

Original Article

Comparative in Vitro Evaluation of Garlic, Red Yeast Rice, Lemon, and Coconut Oil for HMG-CoA Reductase Inhibition and Antioxidant Activity

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ABSTRACT

Objective: This study comparatively evaluated the *in vitro* lipid-modulating and antioxidant profiles of garlic (*Allium sativum*), red yeast rice (*Monascus purpureus*), lemon (*Citrus limon*), and coconut oil.

Methodology: We evaluated prepared samples using concentration-dependent HMG-CoA reductase inhibition and DPPH radical-scavenging assays, along with LDL- and HDL-related endpoints. Apparent IC₅₀ values were estimated where the concentration-response range permitted. We used Fourier-transform infrared spectroscopy for complementary characterization. Lovastatin served as a reference inhibitor.

Results: The dataset demonstrated distinct biochemical profiles. Red yeast rice showed the greatest apparent HMG-CoA reductase inhibition, reaching 72.4% at 100 µg/mL, with an apparent IC₅₀ of 57.3 µg/mL, whereas garlic demonstrated intermediate inhibition (55.2%; IC₅₀, 79.6 µg/mL). Lemon exhibited the strongest apparent DPPH radical-scavenging activity, reaching 82.1% at 100 µg/mL and an apparent IC₅₀ of 51.8 µg/mL. Red yeast rice produced the greatest apparent reduction in the LDL-related endpoint (30.8%), whereas coconut oil showed comparatively limited direct activity. Antioxidant and cholesterol-biosynthesis-related activities were not necessarily concordant.

Conclusion: The investigated natural products exhibited distinct *in vitro* biochemical profiles, with red yeast rice showing prominent HMG-CoA reductase-related activity and lemon showing prominent radical-scavenging activity. However, the thesis identifies the numerical datasets as assumed or simulated; therefore, quantitative findings require verification against original experimental records before presentation as definitive experimental results. These findings do not support conclusions about clinical efficacy.

Introduction

Dysregulated cholesterol metabolism contributes to cardiovascular disease, with elevated low-density lipoprotein cholesterol (LDL-C) recognized as a major modifiable risk factor (1). Hepatic cholesterol biosynthesis is tightly regulated, and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase catalyzes the rate-limiting step in converting HMG-CoA to mevalonate (2). Consequently, inhibiting HMG-CoA reductase is a major pharmacological strategy for reducing cholesterol biosynthesis and the principal mechanism of action of statins (3). Alongside pharmacological interventions, interest has grown in naturally derived products as potential sources of bioactive compounds with lipid-modulating and antioxidant properties (4). Garlic (*Allium sativum*) contains sulfur-containing compounds associated with effects on lipid metabolism and antioxidant activity (5). Red yeast rice, produced by fermenting rice with *Monascus* spp., contains monacolins, particularly monacolin K, a compound structurally identical to lovastatin and capable of inhibiting HMG-CoA reductase (6). However, the composition and bioactive content of natural preparations can vary considerably by source and processing. Lemon (*Citrus limon*) contains vitamin C, flavonoids, phenolic compounds, and other phytochemicals with recognized radical-scavenging potential (7). Its potential biological effects may therefore differ from those of compounds that directly target cholesterol biosynthesis. In contrast, the relationship between coconut oil and lipid metabolism remains complex, particularly because its fatty-acid composition may influence circulating lipoprotein concentrations differently from other dietary fats (8). The present study therefore included coconut oil as a comparative natural dietary product rather than assuming a direct cholesterol-lowering effect. Oxidative stress is another relevant component of metabolic and cardiovascular processes. Accordingly, assessment of antioxidant activity alongside cholesterol-biosynthesis-related activity may provide a broader biochemical characterization of natural products (9). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay provides a simple measure of chemical radical-scavenging capacity, whereas HMG-CoA reductase inhibition evaluates activity at a specific step of cholesterol biosynthesis (10). These assays therefore represent complementary, rather than interchangeable, biological endpoints. Despite considerable research on individual natural products, direct comparisons between different preparations under standardized experimental conditions remain limited. The present study was designed to comparatively evaluate garlic, red yeast rice, lemon, and coconut oil using HMG-CoA reductase inhibition, DPPH radical-scavenging activity, and LDL- and HDL-related biochemical endpoints. This approach aimed to characterize their distinct in vitro biochemical profiles and determine whether antioxidant and cholesterol-biosynthesis-related activities occur concordantly or reflect different functional properties. Importantly, these *in vitro* endpoints provide biochemical evidence only and should not be interpreted as direct evidence of clinical cardiovascular benefit.

Material and Methods

2.1 Study Design

We conducted an in vitro experimental study to comparatively evaluate the antioxidant and cholesterol-biosynthesis-related activities of four natural products: garlic (*Allium sativum*), red yeast rice (*Monascus purpureus*), lemon (*Citrus limon*), and coconut oil. The experimental workflow comprised sample preparation, extraction or stock preparation, DPPH radical-scavenging assay, lipid-related analysis, concentration-dependent response assessment, IC₅₀ estimation, FTIR characterization, and statistical analysis.

2.2 Sample Preparation and Extraction

Garlic and red yeast rice were cleaned, dried, and processed into homogeneous powders before extraction (11). Lemon was prepared according to the experimental protocol, whereas coconut oil was analyzed directly as an oil matrix and therefore did not undergo conventional solid-matrix extraction (12). We followed appropriate precautions throughout sample preparation to minimize contamination and sample-identification errors. For the solid botanical materials, we subjected accurately weighed samples to

solvent extraction under controlled experimental conditions, including extraction time, temperature, extraction technique, and sample-to-solvent ratio (13). The resulting extracts were filtered to remove residual plant material and subsequently concentrated before analysis. Coconut oil was prepared as a stock solution in an assay-compatible solvent system. Extraction yield was calculated as the percentage ratio of the mass of concentrated or dried extract to the initial mass of dry sample: Extraction yield (%) = extract/Mass × 100

Stock solutions of the prepared extracts were subsequently diluted to obtain the required concentrations for the analytical assays.

2.3 DPPH Radical-Scavenging Assay

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay (14). The assay is based on the reduction of the stable DPPH radical in the presence of radical-scavenging compounds, which decreases absorbance. We tested garlic, red yeast rice, lemon, and coconut oil preparations at different concentrations using appropriate controls, samples, and blanks. Where necessary, we included sample blanks to correct for intrinsic color or turbidity.

Radical-scavenging activity was calculated as:

DPPH inhibition (%) = $\frac{A_c - A_s}{A_c} \times 100$

Ac represents the absorbance of the control and As represents the absorbance of the sample. The resulting percentage inhibition values were used for concentration-response analysis and, where appropriate, to estimate IC₅₀ values (15).

2.4 Lipid-Related and HMG-CoA Reductase Assays

The lipid-related activity of the four preparations was evaluated using an LDL/LDL-C-related assay with appropriate untreated controls and treatment groups (16). We tested each preparation at different concentrations, and we performed reactions under the same conditions; calibration, detection wavelength, and calculations followed the validated assay procedure or the manufacturer's instructions. HDL-related responses were similarly evaluated and expressed relative to the untreated control.

HMG-CoA reductase inhibition was assessed as a principal biochemical endpoint because the enzyme catalyzes the rate-limiting step of endogenous cholesterol biosynthesis (17). The assay used spectrophotometric monitoring of NADPH oxidation at 340 nm, with reduced absorbance interpreted as evidence of enzyme inhibition under the assay conditions. Lovastatin was included as a reference inhibitor (18).

2.5 Concentration-Response Analysis and IC₅₀ Estimation

We performed concentration-dependent experiments using multiple concentrations of each natural preparation. We plotted responses from the DPPH, lipid-related, and enzyme-inhibition assays against sample concentration to evaluate concentration-response relationships (19). Where the experimental concentration range adequately encompassed a 50% response, the IC₅₀ was estimated by fitting an appropriate dose-response or regression model. IC₅₀ values were not interpreted when the available concentration range was insufficient to establish a 50% response (20) reliably.

2.6 FTIR Characterization

Fourier-transform infrared (FTIR) or attenuated total reflectance FTIR (ATR-FTIR) analysis was performed to provide complementary chemical characterization of the investigated preparations. A background spectrum was recorded before sample analysis, followed by spectrum acquisition under the instrument's predefined operating conditions. Major absorption bands were interpreted with reference to established spectral databases to identify probable functional-group vibrations. FTIR analysis was used for qualitative characterization rather than

definitive identification of individual compounds (21).

2.7 Statistical Analysis

Data were organized according to treatment, concentration, and replicate. Quantitative results were expressed as mean ± standard deviation (SD). Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by an appropriate post-hoc multiple-comparison test where applicable. A *p*-value < 0.05 was considered statistically significant.

Results

3.1 Extraction Yield

The extraction procedures produced different yields among the three solid botanical materials. Red yeast rice showed the highest extraction yield (20.80%), followed by garlic (15.60%) and lemon (13.80%). Coconut oil was analyzed directly as an oil matrix and therefore was not assigned a conventional extraction yield. Table 1 presents the comparative extraction yields.

Table 1. Extraction yield of the investigated natural products

Natural product	Initial sample mass (g)	Extract obtained (g)	Extraction yield (%)
Garlic (Allium sativum)	50.0	7.80	15.60
Red yeast rice (Monascus purpureus)	50.0	10.40	20.80
Lemon (Citrus limon)	50.0	6.90	13.80
Coconut oil	–	–	N/A

N/A, not applicable.

3.2 DPPH Radical-Scavenging Activity

All four natural preparations demonstrated concentration-dependent increases in DPPH radical-scavenging activity across the tested concentration range of 20–100 µg/mL. Lemon consistently demonstrated the greatest radical-scavenging response, increasing from 24.9% at 20 µg/mL to 82.1% at 100 µg/mL. Red yeast rice showed the second-highest activity, increasing from 21.6% to 77.2%, while garlic increased from 17.8% to 66.9%. Coconut oil demonstrated comparatively lower activity, increasing from 7.2% to 34.8% over the same concentration range (Table 2).

At 100 µg/mL, the DPPH radical-scavenging activities were 82.1%, 77.2%, 66.9%, and 34.8% for lemon, red yeast rice, garlic, and coconut oil, respectively (Table 2).

Table 2. Concentration-dependent DPPH radical-scavenging activity

Concentration (µg/mL)	Garlic (%)	Red yeast rice (%)	Lemon (%)	Coconut oil (%)
20	17.8	21.6	24.9	7.2
40	29.8	35.7	40.8	12.9
60	43.6	51.8	57.6	19.8
80	55.7	65.3	70.4	27.1
100	66.9	77.2	82.1	34.8

3.3 DPPH IC₅₀

The concentration-response profiles permitted estimation of DPPH IC₅₀ values for all four preparations. Lemon showed the lowest estimated IC₅₀ (51.8 µg/mL), followed by red yeast rice (57.9 µg/mL), garlic (68.7 µg/mL), and coconut oil (145.6 µg/mL) (Table 3). Thus, within the assay conditions used, the preparations exhibited distinct apparent radical-scavenging potencies.

Table 3. Estimated DPPH IC₅₀ values

Natural product	DPPH IC ₅₀ (µg/mL)
Garlic	68.7
Red yeast rice	57.9
Lemon	51.8
Coconut oil	145.6

Because DPPH IC₅₀ values can be substantially affected by assay conditions, including solvent composition, reagent concentration, reaction time, and the concentration range tested, these values should be interpreted within the experimental conditions of the present study rather than used for unrestricted comparison across different studies.

3.4 LDL-Related Activity

The LDL-related assay demonstrated a concentration-dependent reduction in the relative LDL-related index for all four preparations, although the magnitude of response differed considerably. At 100 µg/mL, red yeast rice produced the largest reduction in the assay-specific LDL-related endpoint (30.8%), followed by garlic (17.6%), lemon (11.4%), and coconut oil (3.5%). Lovastatin, used as the reference inhibitor, produced a 38.2% reduction (Table 4).

The concentration-dependent response is shown in Table 4. Red yeast rice exhibited the greatest change across the concentration range, with its relative index decreasing from 91.7% at 20 µg/mL to 69.2% at 100 µg/mL. Garlic demonstrated an intermediate response, whereas lemon and particularly coconut oil showed smaller changes.

Table 4. Concentration-dependent LDL-related response

Treatment	Control	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Garlic	100.0	95.8	92.4	88.9	85.7	82.4
Red yeast rice	100.0	91.7	84.9	78.3	73.2	69.2
Lemon	100.0	97.3	95.4	93.1	90.7	88.6
Coconut oil	100.0	99.1	98.2	97.3	96.9	96.5

Table 5. LDL- and HDL-related responses at 100 µg/mL

Treatment	Relative LDL-related index	Reduction vs. control (%)	Relative HDL-related index	Increase vs. control (%)
Control	100.0	—	100.0	—
Garlic	82.4	17.6	106.8	6.8
Red yeast rice	69.2	30.8	108.9	8.9
Lemon	88.6	11.4	107.5	7.5
Coconut oil	96.5	3.5	112.6	12.6
Lovastatin	61.8	38.2	108.1	8.1

The HDL-related endpoint also showed increases relative to the untreated control. Coconut oil demonstrated the largest assay-specific increase (12.6%), followed by red yeast rice (8.9%), lemon (7.5%), and garlic (6.8%); lovastatin showed an 8.1% increase (Table 5). These changes represent responses within the analytical assay and should not be interpreted as evidence of clinical improvement in circulating HDL-C.

3.5 HMG-CoA Reductase Inhibition

HMG-CoA reductase inhibition increased progressively with increasing concentration for all four preparations. Red yeast rice demonstrated the greatest inhibitory response throughout the tested concentration range, increasing from 20.6% at 20 µg/mL to 72.4% at 100 µg/mL. Garlic demonstrated an intermediate response, increasing from 12.8% to 55.2%.

Lemon and coconut oil showed lower inhibition, reaching 31.7% and 19.6%, respectively, at 100 µg/mL (Table 6).

Table 6. Concentration-dependent HMG-CoA reductase inhibition

Concentration (µg/mL)	Garlic (%)	Red yeast rice (%)	Lemon (%)	Coconut oil (%)
20	12.8	20.6	7.4	4.2
40	24.7	35.9	13.8	8.1
60	37.1	49.8	20.6	12.2
80	46.9	62.7	26.3	15.8
100	55.2	72.4	31.7	19.6

At the highest tested concentration, Red yeast rice produced 72.4% inhibition, compared with 55.2% for garlic, 31.7% for lemon, and 19.6% for coconut oil. The reference compound lovastatin exhibited an estimated IC₅₀ of 24.6 µg/mL, compared with 57.3 µg/mL for red yeast rice, 79.6 µg/mL for garlic, 157.4 µg/mL for lemon, and 258.7 µg/mL for coconut oil (Table 7).

Table 7. Estimated HMG-CoA reductase IC₅₀ values

Treatment	HMG-CoA reductase IC ₅₀ (µg/mL)
Lovastatin	24.6
Red yeast rice	57.3
Garlic	79.6
Lemon	157.4
Coconut oil	258.7

3.6 Protein Concentration

The assay dataset also included estimated total protein concentrations for the investigated preparations. Red yeast rice had the highest value (2.37 ± 0.12 mg/mL), followed by garlic (1.84 ± 0.09 mg/mL), lemon (1.26 ± 0.07 mg/mL), and coconut oil (0.18 ± 0.02 mg/mL) (Table 8).

Table 8. Total protein concentration of the investigated preparations

Natural product	Protein concentration (mg/mL)
Garlic	1.84 ± 0.09
Red yeast rice	2.37 ± 0.12
Lemon	1.26 ± 0.07
Coconut oil	0.18 ± 0.02

Discussion

The present study provides a comparative *in vitro* assessment of garlic (*Allium sativum*), red yeast rice (*Monascus purpureus*), lemon (*Citrus limon*), and coconut oil using complementary biochemical endpoints related to antioxidant activity and cholesterol metabolism. The investigated products demonstrated distinct activity profiles rather than a uniform pattern across all assays. Red yeast rice showed the greatest apparent HMG-CoA reductase inhibition and LDL-related reduction, whereas lemon demonstrated the strongest DPPH radical-scavenging activity. Garlic exhibited an intermediate profile across both endpoints, while coconut oil showed comparatively limited direct activity in the DPPH and HMG-CoA reductase assays. These findings support the concept that antioxidant capacity and inhibition of cholesterol biosynthesis represent related but mechanistically distinct biochemical properties. The relatively high extraction yield observed for red yeast rice (20.8%) compared with garlic (15.6%) and lemon (13.8%) may reflect differences in matrix composition and extraction efficiency. However, extraction yield alone cannot be interpreted as an indicator of biological potency because the quantity of recovered extract does not necessarily correspond to the concentration of bioactive constituents. The greater apparent HMG-CoA reductase inhibition observed with red yeast rice is biologically plausible because red yeast rice contains monacolins, particularly monacolin K, which is structurally identical to lovastatin and acts on HMG-CoA reductase (22). Consistent with this profile, red yeast rice

produced 72.4% apparent inhibition at 100 µg/mL and the lowest apparent IC₅₀ among the tested natural products (57.3 µg/mL). It also produced the greatest reduction in the LDL-related assay endpoint (30.8%).Lemon exhibited the strongest apparent antioxidant activity, with DPPH radical scavenging increasing progressively with concentration and reaching 82.1% at 100 µg/mL. Its apparent IC₅₀ of 51.8 µg/mL was also the lowest among the natural products investigated. This profile is consistent with antioxidant constituents reported for citrus products, including vitamin C, flavonoids, and phenolic compounds (23, 24). In contrast, lemon showed comparatively weak activity against HMG-CoA reductase and produced only an 11.4% reduction in the LDL-related endpoint (25).The contrasting results therefore emphasize that strong chemical radical-scavenging activity does not necessarily translate into direct inhibition of cholesterol biosynthesis (26). Garlic demonstrated an intermediate biochemical profile, showing both DPPH radical-scavenging activity and HMG-CoA reductase inhibition (27). At 100 µg/mL, its apparent DPPH inhibition was 66.9%, while HMG-CoA reductase inhibition was 55.2%, with an apparent HMG-CoA reductase IC₅₀ of 79.6 µg/mL. Its LDL-related reduction was also intermediate at 17.6%. This pattern suggests that garlic may have multiple potentially relevant biochemical activities; however, the magnitude of these effects can vary substantially with garlic preparation, extraction conditions, and the concentration of sulfur-containing constituents (28). Consequently, activity observed with a particular extract should not automatically be generalized to other garlic preparations.Coconut oil displayed comparatively limited apparent activity in the direct DPPH and HMG-CoA reductase assays, although the proposed HDL-related endpoint showed a relatively greater response. Its apparent HMG-CoA reductase inhibition at 100 µg/mL was 19.6%, while its DPPH scavenging activity was 34.8%. This finding is important because coconut oil has a substantially different chemical composition from the botanical extracts evaluated in the study. Its effects on circulating lipid profiles are complex and cannot be captured by a single enzyme or antioxidant assay (29). The findings of present study indicate that the four natural products possess distinct biochemical profiles rather than a common cholesterol-lowering mechanism. Red yeast rice was primarily associated with HMG-CoA reductase-related activity, lemon with radical-scavenging activity, and garlic with a more intermediate profile across both endpoints. The study therefore illustrates the importance of evaluating natural products using multiple complementary assays rather than relying on a single measure of biological activity. Several limitations should be considered when interpreting these findings. First, the quantitative values reported in the thesis are identified as assumed or simulated datasets, so the original experimental measurements should be verified before presenting the results as definitive experimental observations. In addition, DPPH IC₅₀ values are dependent on assay conditions and should not be directly compared across studies without methodological equivalence. Similarly, an *in vitro* HMG-CoA reductase assay represents activity against a single biochemical target and cannot establish physiological or clinical cholesterol-lowering efficacy. Finally, crude natural-product preparations do not allow attribution of the observed activity to a specific constituent without further chemical characterization and quantitative standardization.Despite these limitations, the study provides a useful comparative framework for investigating natural food-derived products with potentially different biochemical mechanisms. The contrasting profiles of red yeast rice and lemon particularly demonstrate that antioxidant capacity and cholesterol-biosynthesis-related activity should be considered as separate experimental outcomes. Further work using verified raw experimental data, standardized extracts, quantitative characterization of active constituents, and appropriate cellular, animal, or clinical models is needed to establish the biological and therapeutic relevance of these observations.

Conclusion

In conclusion, the present *in vitro* comparative evaluation demonstrated distinct biochemical profiles among garlic (*Allium sativum*), red yeast rice (*Monascus purpureus*), lemon (*Citrus limon*), and coconut oil. Red yeast rice exhibited the greatest apparent HMG-CoA reductase inhibitory activity and LDL-related reduction, whereas lemon demonstrated the strongest DPPH radical-scavenging activity. Garlic showed intermediate activity across both biochemical endpoints, while coconut oil exhibited comparatively limited direct activity in the DPPH and HMG-CoA reductase assays. These findings highlight that antioxidant capacity and cholesterol-biosynthesis-related activity represent distinct biochemical properties and should not be interpreted as interchangeable measures of cardiovascular benefit. Because the quantitative dataset used in this study is identified in the thesis as assumed/simulated, the reported values require verification against the original experimental records before they can be considered definitive. Further studies using standardized extracts, verified quantitative measurements, chemical characterization of active constituents, and appropriate biological models are warranted to establish the reproducibility and physiological relevance of these observations.

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