ripening or native microbial metabolism may exert inhibitory effects, either directly or by modulating substrate-accessibility. These hypotheses remain to be tested explicitly using fractionation of mango extracts by

ripeness, measurement of residual microbial metabolites, and comparison of simple sugar vs complex carbohydrate content.

The observed pattern—marked inhibition of *Lactobacillus* by tea extract alone, modest growth supported by ice-cream, and restoration (towards control) in the tea + ice-cream mixture—may result from interactions between tea polyphenols and dairy constituents. Studies have shown that milk proteins such as caseins and β-lactoglobulin bind tea polyphenols, reducing the free (active) polyphenol fraction and thereby decreasing antimicrobial potency (e.g. milk-tea model: protein binding rate up to ~50% and corresponding drop in antioxidant/potency) (Chen, 2024; Kankis, 2011; Zhou, 2025). Meanwhile, ice-cream's sugars, lactose, milk proteins, and fats serve both as fermentable substrate and protective factor for *Lactobacillus* survival and growth, as documented in probiotic ice-cream studies where bacterial viability remains high in dairy matrices during storage and stress conditions (Mohammadi, 2011; Panwar, 2019). Thus, the net effect of the combination is a balance of detoxification (protein binding) and nutrient supplementation. These results illustrate that '*Viruddha*' combinations may interact chemically to change biological effects — sometimes worsening, sometimes mitigating harm — and underscore that in vitro microbial outcomes are only one dimension of incompatibility.

The fact that milk and orange juice individually supported *Lactobacillus* growth (13×10^5 and 23×10^5 CFU, respectively) but their 1:1 mixture yielded no recoverable colonies is consistent with classical Ayurvedic caution against *kṣīra-āmla* combinations. Mechanistically, mixing acidic orange juice with milk can cause casein coagulation/curdling, serum precipitation, and pH drop (see Abbasi *et al.*, on milk-orange stability) (Abbasi, 2013). The curdling reaction, as described in classic milk coagulation studies, leads to aggregation of casein micelles and separation of curd and whey phases (Sohngen, 1937). Such coagulation may sequester bacterial cells in insoluble curds, restrict diffusion of nutrients, and subject bacteria to local acid stress or exposure to residual organic acids / phytochemicals from the juice. The antimicrobial potential of orange juice itself has been demonstrated in comparative food antimicrobial assays (Mothershaw et al., 2004), while lactic acid bacteria are known to grow in orange-juice media under favourable nutrient supplementation (Pérez et al., 2022; Mothershaw, 2004). However, a critical caveat is that in this assay the plate control also yielded zero colonies, indicating possible technical error (e.g., over-dilution, omission, plate/incubator fault). Thus, the observed inhibition in the milk + orange mixture must be interpreted with caution. We recommend repeating the experiments with independent replicates, including live/dead viability assays, pH measurement, separation and analysis of curd vs supernatant fractions, and controls using citric acid

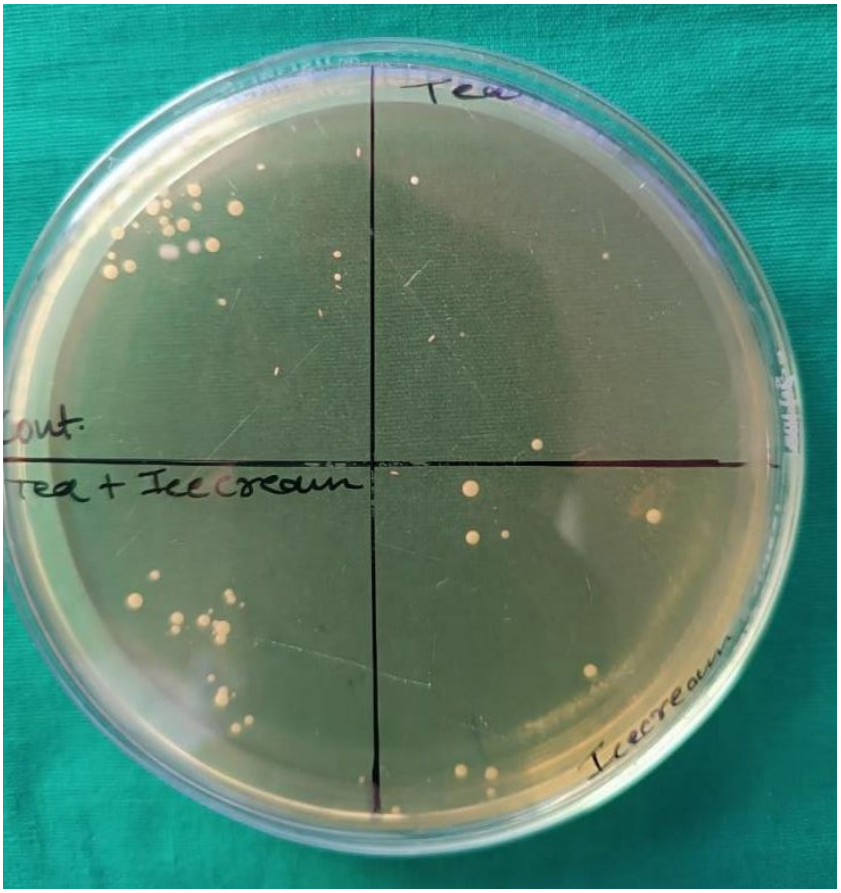

Figure 3: Result of *Lactobacillus* growth seen in plate 03 using samples VV1, VV2 and VV3.

Table 1: Results of *Lactobacillus* growth seen with different samples of *Viruddha Āhāra*.

Sample		Number of colonies formed	CFU
Viruddha Āhāra Sample 1	Honey (MV 1)	0	0
	Ghee (MV 2)	10	10×10^5
	Honey and ghee in equal quantity (MV 3)	15	15×10^5
	Control	29	29×10^5
Viruddha Āhāra Sample 2	Ripened mango (SV 1)	44	44×10^5
	Unripe mango (SV 2)	70	70×10^5
	Over-ripened mango (SV 3)	29	29×10^5
	Control	13	13×10^5
Viruddha Āhāra Sample 3	Tea (VV 1)	4	4×10^5
	Ice cream (VV2)	9	9×10^5
	Tea and ice-cream (VV3)	25	25×10^5
	Control	23	23×10^5
Viruddha Āhāra Sample 4	Milk (SY 1)	13	13×10^5
	Orange (SY 2)	23	23×10^5
	Milk and orange (SY 3)	0	0
	Control	0	0

CFU - Colony Forming Unit

alone to isolate acidity effects separate from juice-milk chemical interactions. Only after ruling out technical artifacts, can one assert a causal link between the milk-orange incompatibility and loss of *Lactobacillus* viability. While the observed in vitro inhibition offers a plausible correlate to the Ayurvedic *Viruddha* concept of *kṣīra-āmla*, extrapolation to in vivo or clinical relevance would demand further studies.

The present study represents a novel attempt to correlate classical Ayurvedic principles of *Viruddha Āhāra* with contemporary microbiological assessment. By evaluating the effect of selected incompatible food combinations on the growth of *Lactobacillus plantarum*, the work establishes an innovative bridge between traditional dietetics and gut microbial science. Use of standardized microbiological techniques such as serial dilution, quadrant plating, and controlled incubation under CO_2 conditions ensured internal validity and reproducibility of the observations. Inclusion of four representative *Viruddha Āhāra* categories-*Matra Viruddha* (honey-ghee), *Sampat Viruddha* (mangoes at different ripening stages), *Vīdhi Viruddha* (tea-ice cream), and *Samyoga Viruddha* (milk-orange)-provided a comprehensive evaluation across diverse incompatibility types. The quantitative enumeration of bacterial colonies (CFU) further strengthened the objectivity of the results and enabled direct comparison with the control group. The findings, particularly the complete inhibition observed with the milk-orange combination, offer experimental support to classical textual cautions regarding such food pairings and open new avenues for exploring their impact on gut microbial balance.

However, certain limitations must be acknowledged. The study employed only a single bacterial species, *L. plantarum*, whereas the human gut harbours a complex consortium of microorganisms whose collective response may differ. The *in vitro* conditions used here do not fully replicate the dynamic physiological environment of the gastrointestinal tract, which includes digestive enzymes, bile, and varying pH levels. Occasional technical variations were noted, such as the absence of growth even in the control group of one plate, suggesting possible procedural or incubation inconsistencies. The study was performed without biological replicates or statistical validation, thereby limiting the strength of causal inference. Additionally, the mixtures were not subjected to physicochemical analysis (pH, curdling behaviour, or chemical composition), which could have clarified the mechanism of inhibition. Finally, while sample sterilisation was ensured before plating, this process itself might have altered certain thermolabile constituents influencing bacterial growth.

In spite of these limitations, the present investigation provides a valuable preliminary insight demonstrating that classical *Viruddha Āhāra* combinations can exert measurable inhibitory effects on beneficial gut flora. These findings underline the need for future research involving multiple microbial species, simulated gastrointestinal models, and detailed biochemical characterization to validate and expand the present observations.

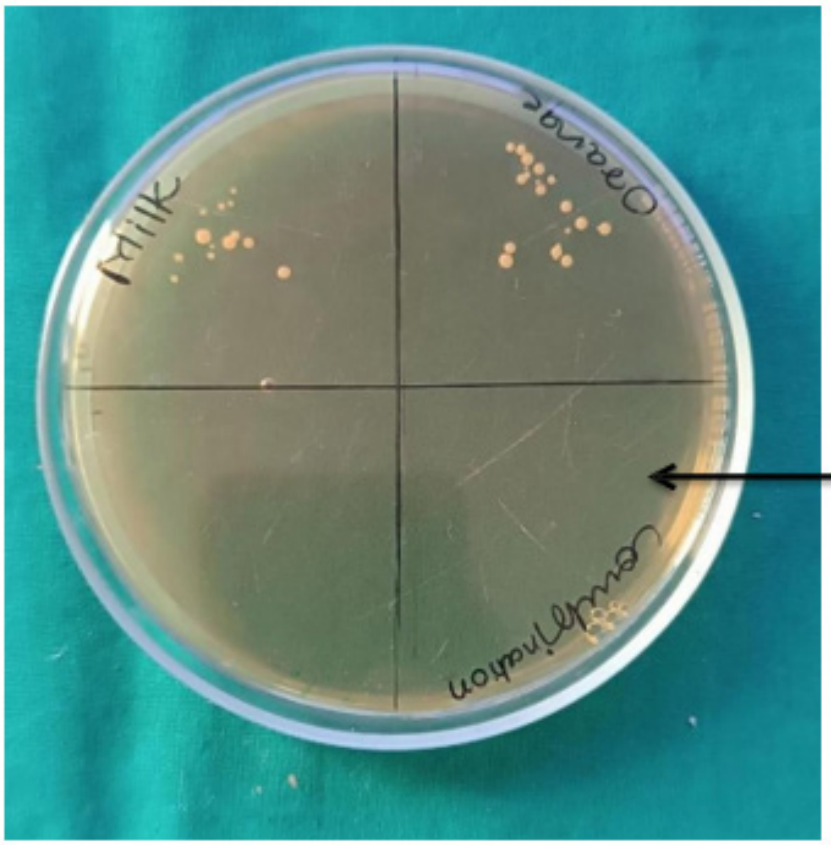

Figure 4: Result of *Lactobacillus* growth seen in plate 04 using samples SY1, SY2 and SY3.

CONCLUSION

Overall, the present study offers an innovative and conceptually strong experimental attempt to validate *Viruddha Āhāra* principles using microbial models. The findings suggest that certain classical incompatible food combinations may indeed create an adverse environment for beneficial *lactobacilli*. However, limited replicates, absence of biochemical characterization, and minor technical inconsistencies constrain the strength of causal inference. Future work using multi-species models, simulated gastrointestinal conditions, and detailed physicochemical profiling could substantiate and refine these findings.

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ABBREVIATIONS

CFU: Colony Forming Unit; **CO_2:** Carbon dioxide; **NaCl:** Sodium chloride; **MRS agar:** De Man - Rogosa - Sharpe agar; **PBS:** Phosphate buffer solution.

CONFLICT OF INTEREST

All the authors do not have any conflicting interests among them for the present research work.

FUNDING

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SUMMARY

Āhāra (food) is one of the three fundamental pillars of health in Ayurveda, and its proper combination plays a vital role in maintaining physiological balance. Incompatible food combinations, known as *Viruddha Āhāra*, are said to impair digestion (*Agni*), promote toxin formation (*Āma*), and disturb systemic metabolism. Classical texts describe several forms of incompatibility - such as *Matra, Sampat, Vidhi*, and *Samyoga Viruddha* - but their biological basis remains poorly understood.

Considering the vital role of gut microbiota, particularly *Lactobacillus* species, in digestion and immune regulation, this study aimed to explore the influence of select *Viruddha Āhāra* combinations on the growth of *Lactobacillus plantarum* under in-vitro conditions.

Four representatives *Viruddha Āhāra* types were tested: (i) *Matra Viruddha* - honey and ghee in equal proportion, (ii) *Sampat Viruddha* - unripe, ripe, and overripe mango, (iii) *Vidhi Viruddha* - tea with ice cream, and (iv) *Samyoga Viruddha* -milk with orange. Each sample and its individual components were sterilized and tested against standardized *L. plantarum* cultures using MRS medium, serial dilution, and quadrant plating techniques.

The results revealed distinct effects. Honey completely inhibited bacterial growth, whereas ghee and the honey-ghee mixture supported moderate proliferation compared to control, likely due to honey's antimicrobial properties and ghee's protective lipid matrix. Among mango samples, unripe mango exhibited the highest *Lactobacillus* growth, followed by ripe and overripe fruits, possibly reflecting differences in polyphenol and carbohydrate profiles. Tea markedly inhibited bacterial growth, but when combined with ice cream, colony counts nearly equalled the control, suggesting protein-polyphenol binding mitigates tea's inhibitory effects. Notably, the milk-orange mixture caused complete growth inhibition, corroborating Ayurvedic caution against *kṣīra-āmla* combinations, although technical inconsistencies warrant cautious interpretation.

This experimental study provides preliminary microbiological evidence supporting classical *Viruddha Āhāra* concepts. While results highlight potential inhibitory effects of certain combinations on beneficial gut flora, limitations - such as single-species testing, lack of physicochemical analysis, and *in vitro* constraints - necessitate further validation through multi-species and gastrointestinal simulation models. Nevertheless, the findings bridge Ayurveda and microbiology, offering a novel approach to scientifically re-examine traditional dietary principles.

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