Trop J Nat Prod Res, August 2025; 9(8): 3969 – 3974 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of NPB Capsules Consisting of *Allium sativum, Hibiscus sabdariffa, Alisma plantago-aquatica,* and *Gynostemma pentaphyllum* on Lipid Metabolism Disorders

Phung V. Bang[1], Hoang L. Hiep[1], Nguyen H. Ngan[2], Le H. Phu[1]*

[1] *Military Institue of Traditional Medicine, 442 Kim Giang, Dinh Cong, Hanoi 11718, Vietnam*
[2] *Vietnam Military Medical University, 160 Phung Hung, Ha Dong, Hanoi 12108, Vietnam*

ARTICLE INFO

Article history:
Received 04 June 2025
Revised 28 July 2025
Accepted 03 August 2025
Published online 01 September 2025

ABSTRACT

Dyslipidemia is a common metabolic disorder marked by elevated total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), and/or reduced high-density lipoprotein cholesterol (HDL-C). It is a key contributor to atherosclerosis. This study evaluated the therapeutic effects of NPB capsules in a murine model of dyslipidemia. The murine model was used to assess NPB capsules' ability to modulate lipid dysregulation by inhibiting cholesterol esterase (CE) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzymes. *In vitro* assays showed that NPB capsules significantly inhibited cholesterol esterase (26.94%) and HMG-CoA reductase (41.98%). *In vivo*, NPB capsules reduced intestinal cholesterol absorption and hepatic lipogenesis, improving serum lipid profiles. These findings indicate NPB capsules' potential as an herbal candidate for dyslipidemia management and support the utility of further clinical investigation.

Keywords: *Allium sativum, Hibiscus sabdariffa, Alisma plantago-aquatica, Gynostemma pentaphyllum, Dyslipidemia, in vivo test.*

Introduction

Dyslipidemia is a metabolic disorder characterized by abnormal lipid profiles, including elevated TG, TC, and LDL-C, or reduced HDL-C.[1-3] It is often associated with cardiovascular, endocrine, and metabolic diseases and poses significant health risks.[4-6] Etiologically, dyslipidemia is classified as primary, caused by genetic mutations affecting lipid synthesis or clearance, or secondary, resulting from poor diet, a sedentary lifestyle, or excessive alcohol intake.[7-10] Treatment strategies typically include lifestyle modification and lipid-reducing drugs.[11] Recently, bioactive compounds from medicinal plants have gained attention as alternatives to synthetic drugs due to their efficacy and safety profiles.[6,12-18] In Vietnamese traditional medicine, herbal remedies have long been used to manage cardiovascular conditions, including atherosclerosis.[19,20] Garlic (*Allium sativum* L., family Alliaceae) exhibits various biological activities, including cholesterol-lowering, antihypertensive, antimicrobial, anti-atherosclerotic, and anticancer effects.[21,22] Garlic can be fermented into black garlic, which is characterized by a sweet taste, soft texture, and increased bioactivity due to the Maillard and Browning reactions and the alliin biosynthesis pathway.[23,24] Sulfur-containing amino acids, such as methionine and cysteine, are converted into water-soluble organosulfur compounds, such as S-allyl-L-cysteine (SALC), alliin, and tetrahydro-β-carboline (THβC) derivatives.[24] These compounds support garlic's antioxidant and lipid-modulating properties. SALC, in particular, protects DNA from oxidative damage, scavenges free radicals, and inhibits lipid peroxidation. [24,25] Black garlic may also reduce LDL-C oxidation, raise HDL-C, increase antioxidant enzyme activity, and prevent vascular endothelial injury, resulting in anti-atherosclerotic effects.[26,27]

*Corresponding author. Email: hongphu70@yahoo.com
Tel: +84-985735088

Citation: Bang PV, Hiep HL, Ngan NH, Phu LH. Effect of NPB capsules consisting of *Allium sativum, Hibiscus sabdariffa, Alisma plantago-aquatica*, and *Gynostemma pentaphyllum* on lipid metabolism disorders. Trop J Nat Prod Res. 2025; 9(8): 3969 – 3974 https://doi.org/10.26538/tjnpr/v9i8.61

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Roselle (*Hibiscus sabdariffa* L., Malvaceae) is rich in flavonoids, anthocyanidins, organic acids, and phenolic acids, including protocatechuic acid (PCA), a key bioactive compound.[28,29] Roselle calyx extract demonstrates lipid-lowering and anti-atherosclerotic effects of reducing TG, TC, and LDL-C levels in serum, as well as inhibiting foam cell formation and vascular smooth muscle calcification in animal models.[30] Clinical studies have also confirmed significant lipid profile improvements in dyslipidemic adolescents after daily roselle supplementation.[31] PCA is considered a major contributor to these effects.[32] Furthermore, roselle exhibits antioxidant, antihypertensive, antidiabetic, hepatoprotective, and broad-spectrum antimicrobial properties.[31,32]

Gynostemma pentaphyllum (jiaogulan), a member of the Cucurbitaceae family, contains flavonoids and over 100 saponins, primarily gypenosides, some of which are structurally similar to ginsenosides.[33,34] These compounds contribute to *G. pentaphyllum*'s lipid-lowering, antihyperglycemic, hepatoprotective, and antioxidant effects. In high-fat diet-induced models, *G. pentaphyllum* reduced serum TG, TC, and LDL-C levels without affecting HDL-C.[35] These effects derive from adenosine monophosphate-activated protein kinase (AMPK) activation and the downregulation of key adipogenic genes, including peroxisome proliferator-activated receptor-γ (PPARγ), sterol regulatory element-binding protein-1c (SREBP-1c), and fatty acid synthase (FAS), while lipid oxidation is increased via carnitine palmitoyltransferase (CPT) and hormone-sensitive lipase (HSL).[36] Whole saponin extracts also promote fecal cholesterol excretion and modulate genes related to lipid metabolism. *G. pentaphyllum* also exerts antihypertensive, cardioprotective, neuroprotective, and immunomodulatory effects.[37]

Alisma plantago-aquatica L. (Alismataceae) contains bioactive triterpenoids, such as alisol A and alisol B 23-acetate, as well as alkaloids, choline, vitamin B12, and essential oils.[38,39] It is traditionally used in Chinese and Japanese medicine as a diuretic and promotes natriuresis via renal sodium-chloride co-transport.[40,41] Studies have affirmed its anti-inflammatory, antibacterial, hepatoprotective, and immunomodulatory effects.[40] *In vitro* and *in vivo* experiments indicate that its ethanol extract inhibits adipogenesis and exerts lipid-reducing effects.[42] In hyperlipidemic mice, the oral administration of 2.26 g/kg/day significantly reduced serum TC and TG and increased HDL-C, suggesting the potential modulation of hepatic cholesterol synthesis and metabolism.[43]

Existing studies have demonstrated that herbal ingredients such as *A.*

sativum, *H. sabdariffa*, *G. pentaphyllum*, and *A. plantago-aquatica* offer lipid-lowering, antihyperglycemic, and anti-atherosclerotic properties, operating through diverse mechanisms. In combination, these herbs may exert synergistic effects. In particular, *A. plantago-aquatica* contributes diuretic and natriuretic actions that complement the metabolic benefits of the other herbs. However, the traditional uses of these herbs remain limited by their low efficacy and inconvenience to patients. This study was conducted to evaluate the effects of NPB capsules comprising *A. sativum*, *H. sabdariffa*, *A. plantago-aquatica*, and *G. pentaphyllum* on lipid metabolism disorders in mice.

Materials and Methods

Chemicals and Equipment

The chemicals and equipment used include poloxamer 407 (P-407) (Sigma-Aldrich) for animal model induction purposes and blood assay kits for quantifying TC, TG, HDL-C, and LDL-C, sourced from DIALAB GmbH (Austria). The biochemical analysis was conducted with a BTS-350 analyzer (Spain), while hematological analysis was performed with an automated KX21 analyzer (Sysmex, Japan). A rounded needle for oral administration to rodents and an animal surgical kit (Japan) were also employed.

Plant Material Collection

Roselle (*H. sabdariffa)* was collected in October 2019 in Ho Chi Minh City. *A. plantago-aquatica* was collected in April and May 2019 in Kim Son District, Ninh Binh. Black garlic, fermented from solitary garlic (*A. sativum* L.) harvested in 4–5 years in 2022 in Phu Yen, Son La. The 500g packages of black garlic were manufactured on December 16, 2022, with an expiration date of December 16, 2024. *G. pentaphyllum* was collected for 6–7 years in 2020 in Quan Ba, Ha Giang. Thang Long Pharmaceutical Company provided the herbal materials, which met the standards of the Vietnam Pharmacopoeia V. Standard samples were stored at the Botany Department, Institute of Ecology and Biological Resources, Vietnam. All materials were dried, ground, and stored in sealed nylon bags in a dry, cool place for use in research.

Extraction and Standardization of H. sabdariffa

H. sabdariffa was extracted once ultrasonically with 30% ethanol as the solvent and an herbal material to solvent ratio of 1:12. The extraction was performed at 70°C for 30 minutes. The extract was then concentrated at 60–70°C, yielding a liquid extract with an herbal material to solvent ratio of 1:3. The extract was allowed to settle overnight, then decanted. The remaining residue was dissolved in 30% ethanol at a 1:1 ratio, allowed to settle overnight, and decanted again. This process was repeated twice more. The extracts obtained from this repeated process were combined with the initial liquid extract to yield a final 1:3 liquid extract. The standardized *H. sabdariffa* extract was prepared by uniting the 1:3 liquid extract with excipients consisting of a 60:40% mixture of aerosil and maltodextrin at an excipient-to-solids ratio of 0.3. The spray-drying solution was 12% solid material, with an input temperature of 130°C and an output temperature of 102–105°C. The feed rate was 30 mL/min. The final product was a dry extract. The PCA content of the dry extract was quantified and was no less than 300 µg/g.

Extraction and Standardization of A. sativum

Ultrasonic extraction was conducted twice with purified water as the solvent and an herbal material to solvent ratio of 1:10 at 50°C for 70 minutes per extraction. The extract was concentrated into a liquid extract with approximately 25% moisture content. Starch was used as the excipient at a ratio of 20% of the solid content in the extract. The mixture was then dried at 90°C for 13 hours. The dry extract was standardized using the drying method. The content of SALC in the dry extract was quantified and was no less than 350 µg/g.

Extraction and Standardization of A. plantago-aquatica

Ultrasonic extraction was conducted twice with 90% ethanol as the solvent and an herbal material to solvent ratio of 1:5 at 60°C for 60 minutes per extraction. The extract was concentrated into a liquid extract with approximately 25% moisture content. Starch was used as the excipient at a ratio of 20% relative to the solid content in the extract. The mixture was dried at 90°C for 13 hours. The dry extract was standardized using the drying method. The content of alisol A in the dry extract was quantified and was no less than 2.0 mg/g.

Extraction and Standardization of G. pentaphyllum

Ultrasonic extraction was performed once with 80% ethanol as the solvent and an herbal material to solvent ratio of 1:20 at 60°C for 60 minutes. The extract was concentrated into a liquid extract with approximately 25% moisture content. Starch was used as the excipient at a ratio of 20% relative to the solid content in the extract. The mixture was dried at 90°C for 13 hours. The dry extract was standardized using the drying method. The content of ginsenoside Rb1 in the dry extract was quantified and was no less than 1.0 mg/g.

Formulation

The NPB capsule incorporated four herbal extracts: black garlic (fermented *A. sativum*), *H. sabdariffa*, *G. pentaphyllum*, and *A. plantago-aquatica*, as shown in Table 1. The excipients (cornstarch, calcium carbonate, magnesium stearate, and talc) were sufficient for one capsule to ensure proper formulation consistency, stability, and ease of administration. The SALC content in one capsule was no less than 0.04 mg; the PCA content in one capsule was no less than 0.03 mg; the ginsenoside Rb1 content in one capsule was no less than 0.07 mg; and the alisol A content in one capsule was no less than 0.15 mg.

Table 1: NPB capsule ingredients.

Ingredients	Amount (mg)
A. sativum (Black garlic)	120
H. sabdariffa	100
G. pentaphyllum	90
A. plantago-aquatica	90
Excipients	
Corn starch Calcium carbonate Magnesium stearate Talc	Quantity sufficient for one capsule

Animals

Adult Swiss albino mice of both sexes, weighing 20 ± 2g, were provided by the Animal Unit-Military Medical Academy and maintained under laboratory conditions for at least a week before the experiment began. Laboratory animals were fed standard food and provided with free access to water. This study was conducted with the approval of the Scientific Council of the Military Medical University and in compliance with the ethical standards of medical research (Ethical permission number IACUC-2203/22, issued March 22, 2022).

Evaluation of CE Inhibition

The inhibitory effect on the CE enzyme was assessed using a method based on Asmaa *et al*.'s procedure, with spectrophotometric measurements taken at 25 ± 0.5°C.[44] The lyophilized CE was dissolved in 1 mL of 100 mM sodium phosphate buffer at a pH of 7.0, then divided into 200 µL portions and stored at −80°C. Before use, each portion was thawed and diluted to 5 µg/mL with 100 mM sodium phosphate buffer. The reaction volume was 1 mL, with 20 µL of various inhibitors (10 mg/mL in acetonitrile solution) pre-incubated with 500 µL of Triton X-100 (5% w/w), 20 µL of *p*-nitrophenyl butyrate (0.05 M in acetonitrile solution), and 40 µL of acetonitrile solution (2%) in 400 µL of the test buffer solution (100 mM sodium phosphate and 100 mM NaCl at a pH of 7.0) mixed thoroughly for 5 min at 25°C. The reaction was initiated by adding 20 µL of CE (5 µl/mL). After incubation for 15 min, the released *p*-nitrophenoxide was determined by measuring the absorbance at 405 nm. Enzyme activity without inhibition was determined by adding the acetonitrile solution instead of the inhibitor solution. Sodium fluoride (NaF) was used as a positive control. The inhibition rate was calculated with Formula 1,

$$Inhibition\ rate = \frac{(A-B)}{A} x\ 100\% \quad (1)$$

where A and B are the absorbances corresponding to enzyme hydrolysis

without and with the inhibitor, respectively. Each test sample was performed in triplicate to obtain an average value.

Evaluation of HMG-CoA Reductase Inhibition

The inhibition of HMG-CoA reductase was determined with spectrophotometric measurements.[45] The HMG-CoA reductase enzyme assay kit was purchased from Sigma-Singapore. The stock HMG-CoA reductase solution was 0.5–0.75 mg/mL. A 50-µg sample was mixed with a reaction mixture containing nicotinamide adenine dinucleotide phosphate (400 µM), the substrate HMG-CoA (400 µM), and potassium phosphate buffer (100 mM at a pH of 7.4) containing potassium chloride (120 mM), ethylenediaminetetraacetic acid (1 mM), and dithiothreitol (5 mM). Then, 2 µL of HMG-CoA reductase was added. The reaction mixture was incubated at 37°C, and the absorbance was measured at 340 nm after 10 min. Simvastatin was used as a positive control, and distilled water was used as a negative control. The percentage of HMG-CoA reductase inhibition by the test sample was calculated with Formula 2.

$$\%\ inhibition = \frac{\Delta absorbance\ control - \Delta absorbance\ test}{\Delta absorbance\ control} x\ 100\% \quad (2)$$

Evaluation of the Effects of NPB Capsules on Endogenous Lipid Disorders

The endogenous dyslipidemia model with P-407 was employed and modified according to Millar *et al*.[46] The 2% P-407 solution was prepared by dissolving 0.4 g of P-407 in 20 mL of physiological saline and refrigerating it overnight to increase P-407 solubility.

The Swiss mice meeting the experimental standards were randomly divided into five groups of 10 mice.

+ Group 1 (physiological control): Mice received distilled water daily, and on the 7th day, received an intraperitoneal injection of physiological saline at 10 ml/kg.

+ Group 2 (model group): Mice received distilled water daily, and on the 7th day, received an intraperitoneal injection of P-407 at a dose of 200 mg/kg.

+ Group 3 (NPB 576): Mice received the test drug at a dose of 576 mg/kg/day daily, and on the 7th day, received an intraperitoneal injection of P-407 at a dose of 200 mg/kg.

+ Group 4 (NPB 1152): Mice received the test drug at a dose of 1152 mg/kg/day daily, and on the 7th day, received an intraperitoneal injection of P-407 at a dose of 200 mg/kg.

+ Group 5 (Atorvastatin 15): Mice received atorvastatin at a dose of 15 mg/kg daily, and on the 7th day, received an intraperitoneal injection of P-407 at a dose of 200 mg/kg.

On the 8th day, 24 hours after the P-407 injection, blood samples were collected from all mice to test the TG, TC, and HDL-C lipid parameters. The non-HDL-C index (non-HDL-C) was calculated with Formula 3.

Non-HDL-C = TC − HDL-C (3)

The atherogenic index (AI) was calculated with Formula 4.

AI = (TC − HDL-C)/HDL-C (4)

Statistical Analysis

The data were analyzed with biomedical statistical methods, and comparisons were made via ANOVA in SPSS software version 16.0. The results are presented as mean ± standard deviation ($\overline{X}$ ± SD). A *p*-value of less than 0.05 was considered statistically significant.

Results and Discussion

Inhibitory Effects of NPB on CE and HMG-CoA Reductase

The percentage of CE inhibition is presented in Table 2. This study used NaF as a positive control that significantly inhibited CE (38.45 ± 3.42% reduction). The enzyme CE plays an important role in cholesterol metabolism. Demonstrating test substances' inhibition of this enzyme is vital to evaluating their potential to reduce cholesterol levels. The test sample exhibited 26.94% inhibition of CE, indicating its potential to lower cholesterol through CE inhibition. The percentage of CE inhibition by the test sample derived from medicinal plants was lower than that of NaF. At the same concentration, the test sample's effect is expected to be less potent than that of the synthetic reference drug NaF. In the HMG-CoA reductase inhibition test, simvastatin was used as a positive control and significantly inhibited HMG-CoA reductase (82.15 ± 3.27% reduction). HMG-CoA reductase is an enzyme that plays a crucial role in cholesterol metabolism. Measuring the inhibition of this enzyme by the test substances supports assessing their potential to reduce cholesterol levels. The test sample exhibited a 41.98% inhibition of HMG-CoA reductase, indicating that it lowered cholesterol by inhibiting HMG-CoA reductase. The percentage of HMG-CoA reductase inhibition by the test sample derived from medicinal plants was lower than that of simvastatin. At the same concentration, the test sample's effect is expected to be less potent than that of simvastatin.

Table 2: Inhibition of CE and HMG-CoA reductase (mean ± SD).

Tests	n	Inhibition of CE (%)[a]	Inhibition of HMG-CoA (%)[a]
NPB	06	26.94 ± 2.36	41.98 ± 4.15
NaF[b]	06	38.45 ± 3.42	-
Simvastatin[b]	06	-	82.15 ± 3.27

[a] Data are presented as the mean ± SD of results from three independent experiments. [b] Positive control. (-) Indicates no test.

Effects of NPB on Endogenous Lipid Disorders

As shown in Table 3, TC, TG, and non-HDL-C blood levels were significantly elevated after P-407 intraperitoneal injection. Mice in the P-407 treatment group (Group 2) exhibited markedly increased levels of TC, TG, and non-HDL-C compared to the control group ($p < 0.01$). Conversely, atorvastatin and the two NPB dosage groups (Groups 3, 4, and 5) demonstrated significant reductions in TC, TG, and non-HDL-C. Mice that received NPB at dosages of 576 or 1152 mg/kg/day and those that received atorvastatin at 15 mg/kg/day all showed significantly lower levels of TC, TG, and non-HDL-C compared to the P-407 group ($p < 0.01$).

Table 3: Blood levels of TC, TG, and non-HDL-C in mice.

Group		n	TC (mmol/L)	TG (mmol/L)	Non-HDL-C (mmol/L)
Group 1 (physiological control)	(1)	10	2.14 ± 0.35	0.81 ± 0.13	1.56 ± 0.36
Group 2 (model group)	(2)	10	2.66 ± 0.44	1.01 ± 0.13	2.17 ± 0.39
Group 3 (NPB 576)	(3)	10	2.01 ± 0.42	0.86 ± 0.13	1.45 ± 0.41
Group 4 (NPB 1152)	(4)	10	1.99 ± 0.53	0.85 ± 0.08	1.42 ± 0.52
Group 5 (atorvastatin 15)	(5)	10	2.06 ± 0.30	0.84 ± 0.11	1.48 ± 0.30
p			$p_{-1} < 0.01$; $p_{-2} < 0.01$		

p-values were calculated using one-way ANOVA followed by Tukey's post-hoc test to compare TC, TG, and non-HDL-C levels among the study groups.

P-407 administration also resulted in a reduction in blood HDL-C levels ($p < 0.05$), whereas atorvastatin at 15 mg/kg/day and NPB doses of 576 and 1152 mg/kg/day led to significant increases in HDL-C (Table 4). Compared to the P-407 model group, the mice treated with NPB doses of 576 and 1152 mg/kg/day and atorvastatin 15 mg/kg/day exhibited significantly higher HDL-C levels ($p < 0.05$). The AI was similarly elevated by the P-407 injection ($p < 0.01$), whereas the NPB and atorvastatin showed significant reductions in AI. Mice treated with NPB doses of 576 or 1152 mg/kg/day and those that received atorvastatin at 15 mg/kg/day exhibited decreases in AI compared to the model group ($p < 0.01$).

Table 4: Blood levels of HDL-C and AI in mice.

Group	n	HDL-C (mmol/L)	AI
Group 1 (physiological control) (1)	10	0.58 ± 0.06	2.73 ± 0.77
Group 2 (model group) (2)	10	0.49 ± 0.10	4.51 ± 0.85
Group 3 (NPB 576) (3)	10	0.56 ± 0.04	2.56 ± 0.69
Group 4 (NPB 1152) (4)	10	0.57 ± 0.06	2.49 ± 0.96
Group 5 (atorvastatin 15) (5)	10	0.58 ± 0.05	2.59 ± 0.63
p		$p_{-1} < 0.05$; $p_{-2} < 0.05$	$p_{-1} < 0.01$; $p_{-2} < 0.01$

p-values were calculated using one-way ANOVA followed by Tukey's post-hoc test to compare HDL-C levels and AI among the study groups.

The endogenous lipid disorder model was established by the intraperitoneal injection of non-ionic surfactants, such as Tween 80, Triton WR-1339, or P-407, without dietary supplementation with exogenous lipids. Previous studies have demonstrated that the injected surfactants promote hepatic cholesterol production. P-407 is widely utilized in experimental research as it can induce lipid abnormalities safely and effectively. P-407-induced lipid disorders are associated with changes in several lipid metabolism-related enzymes, including a reduction in hepatic LDL receptors, an increase in the quantity and activity of HMG-CoA reductase, and the suppression of serum lipoprotein lipase (LPL) and cholesterol 7α-hydroxylase. Therefore, P-407 administration results in elevated blood TG and cholesterol levels. This study's findings indicate that intraperitoneal injection of P-407 caused significant lipid abnormalities in mice, including increased levels of TC, TG, non-HDL-C, and AI, as well as decreased HDL-C levels, compared to the control group. The test drug and atorvastatin showed substantial efficacy in managing lipid disorders in the endogenous lipid disorder model.

Dyslipidemia is a metabolic disorder characterized by abnormal lipid levels in the blood, including cholesterol and TG.[1] This condition can result in significant health issues, especially cardiovascular diseases, such as atherosclerosis, coronary artery disease, and stroke. Common forms of dyslipidemia include hypercholesterolemia, in which non-HDL-C, often called "bad" cholesterol, levels are elevated, which can create arterial plaques, reducing blood flow and increasing cardiovascular risk.[2,9] Hypertriglyceridemia, marked by elevated triglycerides in the blood, is often associated with metabolic disorders, obesity, or diabetes. Meanwhile, low levels of HDL-C, or "good" cholesterol, can also increase the risk of heart disease. Dyslipidemia can arise from genetic factors such as familial hypercholesterolemia, unhealthy lifestyle choices such as poor diet, lack of exercise, or smoking, or from underlying medical conditions such as diabetes, obesity, and kidney disease. Treatment typically involves adopting a healthy diet that is low in saturated fats, increasing physical activity to reduce triglycerides and boost HDL-C, and, occasionally, using medications such as statins, fibrates, niacin, and ezetimibe to help regulate lipid levels. As dyslipidemia often presents with no clear symptoms, regular testing of cholesterol and triglyceride levels is essential for early detection and timely intervention.[10]

This study systematically investigated the lipid-lowering effects of a combination of standardized herbal extracts derived from fermented *A. sativum* (black garlic), *H. sabdariffa*, *G. pentaphyllum*, and *A. plantago-aquatica*. These medicinal plants offer valuable therapeutic benefits, particularly for lipid metabolism and cardiovascular health. Garlic, especially in the fermented form of black garlic, contains potent sulfur compounds, such as SALC, that exert antioxidant, lipid-lowering, and anti-atherosclerotic effects.[25,26] It reduces cholesterol levels, protects against endothelial damage, and prevents arterial plaque formation. Roselle (*H. sabdariffa*) is rich in flavonoids and phenolic acids, such as PCA, and is known for its cholesterol-lowering and anti-atherosclerotic properties; clinical studies confirm its ability to reduce TG and non-HDL-C levels while increasing HDL-C levels.[28,29] *G. pentaphyllum*, which is known for its saponins and flavonoids, offers multiple benefits, including reducing blood lipid levels, improving heart health, and supporting immunity. It has been shown to regulate lipid metabolism, reduce TG and cholesterol levels, and protect against fat accumulation.[31-33] Lastly, *A. plantago-aquatica*, which produces triterpenoid compounds, such as alisol A, has diuretic and anti-inflammatory effects and demonstrable lipid-lowering properties, including cholesterol and TG level reduction in experimental models.[39] This study integrates both *in vitro* enzymatic assays and *in vivo* pharmacodynamic models of extracts from these four medicinal plants, offering a robust and multilayered evaluation of these formulations' therapeutic potential against hyperlipidemia. In the enzymatic assays, the herbal formulations were screened for their ability to inhibit CE and HMG-CoA reductase, two crucial enzymes in cholesterol digestion and biosynthesis. The assay results showed that the herbal formulations exhibited significant inhibitory activity, particularly against CE (26.94% reduction) and HMG-CoA reductase (41.98% reduction). These findings indicate a biochemical basis for the lipid-lowering effects observed later *in vivo*. While the inhibition achieved with the herbal treatment was not as pronounced as that achieved by the synthetic positive controls (NaF for CE and simvastatin for HMG-CoA reductase), this result was expected due to the phytochemicals' lower binding affinities and multifaceted mechanisms. The consistency and relative potency of the tested formulations must be assessed for further preclinical and clinical development. The NPB capsules' moderate enzyme inhibition suggests that they contain bioactive secondary metabolites, such as flavonoids, saponins, and polyphenols, that may exert effects via allosteric modulation or indirect regulatory pathways rather than via direct competitive inhibition. These mechanisms could reduce intestinal cholesterol absorption, downregulate hepatic cholesterol synthesis, or upregulate LDL receptor expression.

The modulation of endogenous lipid metabolism in a P-407-induced model was also investigated. The endogenous lipid disorder model was produced via intraperitoneal injection of P-407, which is well established for inducing dyslipidemia by increasing hepatic cholesterol synthesis and suppressing lipid catabolism. Mice in the model group developed pronounced hyperlipidemia, with elevated levels of TC, TG, non-HDL-C, and AI, as well as reduced HDL-C levels. Treatment with NPB (doses of 576 or 1152 mg/kg/day) resulted in a statistically significant reversal of these lipid abnormalities. NPB capsules reduced the pathological increase in TC, TG, and non-HDL-C levels and restored HDL-C to near-normal levels. The observed changes were similar to those achieved by atorvastatin, a benchmark hypolipidemic agent. Moreover, the marked reduction in AI suggests that NPB may have a protective cardiovascular effect, likely through multiple mechanisms, including the inhibition of hepatic HMG-CoA reductase, modulation of LPL activity, and potential stimulation of bile acid excretion. This multi-targeted action reflects the complexity of phytochemical interactions and emphasizes plant extracts' potential as polyvalent therapeutics for metabolic disorders.

Conclusion

NPB capsules comprising standardized extracts from fermented *A. sativum* (black garlic), *H. sabdariffa*, *G. pentaphyllum*, and *A. plantago-aquatica* demonstrated significant lipid-lowering effects in both *in vitro* and *in vivo* models. At 0.2 mg/mL, NPB capsules inhibited cholesterol esterase (26.94 ± 2.36%) and HMG-CoA reductase (41.98 ± 4.15%). In mice with diet-induced dyslipidemia, the oral administration of NPB capsules at 576 or 1152 mg/kg/day significantly reduced TG, TC, non-HDL-C, and AI while increasing HDL-C, with effects comparable to atorvastatin (15 mg/kg/day). The administration of NPB capsules at a dose of 1152 mg/kg/day resulted in more pronounced improvement in endogenous dyslipidemia than that of the 576 mg/kg/day dose. These findings indicate NPB capsules' potential as a natural adjunct or alternative to statins, as the components exert their effects through mechanisms that include enzyme inhibition and lipid profile modulation. Further studies are warranted to elucidate the active constituents, molecular pathways, and clinical efficacy of such capsules.

Conflict of Interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements
We thank the Department of Pharmacology, Institute of Pharmacy Training, Vietnam Military Medical University for creating favorable conditions to help us complete this study.

References

1. Arvanitis M, Lowenstein CJ. Dyslipidemia. Ann Intern Med. 2023; 176(6):ITC81-ITC96. Doi: 10.7326/AITC202306200
2. Vekic J, Zeljkovic A, Stefanovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. Obesity and dyslipidemia. Metabolism. 2019; 92:71-81. Doi: 10.1016/j.metabol.2018.11.005
3. Warditiani NK, Swastini DA, Arisanti CIS, Sari PMNA, Wirasuta IMAG. Pharmacological potential of *Andrographis paniculata* (Burm. f.) Neesin preventing atherosclerosis: A review. Trop J Nat Prod Res. 2021; 5(11):1913-1918. Doi: 10.26538/tjnpr/v5i11.3
4. Su X, Chen X, Wang B. Pathology of metabolically-related dyslipidemia. Clin Chim Acta. 2021; 521:107-115. Doi: 10.1016/j.cca.2021.06.029
5. Salim HM, Kurnia LF, Bintarti TW, Handayani H, Shimabukuro M. Hepatoprotective effects of methanol extract of *Syzygium polyanthum* L. leaves (Salam) on high fat diet. Trop J Nat Prod Res. 2021; 5(12):2092-2095. Doi: 10.26538/tjnpr/v5i12.8
6. Retnaningsih R, Jaeri S, Husni A, Maharani N, Anjani G. The hypolipidemic effects of combination of garlic, red yeast rice, and red ginger on hormonal status and lipid profiles among post-stroke dyslipidemia. Trop J Nat Prod Res. 2025; 9(6):2692-2696. Doi: 10.26538/tjnpr/v9i6.45
7. Su X, Cheng Y, Zhang G, Wang B. Novel insights into the pathological mechanisms of metabolic related dyslipidemia. Mol Biol Rep. 2021; 48(7):5675-5687. Doi: 10.1007/s11033-021-06529-0
8. Suh SH, Kim SW. Dyslipidemia in patients with chronic kidney disease: An updated overview. Diabetes Metab J. 2023; 47(5):612-629. Doi: 10.4093/dmj.2023.0067
9. Pires A, Sena C, Seiça R. Dyslipidemia and cardiovascular changes in children. Curr Opin Cardiol. 2016; 31(1):95-100. Doi: 10.1097/HCO.0000000000000249
10. Kavey REW. Combined dyslipidemia in children and adolescents: A proposed new management approach. Curr Atheroscler Rep. 2023; 25(5):237-245. Doi: 10.1007/s11883-023-01099-x
11. Borghi C, Fogacci F, Agnoletti D, Cicero AFG. Hypertension and dyslipidemia combined therapeutic approaches. High Blood Press Cardiovasc Prev. 2022; 29(3):221-230. Doi: 10.1007/s40292-022-00507-8
12. Trautwein EA, McKay S. The role of specific components of a plant-based diet in management of dyslipidemia and the impact on cardiovascular risk. Nutrients. 2020; 12(9):2671. Doi: 10.3390/nu12092671
13. Kirkpatrick CF, Sikand G, Petersen KS, Anderson CAM, Aspry KE, Bolick JP, Kris-Etherton PM, Maki KC. Nutrition interventions for adults with dyslipidemia: A clinical perspective from the national lipid association. J Clin Lipidol. 2023; 17(4):428-451. Doi: 10.1016/j.jacl.2023.05.099
14. Silva ML, Bernardo MA, Singh J, de Mesquita MF. Cinnamon as a complementary therapeutic approach for dysglycemia and dyslipidemia control in type 2 diabetes mellitus and its molecular mechanism of action: A review. Nutrients. 2022; 14(13):2773. Doi: 10.3390/nu14132773
15. Okokon JE, Nyong ME, Thomas PS, Daniel AO, Enin GN, Udobang JA. Antiobesity activity of extract, fractions and pure compounds from husk of *Zea mays*. Trop J Nat Prod Res. 2021; 5(10):1868-1875. Doi: 10.26538/tjnpr/v5i10.27
16. Siriyong T, Boonya-arunnate T, Ninbodee K, Kaewmuean T, Mettriyasakul T, Jawang K, Thongmongkol P , Chanwanitsakul S, Kaewnoi K, Buachum B, Saeloh D, Noosak C, Voravuthikunchai SP. Effects of traditional Thai herbal formulations in patients with obesity and borderline hyperlipidemia - A preliminary pilot study. Trop J Nat Prod Res. 2022; 6(1):29-33. Doi: 10.26538/tjnpr/v6i1.6
17. Fakayode1 AE, Emma-Okon BO, Morakinyo AE, Fajobi AO, Akinyele KN, Oyedapo OO. Investigations of ameliorative potentials extract of *P. pellucida* on salt-fructose induced dyslipidemia in Wistar rats. Trop J Nat Prod Res. 2023; 7(9):4112-4116. Doi: 10.26538/tjnpr/v7i9.40
18. Binh NTM, Ngan NH, Lan DTH, Hanh NH, Tuan NTH. Anti-hyperlipidemic effect of *Camellia hakodae* Ninh extract in an in vivo rat model. Trop J Nat Prod Res. 2024; 8(11):9199-9205. Doi: 10.26538/tjnpr/v8i11.32
19. Loi DT. Vietnamese medicinal plants and herbs. Hanoi: Medicine Publishing House; 2016. 1300 p.
20. Chi VV. Dictionary of Vietnamese medicinal plants. Hanoi: Medicine Publishing House; 2012. 3200 p.
21. El-Saber Batiha G, Magdy Beshbishy A, G Wasef L, Elewa YHA, A Al-Sagan A, Abd El-Hack ME, Taha AE, M Abd-Elhakim Y, Prasad Devkota H. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. Nutrients. 2020; 12(3):872. Doi: 10.3390/nu12030872
22. Majewski M. *Allium sativum*: facts and myths regarding human health. Rocz Panstw Zakl Hig. 2014; 65(1):1-8.
23. Ahmed T, Wang CK. Black garlic and its bioactive compounds on human health diseases: A review. Molecules. 2021; 26(16):5028. Doi: 10.3390/molecules26165028
24. Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. Black garlic: A critical review of its production, bioactivity, and application. J Food Drug Anal. 2017; 25(1):62-70. Doi: 10.1016/j.jfda.2016.11.003
25. Ajebli M, Eddouks M. Phytotherapy of hypertension: An updated overview. Endocr Metab Immune Disord Drug Targets. 2020; 20(6):812-839. Doi: 10.2174/1871530320666191227104648
26. Javed M, Ahmed W. Black garlic: A review of its biological significance. J Food Biochem. 2022; 46(12):e14394. Doi: 10.1111/jfbc.14394
27. Stępień AE, Trojniak J, Tabarkiewicz J. Anti-cancer and anti-inflammatory properties of black garlic. Int J Mol Sci. 2024; 25(3):1801. Doi: 10.3390/ijms25031801
28. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. - a phytochemical and pharmacological review. Food Chem. 2014; 165:424-443. Doi: 10.1016/j.foodchem.2014.05.002
29. Sim YY, Nyam KL. *Hibiscus cannabinus* L. (kenaf) studies: Nutritional composition, phytochemistry, pharmacology, and potential applications. Food Chem. 2021; 344:128582. Doi: 10.1016/j.foodchem.2020.128582
30. Wahabi HA, Alansary LA, Al-Sabban AH, Glasziuo P. The effectiveness of *Hibiscus sabdariffa* in the treatment of hypertension: a systematic review. Phytomedicine. 2010; 17(2):83-86. Doi: 10.1016/j.phymed.2009.09.002
31. Serban C, Sahebkar A, Ursoniu S, Andrica F, Banach M. Effect of sour tea (*Hibiscus sabdariffa* L.) on arterial hypertension: a systematic review and meta-analysis of randomized controlled trials. J Hypertens. 2015; 33(6):1119-1127. Doi: 10.1097/HJH.0000000000000585
32. Yagi S, Uba AI, Sinan KI, Piatti D, Sagratini G, Caprioli G, Eltigani SM, Lazarova I, Zengin G. Comparative study on the chemical profile, antioxidant activity, and enzyme

inhibition capacity of red and white *Hibiscus sabdariffa* variety calyces. ACS Omega. 2023; 8(45):42511-42521. Doi: 10.1021/acsomega.3c05217
33. Su C, Li N, Ren R, Wang Y, Su X, Lu F, Zong R, Yang L, Ma X. Progress in the medicinal value, bioactive compounds, and pharmacological activities of *Gynostemma pentaphyllum*. Molecules. 2021; 26(20):6249. Doi: 10.3390/molecules26206249
34. Xie P, Luo HT, Pei WJ, Xiao MY, Li FF, Gu YL, Piao XL. Saponins derived from *Gynostemma pentaphyllum* regulate triglyceride and cholesterol metabolism and the mechanisms: A review. J Ethnopharmacol. 2024; 319(Pt 1):117186. Doi: 10.1016/j.jep.2023.117186
35. Dai N, Zhao FF, Fang M, Pu FL, Kong LY, Liu JP. *Gynostemma pentaphyllum* for dyslipidemia: A systematic review of randomized controlled trials. Front Pharmacol. 2022; 13:917521. Doi: 10.3389/fphar.2022.917521
36. Weng X, Lou YY, Wang YS, Huang YP, Zhang J, Yin ZQ, Pan K. New dammarane-type glycosides from *Gynostemma pentaphyllum* and their lipid-lowering activity. Bioorg Chem. 2021; 111:104843. Doi: 10.1016/j.bioorg.2021.104843
37. Nguyen NH, Ha TKQ, Yang JL, Pham HTT, Oh WK. Triterpenoids from the genus *Gynostemma*: Chemistry and pharmacological activities. J Ethnopharmacol. 2021; 268:113574. Doi: 10.1016/j.jep.2020.113574
38. Feng L, Liu TT, Huo XK, Tian XG, Wang C, Lv X, Ning J, Zhao WY, Zhang BJ, Sun CP, Ma XC. *Alisma* genus: Phytochemical constituents, biosynthesis, and biological activities. Phytother Res. 2021; 35(4):1872-1886. Doi: 10.1002/ptr.6933
39. Wu Y, Wang X, Yang L, Kang S, Yan G, Han Y, Fang H, Sun H. Therapeutic effects of *Alisma orientale* and its active constituents on cardiovascular disease and obesity. Am J Chin Med. 2023; 51(3):623-650. Doi: 10.1142/S0192415X23500301
40. Zhang LL, Xu W, Xu YL, Chen X, Huang M, Lu JJ. Therapeutic potential of rhizoma *Alismatis*: A review on ethnomedicinal application, phytochemistry, pharmacology, and toxicology. Ann N Y Acad Sci. 2017; 1401(1):90-101. Doi: 10.1111/nyas.13381
41. Zhang J, Yan X, Jin Q, Chen J, Yang L, Wei W, Qu H, Yao C, Hou J, Gong L, Wu W, Guo DA. Novel triterpenoids from *Alisma plantago-aquatica* with influence on LDL uptake in HepG2 cells by inhibiting PCSK9. Phytomedicine. 2022; 105:154342. Doi: 10.1016/j.phymed.2022.154342
42. Park YJ, Kim MS, Kim HR, Kim JM, Hwang JK, Yang SH, Kim HJ, Lee DS, Oh H, Kim YC, Ryu DG, Lee YR, Kwon KB. Ethanol extract of *Alismatis* rhizome inhibits adipocyte differentiation of OP9 Cells. Evid Based Complement Alternat Med. 2014; 2014:415097. Doi: 10.1155/2014/415097
43. Xu F, Chen J, Zhang Y, Wu Q, Shen Y, Gu W, Liu S, Lu C, Liao H, Bao K. Molecular insight into the mechanism of lipid regulating effect of *Alisma orientalis* based on ACAT. Int J Biol Macromol. 2020; 158:1141-1162. Doi: 10.1016/j.ijbiomac.2020.04.260
44. Asmaa BH, Nayal Ream. *In vitro* screening of the pancreatic cholesterol esterase inhibitory activity of some medicinal plants grown in Syria. Int J Pharmacog Phytochem Res. 2016; 8(8):1432-1436.
45. Baskaran G, Salvamani S, Ahmad SA, Shaharuddin NA, Pattiram PD, Shukor MY. HMG-CoA reductase inhibitory activity and phytocomponent investigation of *Basella alba* leaf extract as a treatment for hypercholesterolemia. Drug Des Devel Ther. 2015; 9:509-517. Doi: 10.2147/DDDT.S75056
46. Millar JS, Cromley DA, McCoy MG, Rader DJ, Billheimer JT. Determining hepatic triglyceride production in mice: comparison of poloxamer 407 with Triton WR-1339. J Lipid Res. 2005; 46(9):2023-2028. Doi: 10.1194/jlr.D500019-JLR200