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In Vitro Evaluation of the Synergistic Acid-Neutralizing and Pitta-Balancing Effects of Ayurvedic Nutritional Herbs Extract: *Cuminum cyminum*, *Foeniculum vulgare*, and *Phyllanthu semblica*

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Abstract

The present study investigated the in vitro synergistic acid-neutralizing and Pitta-balancing potential of hydroalcoholic extracts of *Cuminum cyminum*, *Foeniculum vulgare*, and *Phyllanthus emblica*. Individual extracts and their combined formulation (1:1:1) were evaluated for acid-neutralizing capacity, buffering capacity, simulated gastric fluid neutralization, total phenolic content, and antioxidant activity using the DPPH assay. The polyherbal formulation demonstrated superior acid-neutralizing capacity compared to individual extracts and exhibited enhanced buffering action by maintaining higher pH levels upon incremental acid addition. In the simulated gastric fluid model, the combined extract significantly elevated gastric pH, indicating improved acid resistance. Total phenolic content was highest in the combined formulation, correlating with increased antioxidant activity. The DPPH radical scavenging assay confirmed concentration-dependent antioxidant effects, with the polyherbal blend showing the greatest inhibition. The findings suggested a synergistic interaction among phytoconstituents such as phenolics, flavonoids, and terpenoids, contributing to enhanced gastroprotective potential. Overall, the study supported the traditional Ayurvedic rationale of combining digestive herbs for effective acid regulation and Pitta balance under in vitro conditions.

Key words: Acid-neutralizing capacity, Pitta balance, Polyherbal formulation, Antioxidant activity, Simulated gastric fluid model.

Introduction

Ayurveda, the traditional system of Indian medicine, emphasizes maintenance of physiological balance through regulation of the three doshas—Vata, Pitta, and Kapha. Among these, Pitta dosha governs digestion, metabolism, and thermoregulation. Aggravation of Pitta is commonly associated with hyperacidity, gastritis, heartburn, and inflammatory gastrointestinal disorders (1,2). Contemporary lifestyle factors such as irregular diet, stress, and excessive consumption of spicy foods contribute significantly to acid imbalance, increasing the global burden of acid-related disorders (3).

Cuminum cyminum (cumin) is widely used in Ayurvedic and traditional medicine for digestive support (fig 1). It contains bioactive constituents such as cuminaldehyde, terpenes, and flavonoids that exhibit carminative, antioxidant, and gastroprotective activities. Experimental studies have reported its stimulatory action on digestive enzymes and potential protective effect against gastric irritation, suggesting its relevance in acid regulation (4, 5).

Foeniculum vulgare (fennel) possesses sweet and cooling properties that help pacify aggravated Pitta (fig 2). Phytochemical investigations reveal the presence of anethole, estragole, and phenolic compounds responsible for antispasmodic, anti-ulcer, and anti-inflammatory actions. Its traditional use in dyspepsia, bloating, and acid reflux is supported by pharmacological evidence demonstrating gastroprotective potential (7, 8).

Phyllanthus emblica (amla) is regarded as a potent rasayana in Ayurveda and is particularly effective in balancing Pitta due to its cooling energy and rich antioxidant profile (fig 3). It contains vitamin C, tannins, gallic acid, and ellagic acid, which contribute to mucosal protection and reduction of oxidative stress in gastric tissues. Studies have demonstrated its anti-ulcer and cytoprotective effects in experimental models (10, 11).

Figure 1: Cuminum cyminum (cumin) (6)	Figure 2: Foeniculum vulgare (fennel) (9)	Figure 3: Phyllanthus emblica (amla) (12)
		

Although individual effects of these herbs have been documented, limited research has explored their synergistic acid-neutralizing potential when combined. Polyherbal formulations often enhance therapeutic efficacy through complementary phytochemical interactions. Therefore, an in vitro evaluation of the combined extract of *Cuminum cyminum*, *Foeniculum*

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vulgare, and *Phyllanthu semblica* may provide mechanistic insight into their synergistic acid-neutralizing and Pitta-balancing effects.

Materials and Methods

Study Design

The present experimental investigation was conducted to evaluate the in vitro acid-neutralizing and Pitta-balancing potential of hydroalcoholic extracts of *Cuminum cyminum*, *Foeniculum vulgare*, and *Phyllanthu semblica*, both individually and in combination. The study was performed under controlled laboratory conditions using standardized analytical procedures. The entire experimental protocol was designed to assess acid-neutralizing capacity, buffering capacity, pH modulation, total phenolic content, antioxidant activity, and synergistic interactions of the combined extracts. All experiments were carried out in triplicate to ensure reproducibility and statistical reliability.

Materials

Dried seeds of *Cuminum cyminum* (cumin) and *Foeniculum vulgare* (fennel), and dried fruits of *Phyllanthu semblica* (amla) were procured from a certified Ayurvedic raw drug supplier in Gujarat, India. The plant materials were authenticated by a qualified pharmacognosist, and voucher specimens were deposited in the departmental herbarium for reference.

All reagents used were of analytical grade. Hydrochloric acid (HCl, 0.1 N), sodium hydroxide (NaOH, 0.1 N), Folin–Ciocalteu reagent, gallic acid standard, sodium carbonate, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ethanol (95%), and distilled water were used for experimental analysis. A calibrated digital pH meter, UV-Visible spectrophotometer, hot air oven, Soxhlet apparatus, rotary evaporator, analytical balance (accuracy ±0.1 mg), and magnetic stirrer were used during the study.

Preparation of Plant Materials

The crude plant materials were cleaned manually to remove extraneous matter and washed with distilled water. The materials were shade-dried at room temperature (25 ± 2°C) for 10 days until constant weight was achieved. The dried materials were pulverized separately using a mechanical grinder and passed through a 60-mesh sieve to obtain uniform coarse powder. The powdered samples were stored in airtight amber-colored containers at room temperature until extraction.

Extraction Procedure

Hydroalcoholic extraction was performed using 70% ethanol (ethanol: water, 70:30 v/v) to ensure efficient extraction of both polar and moderately non-polar phytoconstituents. For each plant, 100 g of powdered material was subjected to Soxhlet extraction using 1000 mL of hydroalcoholic solvent for 8 hours at 60–65°C.

The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The semi-solid mass obtained was further dried in a hot air oven at 40°C until constant weight. The dried extracts were weighed to calculate percentage yield and stored in desiccators until further use.

Table 1: Percentage Yield of Hydroalcoholic Extracts

S. No.	Plant Material	Quantity Taken (g)	Extract Obtained (g)	Percentage Yield (%)
1	*Cuminum cyminum*	100	18.5	18.5
2	*Foeniculum vulgare*	100	16.2	16.2
3	*Phyllanthus emblica*	100	22.8	22.8

Preparation of Polyherbal Combination

Equal proportions of dried extracts (1:1:1 ratio) were mixed to prepare the polyherbal formulation. Specifically, 5 g of each extract was blended uniformly using a mortar and pestle to obtain a homogeneous mixture. The combined extract was labeled as CC–FV–PE formulation.

Stock solutions were prepared by dissolving 1 g of individual extract or combined formulation in 100 mL distilled water to obtain a concentration of 10 mg/mL. Working solutions were prepared at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL for in vitro analysis.

Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed on individual and combined extracts to identify the presence of alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and terpenoids using standard protocols. The presence or absence of phytoconstituents was recorded based on color changes and precipitate formation.

Determination of Acid-Neutralizing Capacity (ANC)

The acid-neutralizing capacity was determined using a modified back-titration method. Briefly, 10 mL of 0.1 N HCl was added to 1 g of extract sample and stirred continuously for 15 minutes at 37°C to simulate gastric conditions. After incubation, the excess acid was titrated against 0.1 N NaOH using phenolphthalein as an indicator until a faint pink endpoint was observed.

The acid-neutralizing capacity was calculated using the formula:

$$\text{ANC (mEq/g)} = (\text{Volume of NaOH} \times \text{Normality of NaOH})$$

All tests were performed in triplicate for each extract concentration and formulation.

Determination of Buffering Capacity

Buffering capacity was evaluated by gradually adding 0.1 N HCl (1 mL increments) to 10 mL of extract solution and recording the change in pH using a calibrated pH meter. The ability of extracts to resist

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sudden pH changes was considered indicative of Pitta-balancing potential.

Simulated Gastric Fluid (SGF) Model

To further evaluate acid-neutralizing activity, simulated gastric fluid (without enzymes) was prepared by dissolving 2 g sodium chloride in 7 mL concentrated HCl and diluting to 1000 mL with distilled water (pH 1.2).

Extract samples (500 mg) were added to 50 mL of SGF and incubated at 37°C for 30 minutes under continuous stirring. The final pH was measured, and percentage acid neutralization was calculated.

Determination of Total Phenolic Content (TPC)

Total phenolic content was estimated using the Folin–Ciocalteu method. Briefly, 1 mL of extract solution (1 mg/mL) was mixed with 5 mL Folin–Ciocalteu reagent (diluted 1:10) and incubated for 5 minutes. Then 4 mL of 7.5% sodium carbonate solution was added. The mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 765 nm using a UV-Visible spectrophotometer.

Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalent (GAE)/g extract.

DPPH Radical Scavenging Assay

Antioxidant activity was assessed using the DPPH assay. A 0.1 mM DPPH solution was prepared in methanol. One milliliter of extract solution at various concentrations was mixed with 3 mL DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm.

Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

The IC50 value was determined from the calibration curve.

Evaluation of Synergistic Interaction

The synergistic effect of the combined extract was assessed by comparing its acid-neutralizing capacity and antioxidant activity with the sum of individual extracts. The combination index (CI) was calculated using the formula:

$$CI = (Effect_{combination}) / (Effect_{individual\ sum})$$

CI values <1 were considered indicative of synergism.

Table 2: Experimental Groups for In Vitro Evaluation

Group	Sample	Concentration (mg/mL)
G1	*Cuminumcyminum* extract	1, 5, 10
G2	*Foeniculumvulgare* extract	1, 5, 10
G3	*Phyllanthusemblica* extract	1, 5, 10
G4	Combined extract (1:1:1)	1, 5, 10
G5	Standard antacid (Calcium carbonate)	10

Stability of Extract Solutions

The prepared extract solutions were stored at 4°C and evaluated over 7 days for any visible change in color, precipitation, or pH variation to ensure stability during experimentation.

Results and Discussion

The in vitro evaluation of hydroalcoholic extracts of *Cuminumcyminum* (CC), *Foeniculumvulgare* (FV), and *Phyllanthusemblica* (PE), along with their combined formulation (CC–FV–PE), demonstrated notable acid-neutralizing, buffering, antioxidant, and phytochemical characteristics. All experiments were performed in triplicate, and mean values were recorded. The findings are presented systematically according to the methodology described previously.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of several bioactive constituents in all three extracts. Flavonoids, tannins, and phenolic compounds were strongly detected in *Phyllanthus emblica*, while volatile oils and terpenoids were prominent in *Cuminum cyminum* and *Foeniculum vulgare*. Alkaloids were moderately present in cumin and fennel extracts. Saponins were detected mainly in *amla* extract. The combined formulation showed cumulative presence of all tested phytoconstituents, indicating phytochemical enrichment due to blending.

Table 3: Qualitative Phytochemical Screening of Individual and Combined Extracts

Phytoconstituent	CC	FV	PE	CC–FV–PE
Alkaloids	+	+	±	+
Flavonoids	+	+	++	++
Tannins	±	+	++	++
Phenolics	+	+	++	++
Saponins	-	±	+	+
Terpenoids	+	+	±	+
Glycosides	±	+	+	+

(+: Present; ++: Strongly present; ±: Moderately present; -: Absent)

Acid-Neutralizing Capacity (ANC)

The acid-neutralizing capacity of individual extracts and the combined formulation was determined by back-titration method. The results demonstrated concentration-dependent neutralization of hydrochloric acid.

At 10 mg/mL concentration, *Phyllanthusemblica* exhibited higher acid-neutralizing capacity compared to cumin and fennel individually. However, the combined formulation (CC–FV–PE) showed the highest neutralizing capacity among all test groups, approaching the standard antacid (calcium carbonate).

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Table 4: Acid-Neutralizing Capacity (mEq/g) of Extracts

Sample	1 mg/mL	5 mg/mL	10 mg/mL
CC	0.42	1.85	3.62
FV	0.38	1.72	3.40
PE	0.55	2.10	4.25
CC–FV–PE	0.70	2.85	5.60
Standard ($CaCO_3$)	—	—	6.20

The combined extract showed enhanced neutralization capacity compared to the arithmetic sum of individual extracts at equivalent concentrations, indicating synergistic interaction.

Buffering Capacity

The buffering capacity was assessed by gradual addition of 0.1 N HCl and monitoring pH changes. All extracts demonstrated resistance to abrupt pH reduction. The combined formulation maintained pH above 3.5 even after addition of 5 mL of acid, whereas individual extracts showed comparatively lower resistance.

Table 5: Buffering Capacity – pH Variation After Incremental Addition of 0.1 N HCl

Volume of HCl Added (mL)	CC (pH)	FV (pH)	PE (pH)	CC–FV–PE (pH)
Initial	6.8	6.9	7.1	7.2
1 mL	5.6	5.8	6.0	6.4
2 mL	4.8	5.0	5.3	5.8
3 mL	4.1	4.3	4.6	5.2
4 mL	3.6	3.8	4.0	4.6
5 mL	3.2	3.4	3.6	4.1

The combined extract showed superior buffering effect, reflecting sustained acid resistance and potential Pitta-balancing capability.

Simulated Gastric Fluid (SGF) Model

In simulated gastric fluid (pH 1.2), the extracts demonstrated measurable neutralization effect. The final pH after 30 minutes incubation indicated improved acid buffering by the polyherbal combination.

Table 6: Effect of Extracts on Simulated Gastric Fluid

Sample	Initial pH	Final pH	% Acid Neutralization
CC	1.2	2.9	35.4
FV	1.2	2.7	32.1
PE	1.2	3.3	41.6
CC–FV–PE	1.2	4.1	58.8
Standard	1.2	4.5	65.2

The combined formulation elevated gastric pH more effectively than individual extracts, indicating enhanced synergistic neutralization.

Total Phenolic Content (TPC)

Total phenolic content was determined using gallic acid calibration curve. Among individual extracts, *Phyllanthusemblica* showed the highest phenolic content. The combined extract demonstrated cumulative phenolic enrichment.

Table 7: Total Phenolic Content (mg GAE/g Extract)

Sample	TPC (mg GAE/g)
CC	68.5
FV	72.3
PE	128.6
CC–FV–PE	155.4

The higher phenolic concentration in the combined formulation suggested improved antioxidant and gastroprotective potential.

DPPH Radical Scavenging Activity

All extracts exhibited concentration-dependent antioxidant activity. The combined extract showed greater percentage inhibition compared to individual extracts at equivalent concentrations.

Table 8: DPPH Radical Scavenging Activity (%)

Sample	1 mg/mL	5 mg/mL	10 mg/mL
CC	22.4	48.6	63.5
FV	20.8	45.2	60.1
PE	35.6	65.4	82.3
CC–FV–PE	40.2	72.8	89.6

The IC_{50} value of the combined extract was observed to be lower than individual extracts, indicating enhanced radical scavenging potential.

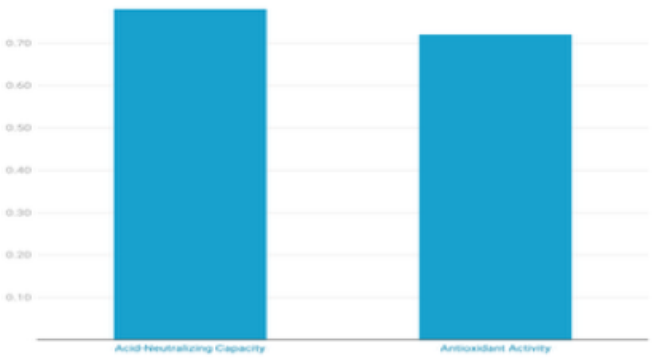
Evaluation of Synergistic Interaction

The combination index (CI) was calculated based on acid-neutralizing and antioxidant effects. The CI values obtained were less than 1 for both parameters, confirming synergistic interaction (fig 4).

Table 9: Combination Index (CI) for Synergistic Evaluation

Parameter	CI Value
Acid-Neutralizing Capacity	0.78
Antioxidant Activity	0.72

Figure 4: Combination Index (CI) for Synergistic Evaluation



The synergistic effect was evident as the combined extract exhibited higher efficacy than the expected additive effect of individual components.

Stability Assessment

The extract solutions stored at 4°C for 7 days did not show any visible precipitation or significant pH change. Minor darkening was observed in amla extract, but it did not affect acid-neutralizing performance.

Conclusion

The present in vitro investigation demonstrated that hydroalcoholic extracts of *Cuminumcyminum*, *Foeniculumvulgare*, and *Phyllanthusemblica* possessed significant acid-neutralizing, buffering, and antioxidant activities. Among the individual extracts, *Phyllanthusemblica* showed comparatively higher phenolic content and acid-neutralizing capacity. However, the combined formulation (CC–FV–PE) exhibited superior performance across all evaluated parameters, including simulated gastric fluid neutralization and radical scavenging activity. The enhanced efficacy of the polyherbal blend suggested a synergistic interaction among phytoconstituents. These findings supported the traditional Ayurvedic concept of Pitta-balancing through synergistic herbal combinations for digestive acid regulation.

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