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ORIGINAL ARTICLE

Hypolipidaemic Effect of an Immature Pomelo (*Citrus Maxima*) Extract in a Tyloxapol-Induced Hyperlipidaemia Mouse Model

Ba Dien Pham,[1] Chi Nhan Ton,[2] Ngoc Chi Lan Nguyen,[2] Minh Hoang Le[2]

1. Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam.
2. Faculty of Traditional Medicine, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam.

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Corresponding author:
MINH HOANG LE
E: lmhoang@ctump.edu.vn
T: +84 973 431 666

Abstract

Background/Aim: Pomelo extracts are rich in bioactive flavonoids and may regulate lipid metabolism. However, studies evaluating the lipid-lowering effects of immature pomelo derived from agricultural by-products remain limited in Vietnam. This study was conducted to investigate the hypolipidaemic activity of a liquid extract obtained from immature pomelo (*Citrus maxima*) in a tyloxapol-induced mouse model of acute hyperlipidaemia.

Methods: Sixty mice were randomly assigned to six groups: normal control, pathological control (tyloxapol-induced hyperlipidaemia), positive control (atorvastatin-treated) and three experimental groups receiving immature pomelo extract orally at doses of 5.17, 10.34 and 15.51 g/kg. Key lipid indicators, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were quantified in serum samples. Data were analysed using appropriate statistical tests, including the Shapiro–Wilk test, independent t-test and Levene's test.

Results: All extract doses significantly reduced total cholesterol, triglycerides and LDL-C compared with the pathological control ($p < 0.001$). The lowest dose produced the greatest reduction in total cholesterol (3.420 mmol/L), while higher doses showed stronger effects on HDL-C restoration, reaching levels similar to those recorded in the normal and atorvastatin-treated groups (2.100 and 1.950 mmol/L; $p > 0.05$).

Conclusion: The liquid extract of immature pomelo demonstrated significant hypolipidaemic activity in this experimental model.

Key words: *Citrus maxima*; Dyslipidaemias; Flavonoids; Hyperlipidaemia model; Tyloxapol.

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Introduction

Dyslipidaemia refers to an imbalance in lipid parameters, typically involving elevated total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C), together with reduced high-density lipoprotein cholesterol (HDL-C) levels. This condition plays a central role in the pathogenesis of atherosclerosis and related cardiovascular disorders.[1] Cardiovascular disorders remain the leading cause of mortality globally, with an estimated 17.9 million deaths reported each year according to the World Health Organisation.[2] Currently, Western medicines such as statins and fibrates are widely used for the treatment of dyslipidaemia; however, their long-term use may cause adverse effects, in-

cluding elevated liver enzymes, rhabdomyolysis, renal vascular obstruction and gastrointestinal disturbances.[3] Therefore, the search for alternative or complementary therapies derived from natural medicinal resources with favourable safety profiles has attracted increasing attention.

According to traditional medicine theory, dyslipidaemia corresponds to the category of "phlegm-dampness syndrome", associated with spleen deficiency, liver stagnation and excessive intake of fatty and sweet foods leading to the accumulation of turbid phlegm. The principal therapeutic approaches include strengthening the spleen, resolving phlegm and eliminating dampness.[4, 5] Several medicinal plants such as mango leaves, shallots, *Gymnanthemum amygdalinum* leaves and particularly immature pomelo—an agricultural by-product commonly discarded during cultivation—have been reported to possess lipid-lowering effects.[6, 7] Pomelo contains various bioactive compounds, mainly flavonoids such as naringin, hesperidin and poncirin, which have been reported to exhibit antioxidant, anti-inflammatory and lipid metabolism–regulating activities.[6, 8, 9] These compounds may exert hypolipidaemic effects through mechanisms including inhibition of HMG-CoA reductase, upregulation of hepatic LDL receptors and modulation of genes involved in fatty acid oxidation.[10]

In Vietnam, systematic studies evaluating the lipid-lowering effects of extracts from immature pomelo in experimental models remain limited.[6] Therefore, this study was conducted to assess the hypolipidaemic activity of a liquid extract derived from immature pomelo using a mouse model of induced hyperlipidaemia, with the aim of providing further scientific evidence supporting its therapeutic potential.

Methods

This study employed a controlled experimental design to investigate the hypolipidaemic effect of an immature pomelo (*Citrus maxima*) extract in a tyloxapol-induced hyperlipidaemia mouse model.

Animals

White mice (*Mus musculus*) of both sexes (5–6 weeks old; body weight 20 ± 2 g) were obtained from the Pasteur Institute in Nha Trang. Animals were maintained under standard laboratory conditions and acclimatised for at least one week prior to experimentation.

The mice were randomly allocated into six groups (n = 10 per group): a normal control group, a pathological control group, three extract-treated groups receiving immature pomelo extract at doses of 5.17, 10.34 and 15.51 g/kg body weight and a positive control group treated with atorvastatin (64 mg/kg). A total of 60 animals were used.

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Can Tho University of Medicine and Pharmacy (Vietnam) and were conducted in accordance with institutional guidelines and international standards for the care and use of laboratory animals.

Liquid extract of immature pomelo (*Citrus maxima*)

The plant material (immature fruits of *Citrus maxima*) was collected from An Giang Province, Vietnam. Botanical identification was performed using DNA barcoding based on matK gene sequencing at the Institute of Biotechnology and Food Technology, Can Tho University. The obtained sequence showed 99.87 % similarity with reference sequences of Citrus maxima in the NCBI database, confirming the taxonomic identity of the plant material.

Dried immature fruits were used for extract preparation. A total of 420 g of dried material was subjected to aqueous extraction by decoction. In the first extraction, the material was decocted in 2000 mL of distilled water at 100 °C for 2 hours. The residue was subsequently re-extracted under identical conditions. The combined extracts were concentrated under reduced pressure to obtain the final extract. A schematic representation of the extraction process is provided in Figure 1.

The resulting extract was subjected to physicochemical and microbiological quality control at the Can Tho Technical Centre of Standards, Metrology and Quality. The extract met quality requirements, with no detectable levels of heavy metals (As, Pb, Hg, Cd), aflatoxins, or pathogenic microorganisms, including *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus*. All analyses were conducted in accordance with relevant quality standards.

The experimental dose was calculated from the traditional human dose of dried immature pomelo (22 g/day). Based on a reference adult body weight of 50 kg, the human equivalent dose was 0.44 g/kg/day. Using the standard interspecies conversion factor of 11.76,[11] the corresponding dose in mice was estimated at 5.17 g/kg/day. The liquid extract was administered orally to mice using a curved, blunt-ended gavage needle inserted carefully into the stomach.

Evaluation of the lipid-lowering effect of immature pomelo liquid extract in a tyloxapol-induced hyperlipidaemia mouse model

An acute hyperlipidaemia model was established using tyloxapol. Mice were acclimatised under standard laboratory conditions for at least one week prior to experimentation. The animals were randomly assigned to six groups (n = 10 per group), including a normal control group, a pathological control group, three extract-treated groups and a positive control group. Hyperlipidaemia was induced by a single intraperitoneal administration of tyloxapol (*Triton WR-1339*) at a dose of 400 mg/kg. Table 1 and Figure 2 summarise the experimental design.

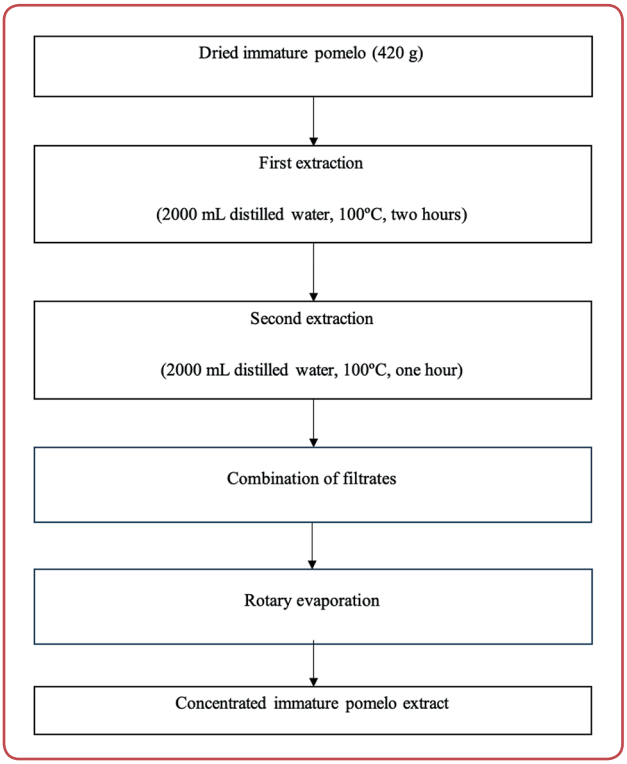

Figure 1: Flowchart of the preparation of immature pomelo (Citrus maxima) water extract

Table 1: Experimental design for evaluating the hypolipidemic effects of immature pomelo extract

Group	Hyperlipidaemia induction	Test sample
Normal control group	0.9 % NaCl (no tyloxapol injection)	Distilled water
Pathological control group	Tyloxapol (Triton WR-1339), 400 mg/kg, administered intraperitoneally (ip), T0307, Sigma-Aldrich, MO, USA; single injection.[12-15]	Distilled water
Extract group 1		Liquid extract of immature pomelo, dose 5.17 g/kg
Extract group 2		Liquid extract of immature pomelo, dose 10.34 g/kg
Extract group 3		Liquid extract of immature pomelo, dose 15.51 g/kg
Positive control group		Atorvastatin 64 mg/kg[13]

Data collection methods

The pathological control group, extract-treated groups and the positive control group received the respective treatments at 60 minutes and 12 hours after tyloxapol injection. All treatments were delivered orally via gavage at a volume of 0.1 mL per 10 g of body weight.

Tyloxapol was dissolved in distilled water and sonicated until completely dissolved prior to administration. The immature pomelo liquid extract and atorvastatin were freshly prepared each day in distilled water at doses of 5.17 g/kg, 10.34 g/kg, 15.51 g/kg and 64 mg/kg, respectively.

At twenty-four hours after tyloxapol injection, the mice were anesthetised by CO_2 inhalation and blood samples (approximately 1 mL) were collected via cardiac puncture. The samples were then centrifuged at 4000 rpm for 20 minutes at room temperature to separate serum. The resulting serum was subsequently analysed for lipid-related parameters, including total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C.

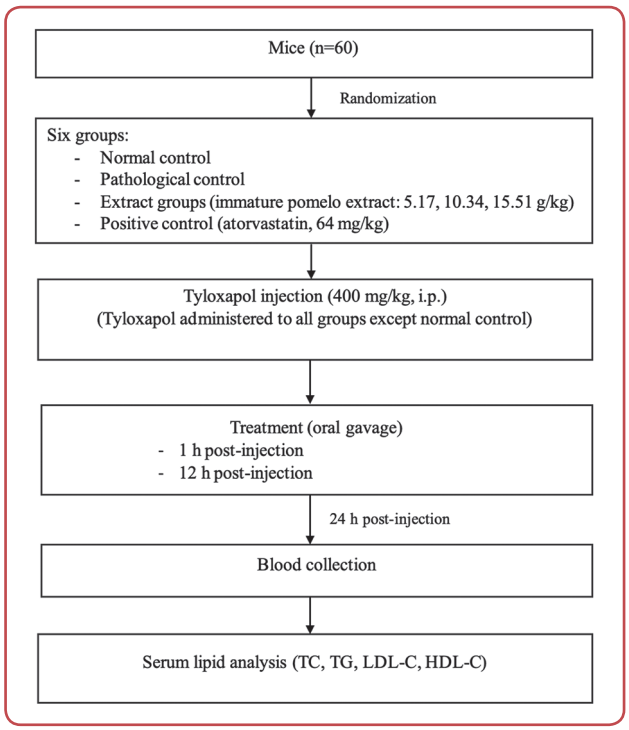

Figure 2: Schematic diagram of the experimental design used to evaluate the hypolipidaemic effect of immature pomelo extract in a tyloxapol-induced hyperlipidaemia mouse model

TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol;

Statistical analysis

Statistical analyses were conducted using SPSS (version 27.0). Data are expressed as mean ± standard deviation (SD) for normally distributed variables, or as median with interquartile range (IQR) for non-normally distributed data. The distribution of continuous variables was assessed using the Shapiro–Wilk or Kolmogorov–Smirnov tests, as appropriate, while homogeneity of variance was examined using Levene's test. For comparisons between two independent groups, the independent samples t-test was applied when parametric assumptions were met; otherwise, the Mann–Whitney U test was used. For multiple group comparisons, the Kruskal–Wallis test was performed, followed by pairwise comparisons where appropriate. Statistical significance was defined at $p < 0.05$.

Results

Induction and confirmation of the hyperlipidaemia model

Injection of tyloxapol successfully induced acute hyperlipidaemia in mice. Compared with the normal control group, the pathological control group exhibited significantly increased levels of total cholesterol, triglycerides and LDL-C, along with a marked decrease in HDL-C ($p < 0.05$). These results indicate that the hyperlipidaemia model was successfully established.

Impact of immature pomelo extract on serum total cholesterol

The impact of immature pomelo extract on serum total cholesterol is summarised in Figure 3. Treatment with the extract significantly reduced total cholesterol levels in all experimental groups compared with the pathological control group ($p < 0.001$). The lowest dose produced the greatest reduction in total cholesterol, whereas higher doses restored cholesterol levels to values comparable with those observed in the normal and positive control groups.

Impact of immature pomelo extract on serum triglycerides

The impact of immature pomelo extract on serum triglycerides is shown in Figure 4. Triglyceride levels were markedly higher in the pathological control group than in the normal control group ($p < 0.001$). Treatment with immature pomelo extract led to a significant reduction in triglyceride levels across all experimental groups relative to the pathological control group ($p < 0.001$). Furthermore, triglyceride levels in the extract-treated groups were comparable to, or lower than, those observed in the positive control group treated with atorvastatin.

Impact of immature pomelo extract on serum LDL-C

The impact of immature pomelo extract on serum LDL-C is shown in Figure 5. LDL-C levels were markedly elevated in the pathological control group compared with the normal control group ($p < 0.001$). Treatment with immature pomelo extract resulted in a significant reduction in LDL-C levels across all experimental groups relative to the pathological control group ($p < 0.001$). Importantly, LDL-C levels in the extract-treated groups did not differ significantly from those in the positive control group ($p > 0.05$), suggesting comparable lipid-lowering efficacy.

Impact of immature pomelo extract on serum HDL-C

The impact of immature pomelo extract on serum HDL-C is shown in Figure 6. HDL-C levels

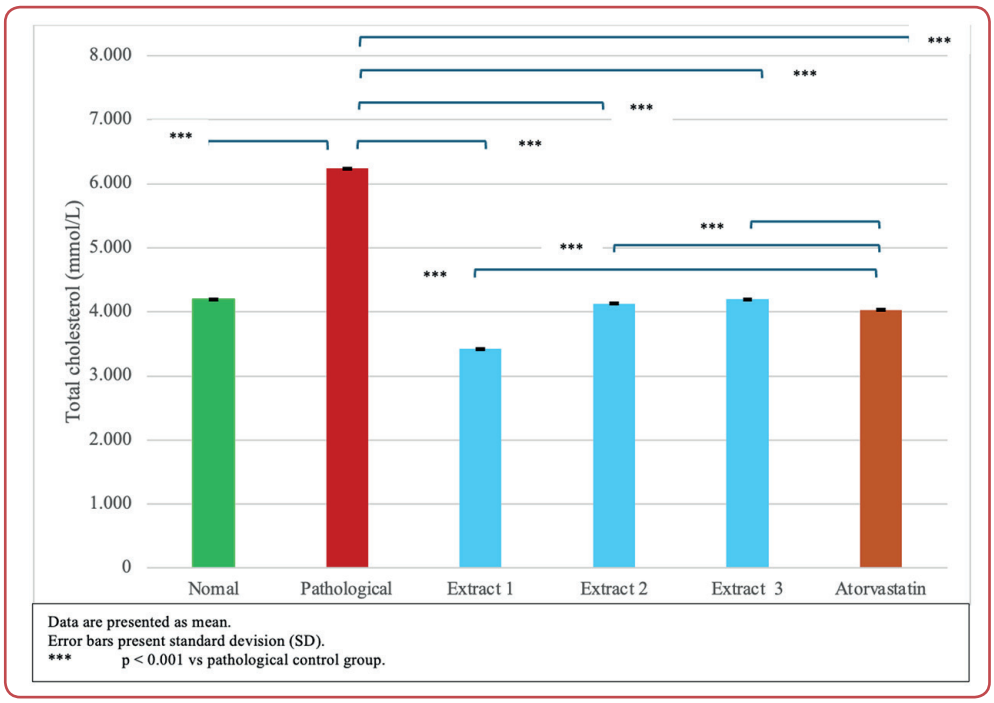

Figure 3: *Effect of immature pomelo extract on serum total cholesterol levels in mice*
Data are presented as mean ± SD

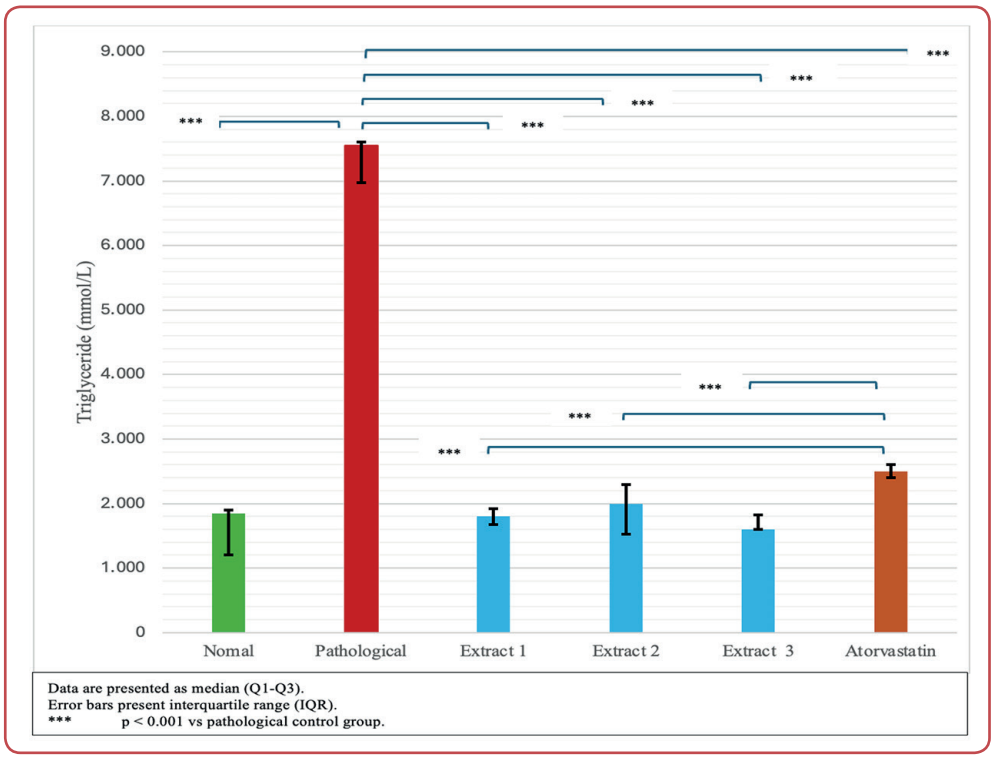

Figure 4: *Effect of immature pomelo extract on serum triglyceride levels in mice*
Data are presented as median (Q1-Q3)

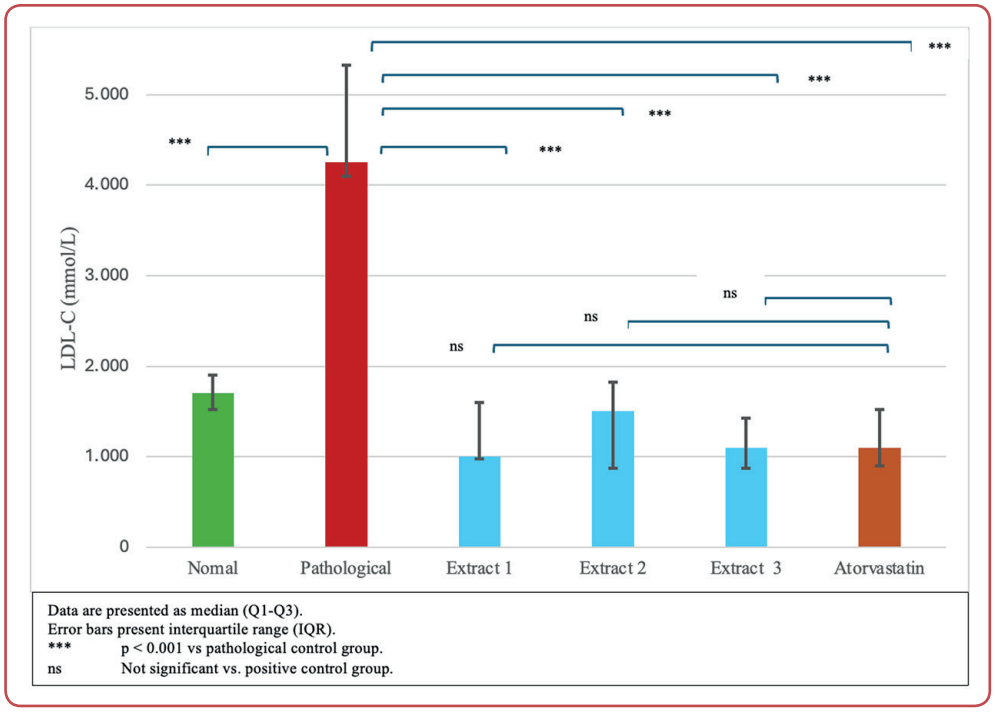

Figure 5: Effect of immature pomelo extract on serum LDL-C levels in mice
Data are presented as median (Q1-Q3)

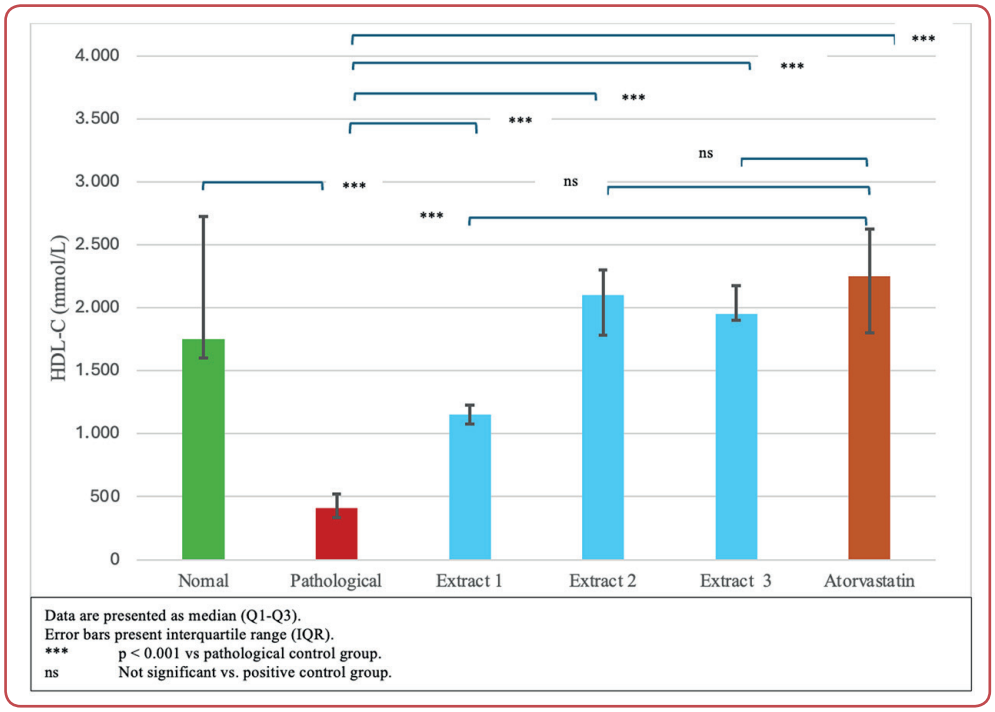

Figure 6:Effect of immature pomelo extract on serum HDL-C levels in mice
Data are presented as median (Q1-Q3)

were significantly lower in the pathological control group than in the normal control group ($p < 0.001$). Treatment with immature pomelo extract markedly increased HDL-C levels in all experimental groups compared with the pathological control group ($p < 0.001$). At higher doses, HDL-C levels approached those of the normal and positive control groups, with no statistically significant differences observed ($p > 0.05$).

Discussion

The present study demonstrates that a liquid extract of immature pomelo produces significant hypolipidaemic effects in a tyloxapol-induced acute hyperlipidaemia mouse model. These findings are consistent with previous reports on *Citrus* species indicating their ability to regulate lipid metabolism.[16] Abnormal lipid metabolism, reflected by increased levels of total cholesterol, triglycerides and LDL-C along with reduced HDL-C, is strongly implicated in the development of atherosclerosis and cardiovascular disease;[17] consequently, plant-derived compounds with lipid-regulating potential have attracted growing therapeutic interest.

In this study, the extract significantly reduced total cholesterol and LDL-C at all tested doses, with LDL-C levels in two treatment groups approaching those observed with atorvastatin. Similar lipid-lowering effects of pomelo preparations have been reported previously.[18] Mechanistically, this effect may be partly explained by naringenin, a metabolite of naringin, which competitively inhibits hepatic HMG-CoA reductase and suppresses endogenous cholesterol biosynthesis in a manner comparable to statins.[19]

A particularly notable finding was the strong triglyceride-lowering activity of the extract. All treatment groups showed markedly lower triglyceride levels than the pathological control and numerically lower values than the atorvastatin group. This observation is consistent with evidence that pomelo flavonoids activate AMPK signalling and PPARα pathways, thereby enhancing fatty-acid oxidation and reducing hepatic triglyceride synthesis.[20] This may be relevant in mixed dyslipidaemia, where statin monotherapy often fails to adequately control triglycerides.

Alongside its effects on LDL-C and triglycerides, the extract enhanced HDL-C levels. HDL-C, commonly known as "good cholesterol," plays a protective role by facilitating the transport of excess cholesterol from peripheral tissues to the liver for elimination, thereby playing a protective role against atherosclerosis.[21] Higher doses restored HDL-C towards physiological ranges, supporting earlier observations that pomelo may enhance reverse cholesterol transport and improve metabolic profiles.[22]

Interestingly, the hypolipidaemic effect of the extract did not show a clear dose–response relationship. In some parameters, lower or moderate doses appeared to produce comparable or even greater effects than the highest dose. This non-linear response may be attributed to the complex mixture of bioactive compounds in the extract, potential saturation of absorption mechanisms, or opposing pharmacological effects at higher concentrations. Similar non-linear dose–response patterns have been reported for plant-derived extracts, where synergistic and antagonistic interactions between constituents may influence the overall biological activity.

While the extract met physicochemical and microbiological quality standards, detailed phytochemical characterisation was not performed in the present study. However, previous studies have shown that immature pomelo contains flavonoids such as naringin, hesperidin and poncirin, which are likely responsible for the observed lipid-lowering effects.[23] Naringin is known to inhibit enzymes involved in the biosynthesis of cholesterol and fatty acids, particularly HMG-CoA reductase and Acyl-CoA-cholesterol acyltransferase (ACAT).[10] In general, flavonoids derived from *Citrus* species have been consistently reported to improve lipid profiles by lowering total cholesterol, LDL-C and triglycerides while increasing HDL-C levels.[16, 24]

Several limitations should be acknowledged. First, this is a preclinical animal study and confirmation in human clinical trials is required. Second, the present study employed a tyloxapol-induced hyperlipidaemia model, which reflects acute alterations in lipid metabolism and does not fully mimic chronic dyslipidaemia in humans. Third, the use of a whole extract highlights the need for standardisation, as the content of bioactive constituents such as naringin may vary depending on extraction procedures.[25] Future studies should therefore focus on identifying active constituents, clarifying molecular mechanisms and developing standardised formulations suitable for clinical evaluation.

Despite these limitations, the translational potential of immature pomelo is supported by recent product-development studies, including capsule formulations derived from this material.[26] Together with the present efficacy and safety findings, these results provide a scientific basis for the utilisation of immature pomelo – an abundant agricultural by-product in the Mekong Delta – as a source of functional lipid-modulating products.

The present study utilised a tyloxapol-induced hyperlipidaemia model, which is widely employed to evaluate the acute lipid-lowering activity of test compounds. However, this model primarily reflects short-term alterations in lipid metabolism through inhibition of lipoprotein lipase and does not fully mimic the complex pathophysiology of chronic dyslipidaemia in humans. Therefore, while the findings demonstrate promising acute hypolipidaemic effects, further studies using chronic models are required to confirm the long-term efficacy and clinical relevance of the extract.

Conclusion

This study demonstrates that a liquid extract of immature pomelo exhibits significant hypolipidaemic activity in a tyloxapol-induced hyperlipidaemia mouse model. The extract effectively reduced total cholesterol, LDL-C and triglyceride levels while improving HDL-C concentrations, indicating a broad lipid-modulating effect. These effects are likely associated with the flavonoid constituents of pomelo, particularly naringin and hesperidin, which have been reported to influence key pathways involved in lipid metabolism.

Given the abundance of immature pomelo as an agricultural by-product in the Mekong Delta, the present findings highlight its potential as a sustainable natural source of lipid-regulating compounds. Although further studies, particularly clinical investigations, are required to confirm these effects in humans, the results provide valuable scientific evidence supporting the development of immature pomelo–derived products for dyslipidaemia management.

Ethics

All experimental procedures involving animals were approved by the Institutional Ethics Committee of Can Tho University of Medicine and Pharmacy (Approval No: 25.004.HV.CTUMP/PCT-HĐĐĐ, dated 1 April 2026).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Ba Dien Pham (BDP):
0009-0000-9464-9975
Chi Nhan Ton (CNT):
0009-0009-5731-2287
Ngoc Chi Lan Nguyen (NCLN):
0000-0002-9659-5410
Minh Hoang Le (MHL):
0000-0002-5968-078X

Author contributions

Conceptualisation: BDP, MHL
Methodology: BDP, MHL
Software: BDP
Validation: BDP, CNT
Formal analysis: BDP
Investigation: BDP

Resources: BDP, MHL
Data curation: BDP
Writing – original draft: BDP
Writing – review and editing: BDP, CNT, NCLN, MHL
Visualisation: BDP
Supervision: MHL, CNT, LNNC
Project administration: BDP
Funding acquisition: MHL.

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