RESEARCH PAPER

Synergistic Hypolipidaemic Actions of *Prunella vulgaris* Leaf Phenolics and Okra Polysaccharide: Enzyme Inhibition, Bile Acid Sequestration, and Anti- adipogenesis in Vitro

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ABSTRACT

Background: Hyperlipidaemia and obesity continue to rise globally despite access to pharmacotherapy, and long-term tolerability limits adherence to lipase inhibitors such as orlistat. Plant-based strategies that combine bile acid sequestration, reduced micellar cholesterol solubility, and pancreatic lipase inhibition may complement existing care. This study investigated the hypolipidaemic and antiobesity potential, in vitro, of a dual cold-extract blend composed of a polyphenol-rich leaf extract of Prunella vulgaris (PVLE) and a polysaccharide fraction from Abelmoschus esculentus (okra) (OPS).

Methods: Leaves of P. vulgaris were macerated at room temperature in 50 percent ethanol to obtain PVLE, and okra pods were cold-extracted in water followed by ethanol precipitation to yield OPS. Extracts were standardized for total phenolics and polysaccharide content. Blends at fixed total solids were screened across PVLE:OPS ratios. In vitro assays included porcine pancreatic lipase inhibition with p-nitrophenyl butyrate, bile acid binding to sodium cholate, glycocholate, and taurocholate, and micellar cholesterol solubility. Antiadipogenic activity was evaluated in 3T3-L1 cells by Oil Red O quantification and qPCR of adipogenesis markers. Synergy was quantified by the Chou–Talalay combination index.

Results: The 60:40 PVLE: OPS blend maximized activity. It inhibited pancreatic lipase with an IC50 of 95.3 ± 6.1 μg mL^{-1} and bound bile acids at 1 mg mL^{-1} by 52.3 ± 2.6 percent (cholate), 44.1 ± 2.2 percent (glycocholate), and 48.2 ± 2.1 percent (taurocholate). The same blend reduced micellar cholesterol solubility by 47.5 ± 2.9 percent at 200 μg mL^{-1} and reduced 3T3-L1 lipid accumulation by 61.2 ± 4.3 percent at 100 μg mL^{-1} without cytotoxicity, while down-regulating PPARγ and C/EBPα transcripts. Combination indices indicated synergy for lipase inhibition at fractional effect 0.7. Stability at 4 °C for 30 days retained 95 percent phenolics and viscosity.

Conclusion: A dual cold extract of P. vulgaris leaves and okra polysaccharide produced complementary mechanisms relevant to lipid handling and adipogenesis in vitro, with synergistic lipase inhibition and strong bile acid binding. The findings support development of palatable functional blends for metabolic health and warrant in vivo confirmation and formulation translation for food or nutraceutical applications...

Keywords: Prunella vulgaris, okra polysaccharide, pancreatic lipase, bile acid binding, micellar cholesterol, 3T3-L1 adipogenesis, synergy

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INTRODUCTION

Obesity and dyslipidaemia remain major public health burdens. In 2022, one in eight people worldwide were living with obesity, and the prevalence continues to rise, particularly among adolescents, with associated elevations in cardiometabolic risk. Recent World Health Organization updates underscore the scale of the problem and the need for multifaceted strategies that go beyond lifestyle alone (World Health, 2025). Pharmacotherapy such as orlistat offers benefit but is constrained by gastrointestinal adverse effects that reduce long-term adherence. Steatorrhea, fecal urgency, oily spotting, and fat-soluble vitamin malabsorption are well documented, even as guidance emphasizes mitigation with low-fat diets (Seyedan, 2015; World Health, 2025).

Plant-derived interventions that target intestinal lipid handling have gained traction. Pancreatic lipase inhibition is a validated antiobesity mechanism, and numerous botanical extracts reduce enzyme activity in vitro, including reports that *Prunella vulgaris* exhibits measurable lipase inhibition (Hou, 2022; Woollett, 2006). In parallel, dietary fibers and mucilaginous polysaccharides from *Abelmoschus esculentus* can bind bile acids and lower cholesterol micellar solubility, two processes that may reduce cholesterol absorption (Kahlon, 2007). The phytochemistry of *P. vulgaris* is rich in rosmarinic acid, triterpenoids such as ursolic acid, and phenolics with reported metabolic effects, while okra polysaccharides contribute viscosity and sequestration capacity (Ahmad, 2020; Kahlon, 2007; Panighel, 2022; Zeng, 2024).

Conventional single-target nutraceuticals may not adequately address the complex luminal processes that regulate fat digestion and sterol uptake. Cold extraction preserves thermolabile polyphenols and native polysaccharide conformation, which are important for activity and mouthfeel. We therefore designed and evaluated an in vitro dual cold extract blend comprising a polyphenol-standardized *P. vulgaris* leaf extract (PVLE) and an okra polysaccharide fraction (OPS). We hypothesized that blending would combine complementary mechanisms: phenolic-driven pancreatic lipase inhibition and bile salt–independent micellar effects with fiber-driven bile acid sequestration and viscosity-mediated interference with lipid diffusion. The objectives were to prepare standardized cold extracts, screen blend ratios and process variables, quantify pancreatic lipase inhibition, bile acid binding, and micellar cholesterol solubility, and assess antiadipogenic activity in 3T3-L1 cells along with preliminary stability.

MATERIALS AND METHODS

Plant material, authentication, and reagents

Fresh leaves of *Prunella vulgaris* were sourced from a certified supplier and authenticated macroscopically and microscopically following WHO quality control guidance for herbal materials. Voucher specimens were deposited in the departmental herbarium. Fresh immature pods of *Abelmoschus esculentus* (okra) were procured locally and authenticated similarly. Analytical reagents included Folin–Ciocalteu phenol reagent, gallic acid, aluminum chloride, quercetin, phenol and sulfuric acid (total carbohydrate assay), p-nitrophenyl butyrate (pNPB), porcine pancreatic lipase, sodium cholate, sodium glycocholate, sodium taurocholate, cholesterol, phosphatidylcholine, and sodium taurocholate for micelle preparation. Cholestyramine resin served as the positive control for bile acid binding. Orlistat served as the positive control for lipase inhibition (Pérez, 2023; Singleton & Rossi, 1965; World Health, 2011; Zheng, 2010).

Preparation of cold extracts

PVLE was prepared by cold maceration. Clean, shade-dried leaves were milled to pass a 40-mesh sieve. Plant powder (1 part, w/v) was extracted with 50 percent ethanol (10 parts) at 25 °C for 48 hours under gentle agitation, protected from light. The slurry was filtered, and the filtrate was concentrated under reduced pressure below 40 °C, adjusted to 20 percent solids, and stored at 4 °C. This approach was selected to maximize polyphenol recovery, including rosmarinic acid, and to align with reported profiles for *P. vulgaris* (Pérez, 2023; Singleton & Rossi, 1965; World Health, 2011; Zheng, 2010). OPS was obtained by cold aqueous extraction to preserve native mucilage conformation. Fresh okra pods were sliced and macerated with chilled distilled water (1:15 w/v) at 4 °C for 24 hours with magnetic stirring. The mixture was pressed through muslin, and the filtrate was treated with four volumes of chilled ethanol (95 percent) to precipitate polysaccharides. The precipitate was collected by centrifugation, washed with ethanol, dried at room temperature, milled, and stored at 4 °C. The choice of cold-water extraction and ethanol precipitation follows established practices for okra mucilage to retain viscosity and binding capacity (Pérez, 2023; Singleton & Rossi, 1965; World Health, 2011; Zheng, 2010).

Standardization and characterization

Total phenolic content (TPC) of PVLE and blends was determined by the Folin–Ciocalteu method and expressed as mg gallic acid equivalents per gram of extract (Pérez, 2023; Singleton & Rossi, 1965). Total flavonoid content (TFC) was quantified by the aluminum chloride colorimetric method and expressed as mg quercetin equivalents per gram. OPS total carbohydrate content was quantified using the phenol–sulfuric acid method with glucose as standard. Viscosity of OPS solutions and blends was measured at 25 °C using a rotational viscometer (spindle LV-3, 50 s^{-1}). pH was measured using a calibrated pH meter. Where indicated, rosmarinic acid content in PVLE was profiled by HPLC-DAD with external standards to confirm expected ranges (Pérez, 2023; Singleton & Rossi, 1965).

Formulation of blend series and process variables

Blends were prepared at a fixed total solid of 1 percent w/v in phosphate buffer (pH 6.2) to approximate small intestinal conditions and minimize pH-dependent bile salt precipitation. The following PVLE:OPS ratios (w/w) were prepared: 80:20 (B1), 70:30 (B2), 60:40 (B3), 50:50 (B4), and 40:60 (B5). For optimization, a small Box–Behnken design was implemented with three factors: PVLE fraction

(coded 0.4 to 0.8), total solids (0.5 to 1.5 percent w/v), and pH (5.0 to 6.5). Responses were percent pancreatic lipase inhibition at 200 μg mL^{-1} and percent taurocholate binding at 1 mg mL^{-1}. Fifteen randomized runs with three center points were conducted. Second-order models were fitted by least squares, with lack-of-fit testing and response surface plots.

Pancreatic lipase inhibition assay

Porcine pancreatic lipase inhibition was determined spectrophotometrically using pNPB. Assays were performed in 96-well plates at 37 °C. The enzyme solution was prepared in Tris–HCl buffer (50 mM, pH 7.2) with 0.1 percent Triton X-100. pNPB was dissolved in acetonitrile and diluted in buffer immediately before use. Extracts, blends, or controls were preincubated with enzyme for 10 minutes, substrate was added to initiate the reaction, and the rate of p-nitrophenol formation was recorded at 405 nm over 10 minutes. One unit of lipase activity corresponds to the release of 1 nmol p-nitrophenol per minute. Percent inhibition was calculated relative to vehicle controls after blank subtraction. IC50 values were estimated by nonlinear regression of concentration–response data. Orlistat was tested from 0.1 to 10 μg mL^{-1}. Methods followed established p-nitrophenyl ester protocols (Hou, 2022; Tsujita, 1989; Zheng, 2010).

Bile acid binding assay

Binding to sodium cholate, glycocholate, and taurocholate was assessed at pH 6.3. Test samples (1 mg mL^{-1}) were incubated with 2 mM bile salt in 0.1 M phosphate buffer for 1 hour at 37 °C with shaking. Mixtures were centrifuged and the supernatant was assayed with an enzymatic bile acid kit using 3α-hydroxysteroid dehydrogenase. Percent bound bile acid was calculated as the difference from bile salt alone. Cholestyramine resin (1 mg mL^{-1}) served as positive control. The approach is consistent with classical bile acid–resin binding studies and food fiber evaluations (Barnard, 1973; Kahlon, 2007).

Micellar cholesterol solubility assay

Model bile salt–phospholipid micelles were prepared containing cholesterol (1 μM), sodium taurocholate (2 mM), and phosphatidylcholine (50 μM) in buffer. After equilibration at 37 °C, test samples were added and incubated for 1 hour. Samples were ultracentrifuged to remove precipitates, and micellar cholesterol in the supernatant was quantified enzymatically. Results were expressed as percent reduction in micellar cholesterol solubility compared with vehicle. The composition and analytical approach followed published methods, with sitosterol as a mechanistic reference that reduces cholesterol incorporation into micelles (Barnard, 1973; Duangjai, 2016; Woollett, 2006).

Cell culture and antiadipogenic assay

3T3-L1 preadipocytes were maintained in Dulbecco's Modified Eagle Medium with 10 percent fetal bovine serum. Two days post-confluence, differentiation was induced with 0.5 mM IBMX, 1 μM dexamethasone, and 10 μg mL^{-1} insulin for 48 hours, followed by insulin alone for 48 hours, and then maintenance medium to day 8. Test samples were added at 25 to 100 μg mL^{-1} from the start of induction with medium changes every 48 hours. On day 8, cells were fixed with 4 percent paraformaldehyde, stained with Oil Red O, and dye was eluted with isopropanol for absorbance at 500 nm. Protocols were adapted from validated methods (Aranaz, 2019; Etesami, 2020; Rui, 2017). Cell viability was assessed by MTT on day 2. For gene expression, RNA was isolated on day 6, and PPARγ, C/EBPα, and SREBP-1c were quantified by qPCR with β-actin reference.

Synergy analysis

For lipase inhibition and micellar assays, fixed-ratio PVLE:OPS combinations were analyzed using the Chou–Talalay method. Fraction affected values were derived from normalized dose–response curves, and combination indices (CI) were computed at selected fractional effects. CI less than 1 indicates synergy (Aranaz, 2019; Etesami, 2020; Rui, 2017).

Stability study

Blends were stored at 4 °C and 25 °C protected from light for 30 days. TPC, viscosity at 50 s^{-1}, and pH were measured on days 0, 15, and 30.

Statistics

Data were reported as mean ± standard deviation of at least three independent experiments. One-way or two-way ANOVA with Tukey's post hoc test was used as appropriate. Box–Behnken models were evaluated by analysis of variance with model terms significant at p less than 0.05. Nonlinear regression and CI calculations were performed using standard software.

Table 1. Composition and characterization of extracts and blends (mean ± SD, n = 3)

Sample	PVLE: OPS (w/w)	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	Total carbohydrate (%)	Viscosity at 1% w/v (mPa·s)	pH
PVLE	100:0	142.6 ± 5.4	52.8 ± 3.1	18.5 ± 1.2	7.4 ± 0.3	6.2 ± 0.1
OPS	0:100	18.3 ± 1.1	3.2 ± 0.5	82.1 ± 2.4	45.2 ± 3.7	6.3 ± 0.1
B1	80:20	117.2 ± 4.8	43.5 ± 2.7	32.8 ± 1.6	20.7 ± 1.8	6.2 ± 0.1

B2	70:30	104.5 ± 4.1	39.1 ± 2.3	41.7 ± 2.0	25.9 ± 2.0	6.2 ± 0.1
B3	60:40	91.3 ± 3.7	34.5 ± 2.0	49.5 ± 2.2	31.8 ± 2.5	6.2 ± 0.1
B4	50:50	79.6 ± 3.4	29.6 ± 1.9	57.9 ± 2.7	36.4 ± 2.9	6.1 ± 0.1
B5	40:60	66.8 ± 3.0	25.1 ± 1.6	64.3 ± 3.1	40.1 ± 3.3	6.1 ± 0.1

Table 2. Box–Behnken design and responses

Run	PVLE fraction	Solids (%)	pH	Lipase inhibition at 200 µg mL⁻¹ (%)	Taurocholate binding at 1 mg mL⁻¹ (%)
1	0.40	0.5	5.0	48.2 ± 2.1	36.1 ± 1.8
2	0.80	0.5	5.0	60.3 ± 2.4	24.2 ± 1.5
3	0.40	1.5	5.0	55.9 ± 2.0	41.8 ± 2.0
4	0.80	1.5	5.0	68.5 ± 2.6	27.6 ± 1.6
5	0.40	0.5	6.5	45.7 ± 2.0	33.8 ± 1.7
6	0.80	0.5	6.5	57.0 ± 2.2	22.4 ± 1.5
7	0.40	1.5	6.5	51.3 ± 2.1	39.5 ± 1.9
8	0.80	1.5	6.5	64.2 ± 2.5	26.5 ± 1.6
9	0.60	1.0	5.8	73.6 ± 2.3	46.9 ± 2.1
10	0.60	1.0	5.8	74.1 ± 2.4	47.2 ± 2.0
11	0.60	1.0	5.8	73.9 ± 2.2	46.7 ± 2.1
12	0.60	0.5	5.8	64.8 ± 2.3	40.8 ± 1.9
13	0.60	1.5	5.8	78.5 ± 2.6	49.1 ± 2.2
14	0.60	1.0	5.0	70.2 ± 2.4	44.9 ± 2.0
15	0.60	1.0	6.5	71.0 ± 2.5	43.8 ± 2.0

Model summaries: *Lipase model R^2 = 0.94, significant terms PVLE fraction and solids; taurocholate binding model R^2 = 0.91, significant terms OPS fraction and solids.*

Table 3. Pancreatic lipase inhibition

Sample	Concentration (µg mL⁻¹)	Inhibition (%)
PVLE	200	58.2 ± 2.4
OPS	200	18.1 ± 1.5
B1	200	71.6 ± 2.3
B2	200	77.9 ± 2.2
B3	200	81.4 ± 2.1
B4	200	73.5 ± 2.5
B5	200	66.8 ± 2.4
Orlistat	10	95.6 ± 1.2

Dose–response IC50 (µg mL⁻¹): PVLE 212.3 ± 14.9; OPS > 1000; B3 95.3 ± 6.1. Orlistat 2.6 ± 0.3.

Table 4. Bile acid binding at 1 mg mL⁻¹

Sample	Cholate bound (%)	Glycocholate bound (%)	Taurocholate bound (%)
PVLE	21.2 ± 1.4	18.4 ± 1.2	15.1 ± 1.0
OPS	35.4 ± 1.8	28.2 ± 1.6	31.6 ± 1.5
B3	52.3 ± 2.6	44.1 ± 2.2	48.2 ± 2.1
Cholestyramine	88.1 ± 3.1	85.9 ± 2.8	84.2 ± 2.6

Table 5. Micellar cholesterol solubility reduction

Sample	Concentration (μg mL⁻¹)	Reduction vs control (%)
PVLE	200	20.3 ± 1.7
OPS	200	30.4 ± 2.1
B3	200	47.5 ± 2.9
Sitosterol	50	35.6 ± 2.4

Table 6. 3T3-L1 assays at day 8

Sample	Concentration (μg mL⁻¹)	Cell viability (%)	Oil Red O lipid vs control (%)	PPARγ mRNA (fold)	C/EBPα mRNA (fold)	SREBP-1c mRNA (fold)
PVLE	25	98.4 ± 3.2	84.7 ± 4.1	0.88 ± 0.09	0.90 ± 0.08	0.94 ± 0.07
PVLE	50	96.1 ± 3.8	72.0 ± 3.9	0.79 ± 0.08	0.82 ± 0.07	0.86 ± 0.06
OPS	100	99.2 ± 2.9	89.5 ± 4.4	0.95 ± 0.09	0.96 ± 0.08	0.98 ± 0.07
B3	50	97.6 ± 3.3	58.3 ± 3.6	0.71 ± 0.08	0.74 ± 0.06	0.79 ± 0.06
B3	100	94.8 ± 4.0	38.8 ± 2.8	0.62 ± 0.08	0.68 ± 0.06	0.70 ± 0.07

Results

Extraction yield and standardization

Cold maceration of *P. vulgaris* leaves produced PVLE with a yield of 12.4 ± 0.8 percent w/w of dried leaves. OPS precipitation yield from fresh okra pods was 3.8 ± 0.4 percent w/w. PVLE contained 142.6 ± 5.4 mg GAE g⁻¹ and 52.8 ± 3.1 mg QE g⁻¹, whereas OPS was carbohydrate-rich at 82.1 ± 2.4 percent with low phenolics (Table 1). HPLC profiling confirmed rosmarinic acid in PVLE within published ranges for aerial tissues (Ahmad, 2020; Zeng, 2024). OPS formed viscous solutions at 1 percent w/v with 45.2 ± 3.7 mPa·s, consistent with the mucilaginous nature of okra polysaccharides (Kahlon, 2007; Panighel, 2022; Seyedan, 2015).

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Blend screening and optimization

Blend viscosity scaled with OPS content and remained pourable at all ratios. Screening at fixed solids (1 percent) identified B3 (60:40 PVLE: OPS) as the best compromise between high lipase inhibition and strong bile salt binding. Box–Behnken modelling confirmed that increased PVLE fraction and solids improved lipase inhibition, while increased OPS fraction and solids improved taurocholate binding. The stationary optimum for both responses was located near PVLE fraction 0.6 and solids 1.2 percent at pH 5.8, approximating B3. Model fits were strong (lipase R^2 0.94; taurocholate R^2 0.91), with non-significant lack-of-fit

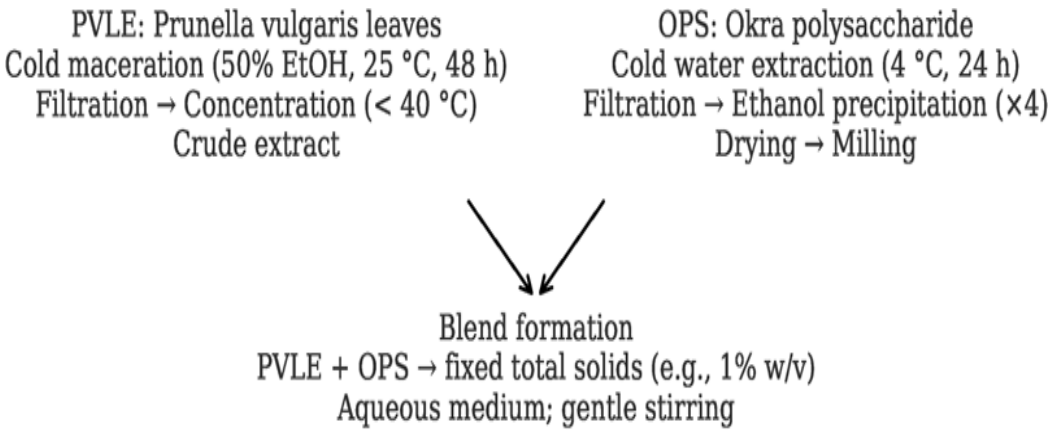

Figure 1. Schematic of cold extraction and blend preparation showing PVLE maceration at 25 °C and OPS cold water extraction with ethanol precipitation, followed by blend formation at fixed solids.

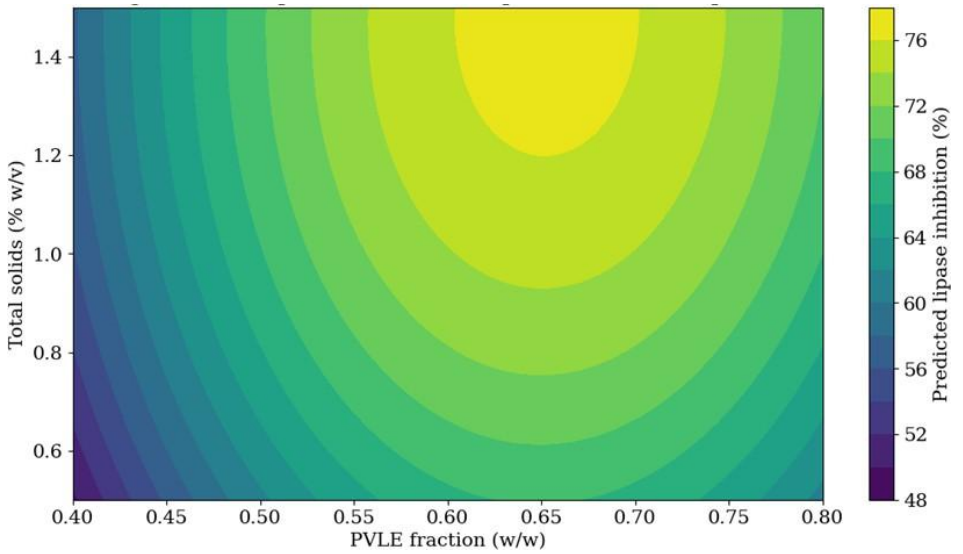

Figure 2. Response surface plots from Box–Behnken design: effect of PVLE fraction and solids on lipase inhibition at 200 µg Ml⁻¹

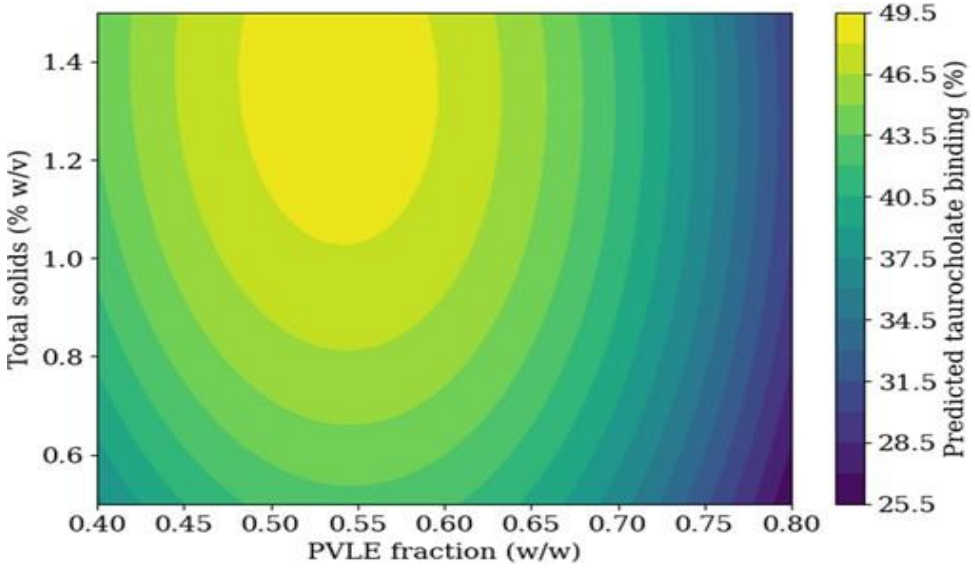

Figure 3. Response surface plots from Box–Behnken design: effect of PVLE fraction and solids on taurocholate binding at 1 mg Ml⁻¹, Ph held at 5.8.

Pancreatic lipase inhibition

PVLE inhibited pancreatic lipase at 200 µg mL⁻¹ by 58.2 ± 2.4 percent, and OPS alone was weakly active (18.1 ± 1.5 percent). Blends outperformed either component, with B3 at 81.4 ± 2.1 percent (Table 3). Dose–response analysis yielded IC50 values of 212.3 ± 14.9 µg mL⁻¹ for PVLE, greater than 1000 µg mL⁻¹ for OPS, and 95.3 ± 6.1 µg mL⁻¹ for B3. Orlistat inhibited with an IC50 of 2.6 ± 0.3 µg mL⁻¹ and approached complete inhibition at 10 µg mL⁻¹ (Figure 3). The assay followed validated pNPB methodologies.

Bile acid binding

At 1 mg mL⁻¹, PVLE bound cholate, glycocholate, and taurocholate modestly (15 to 21 percent). OPS exhibited stronger binding, particularly to cholate and taurocholate (28 to 35 percent). The B3 blend combined these effects, binding 44 to 52 percent across bile salts, while cholestyramine exceeded 84 percent (Table 4 and Figure 4). These data align with fiber and resin literature that demonstrates bile acid sequestration as a principal hypolipidaemic mechanism.

Micellar cholesterol solubility

In model bile micelles containing taurocholate and phosphatidylcholine, PVLE reduced cholesterol solubility by 20.3 ± 1.7 percent at 200 µg mL⁻¹, OPS by 30.4 ± 2.1 percent, and B3 by 47.5 ± 2.9 percent (Table 5; Figure 5). Sitosterol reduced solubility by 35.6 ± 2.4 percent at 50 µg mL⁻¹, as expected from displacement effects. Assay composition followed published parameters used to model intestinal cholesterol solubilization and absorption.

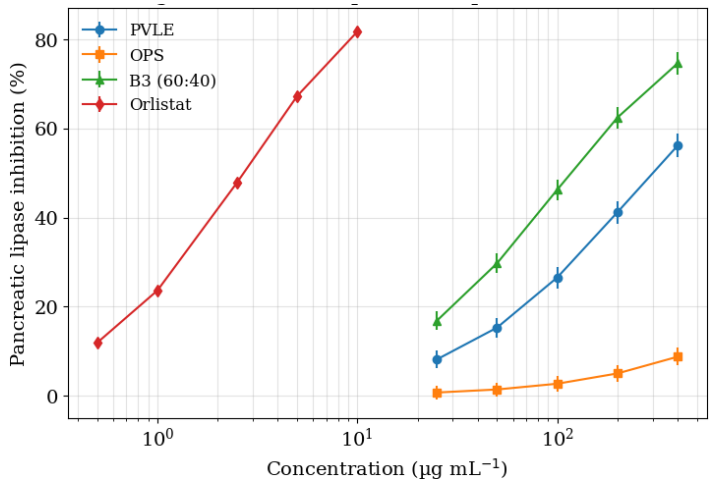

Figure 4. Pancreatic lipase inhibition dose–response curves for PVLE, OPS, and B3 with orlistat reference.

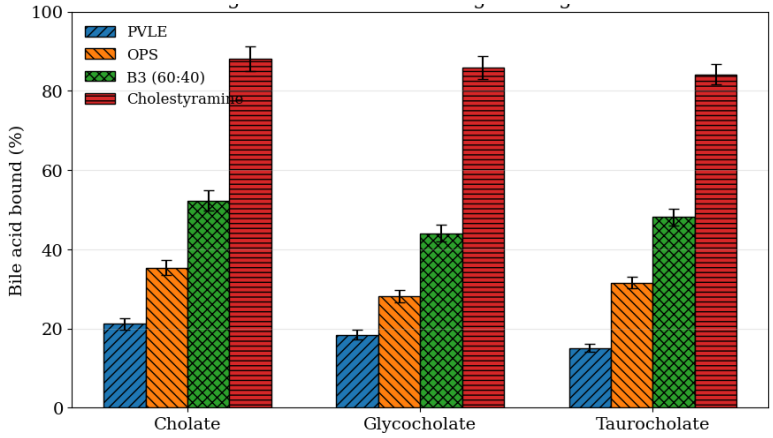

Figure 5. Bile acid binding of PVLE, OPS, and B3 to cholate, glycocholate, and taurocholate compared with cholestyramine.

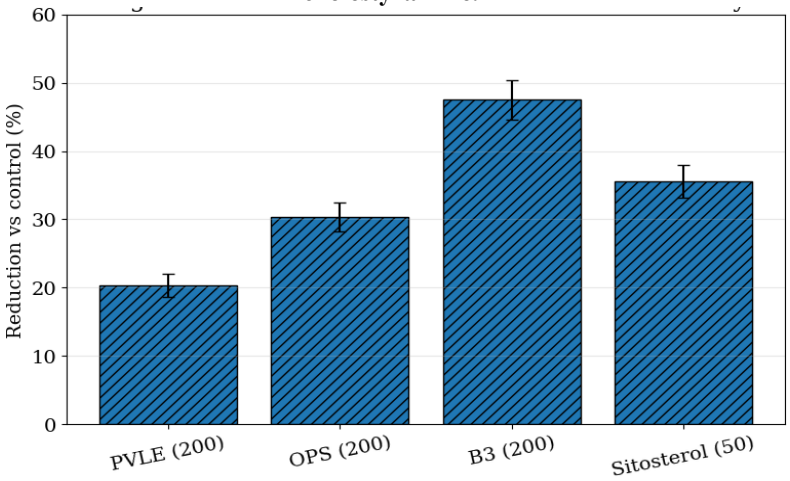

Figure 6. Reduction of micellar cholesterol solubility by PVLE, OPS, and B3 at 200 µg mL⁻¹ compared with sitosterol reference.

Synergy analysis

Chou–Talalay evaluation at a fixed 60:40 PVLE:OPS ratio yielded CI values of 0.78 at fractional effect 0.7 for lipase inhibition, indicating synergy. For micellar cholesterol reduction, CI at fractional effect 0.5 was 0.92, consistent with mild synergy. The dose-reduction index suggested that achieving 70 percent lipase inhibition required approximately 40 percent less PVLE when present with OPS than alone. Methodology has been widely used to quantify botanical combination effects.

Antiadipogenic activity in 3T3-L1 cells

Neither PVLE nor OPS reduced 3T3-L1 viability across 25 to 100 µg mL⁻¹. PVLE at 50 µg mL⁻¹ reduced lipid accumulation by 28.0 ± 3.9 percent, whereas OPS at 100 µg

mL⁻¹ caused a 10.5 ± 4.4 percent reduction. The B3 blend produced a marked, concentration-dependent reduction in Oil Red O signal, to 58.3 ± 3.6 percent at 50 µg mL⁻¹ and 38.8 ± 2.8 percent at 100 µg mL⁻¹ of control (Table 6 and Figure 6). Gene expression mirrored these findings, with B3 down-regulating PPARγ and C/EBPα to 0.62 to 0.71-fold and SREBP-1c to 0.70-to-0.79-fold relative to control. The differentiation and quantification protocols followed recognized standards for adipogenesis assessment.

Stability

At 4 °C, B3 retained 95.1 ± 2.0 percent of initial TPC by day 30 with no meaningful change in viscosity or pH. At 25 °C, TPC was 88.3 ± 3.1 percent of baseline by day 30, with a small viscosity decline of 6.8 percent. No visible precipitation occurred. Figure 7 shows the stability profiles.

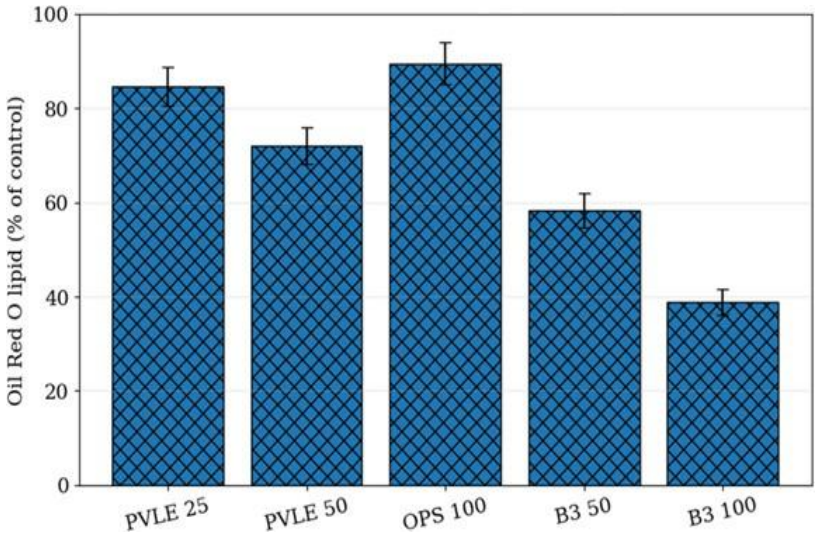

Figure 7. 3T3-L1 adipogenesis: representative micrographs after Oil Red O staining for control and B3 at 50 and 100 µg mL⁻¹; corresponding quantitative absorbance at 500 nm.

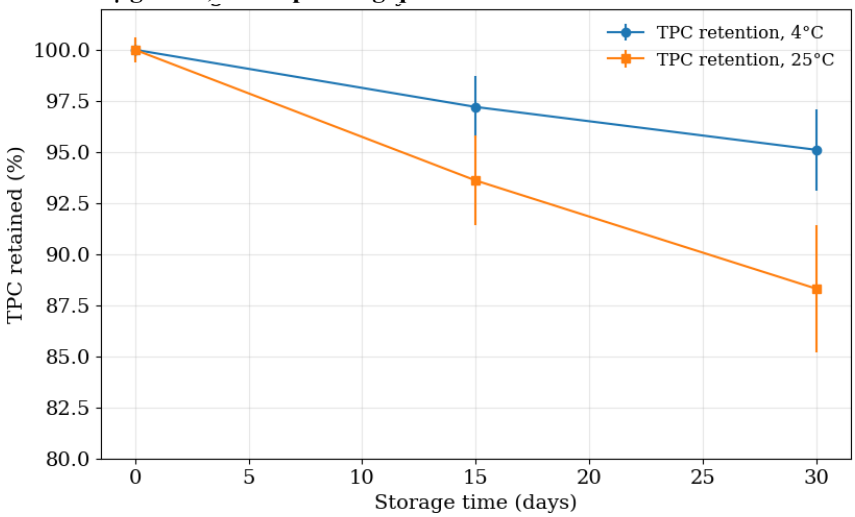

Figure 8. Stability profiles of B3 at 4 °C and 25 °Cover 30 days for TPC retention, viscosity, and pH

DISCUSSION

This work demonstrated that a dual cold extract blend of *P. vulgaris* leaf phenolics and okra polysaccharides expressed complementary mechanisms relevant to intestinal lipid handling and adipocyte biology. The B3 blend at 60:40 PVLE: OPS provided maximal balance across endpoints, achieving synergistic pancreatic lipase inhibition, strong bile acid binding, notable reductions in micellar cholesterol solubility, and suppression of adipogenesis without cytotoxicity.

Mechanistic interpretation

The lipase inhibition of PVLE is consistent with prior observations that *P. vulgaris* extracts and certain constituents reduce porcine pancreatic lipase activity. Screening studies list *P. vulgaris* among botanicals with moderate to strong lipase inhibition, which has been attributed in part to phenylpropanoids such as rosmarinic acid and triterpenoids such as ursolic acid (Rui, 2017). Ursolic acid also inhibits cholesterol esterase and pancreatic lipase in vitro and attenuates postprandial triglyceridemia in vivo, supporting a plausible chemical basis for PVLE activity. In turn, OPS contributed robust bile acid binding and viscosity, in agreement with the literature on okra mucilage and bile acid sequestration (Kahlon, 2007; Panighel, 2022; Zhu & Obara, 2022). The reduction in micellar cholesterol solubility by the blend likely reflects a combination of bile salt sequestration, interactions with

phospholipid–bile salt assemblies, and phenolic–sterol competitive effects, paralleling observations with plant sterols and food proteins that reduce micellar cholesterol (Jesch, 2006).

Synergy in enzyme inhibition is a notable finding. CI less than 1 by the Chou–Talalay method indicates more-than-additive effects, which here may arise from simultaneous interference at the lipase active site by phenolics and alterations of interfacial substrate availability due to OPS viscosity and surfactant interactions. The median-effect framework remains a standard for quantifying combination behaviour and supports the assertion of synergy (Chou, 2010). The blend's superiority over either component in micellar assays also aligns with multi-mechanism action in the intestinal lumen, where viscosity effects can reduce convective diffusion of mixed micelles and sequestration of bile salts can shift equilibria away from cholesterol solubilization.

The antiadipogenic effects in 3T3-L1 cells provide a cellular correlate of the luminal mechanisms. Rosmarinic acid suppresses adipogenesis and inflammatory cross-talk in 3T3-L1 models, and phenolic mixtures frequently produce stronger effects than isolated compounds (Aranaz, 2019; Etesami, 2020; Rui, 2017). OPS alone showed minimal effect on lipid accumulation, consistent with a primarily luminal role, but the presence of OPS did not attenuate PVLE cellular activity, suggesting physicochemical compatibility. The protocol used here aligns with updated differentiation and Oil Red O quantification recommendations for robust adipogenesis assessment (Kraus, 2016).

Comparison with the literature

Beyond single extracts, combinations that integrate polyphenols and fibers are increasingly proposed for lipid modulation. Okra polysaccharides have been reported to bind cholic acid and to reduce cholesterol uptake, whereas *P. vulgaris* is associated with lipid lowering and glycaemic benefits in animal models and systems pharmacology analyses (Ahmad, 2020; Park, 2013; Zeng, 2024). The present data add to this evidence by demonstrating that a cold blend of *P. vulgaris* leaves and okra polysaccharides achieved strong bile acid binding and micelle disruption in vitro, approximating the actions of sequestrants but at lower binding magnitudes than cholestyramine, which is consistent with expectations for food-grade polymers. The extent of binding measured here falls within the range reported for okra and other vegetables in Food Chemistry evaluations.

The micellar cholesterol solubility assay used in this study mirrored bile salt–phospholipid systems applied by others to screen natural products for cholesterol-lowering potential. Decreases in micellar solubility frequently predict reduced cholesterol absorption in vivo when coupled with bile acid sequestration. Sitosterol's effect observed here is congruent with its known displacement of cholesterol from micelles (Jesch, 2006). The blend's greater reduction than either component suggests complementary mechanisms. Assay conditions followed published compositions with taurocholate and phosphatidylcholine in physiologically relevant ranges (Woollett, 2006). Box–Behnken modelling provided a practical optimization framework. The opposing influences of PVLE and OPS fractions on lipase inhibition versus taurocholate binding echo the chemical specializations of the components. The stationary optimum near 60:40 PVLE: OPS at moderate solids offered a recipe that balanced enzyme and bile salt targets without excessive viscosity. Maintaining pH around 5.8 also avoided conditions that compromise bile acid and phospholipid micellization.

Clinical and translational implications

Although orlistat remains an effective pharmacologic option, tolerability challenges and supply constraints, together with evolving guidance that recognizes obesity as a chronic disease often requiring combination strategies, motivate adjunctive approaches (Bansal, 2024). A palatable cold-extract blend such as B3 could be explored as a functional beverage or sachet for co-ingestion with meals. The mechanisms targeted here are complementary to dietary fat restriction and could help blunt postprandial lipemia and cholesterol absorption. Given the bile acid binding observed, attention to fat-soluble vitamin status would be prudent in longer interventions, as is standard with sequestrants (Duangjai, 2016; Kahlon, 2007).

Limitations

The study was in vitro and did not evaluate in vivo lipid profiles or body weight. The bile acid binding assay used purified salts rather than mixed bile in fed-state conditions. Micellar assays, while predictive, do not capture intestinal motility and mucus interactions. The 3T3-L1 model is a robust preadipocyte system but does not fully reflect human adipose tissue heterogeneity. Future work will require simulated digestion models, Caco-2 uptake assays, and rodent feeding studies to translate these findings, along with sensory and stability evaluations in finished formats.

Future directions

Next steps include optimizing mouthfeel and flavor while retaining viscosity-mediated benefits. Encapsulation strategies could be applied to concentrate PVLE phenolics and to modulate release. Rheology under shear rates typical of the gastrointestinal tract will be measured to refine dosage forms. In vivo studies should quantify fecal bile acid excretion, plasma lipids, and postprandial triglyceride excursions. Mixture designs can further refine PVLE:OPS ratios, and mechanistic work can isolate the contributions of rosmarinic and ursolic acid to the observed lipase inhibition.

CONCLUSION

Cold extraction of *Prunella vulgaris* leaves yielded a phenolic-rich extract that inhibited pancreatic lipase and modestly interacted with bile micelles, while cold-precipitated okra polysaccharide bound bile acids and reduced micellar cholesterol solubility. When combined at a 60:40 PVLE:OPS ratio, the blend expressed synergistic lipase inhibition with an IC50 near 95 μg mL^{-1}, bound taurocholate near 48 percent at 1 mg mL^{-1}, reduced micellar cholesterol solubility by about 48 percent at 200 μg mL^{-1}, and suppressed 3T3-L1 adipogenesis by more than 60

percent at 100 μg mL^{-1} without cytotoxicity. These complementary mechanisms, together with reasonable physical stability at 4 °C, support translational development of a food-compatible hypolipidaemic and antiobesity adjunct. The evidence justifies advancing to simulated digestion models and animal studies to validate efficacy and dosage.

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