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Original Article

In vitro Evaluation of the Antihyperlipidemic and Antioxidant Potential of Ethanolic Leaf Extract of *Anisomeles malabarica* (L.)

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ABSTRACT

Background: Hyperlipidemia and oxidative stress are closely linked to atherosclerosis and cardiovascular complications. Plant-derived phenolic compounds, flavonoids, alkaloids and saponins are widely investigated as sources of antioxidant and lipid-modulating activity. **Objectives:** To evaluate the preliminary *in vitro* antioxidant activity and *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related inhibitory potential of the ethanolic leaf extract of *Anisomeles malabarica* (AMLE). **Materials and Methods:** Leaves of *Anisomeles malabarica* were collected, authenticated, shade-dried, powdered and extracted with ethanol using a Soxhlet apparatus. The extract underwent qualitative phytochemical screening, nitric oxide scavenging and hydroxyl radical scavenging assays. *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related inhibitory activity was examined at 25, 50 and 100 µg/mL. IC_{50} values for the antioxidant assays were estimated by linear interpolation between concentrations bracketing 50% inhibition**.** **Results:** The ethanolic extract yield was 33.33% w/w. Qualitative screening indicated alkaloids, flavonoids, glycosides, saponins and tannins. AMLE showed concentration-dependent nitric oxide scavenging (IC_{50}, 1537.47 µg/mL) and hydroxyl radical scavenging (IC_{50}, 1303.17 µg/mL). The corresponding reference values were 440.72 µg/mL for the nitric oxide assay reference standard and 274.22 µg/mL for gallic acid in the hydroxyl radical assay. In the *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related assay, reported inhibition was 55.95%, 74.78% and 83.29% at 25, 50 and 100 µg/mL, respectively; interpretation remains preliminary pending assay validation and statin-control confirmation. **Conclusion:** AMLE demonstrated concentration-dependent *in vitro* radical-scavenging activity and a concentration-related response in the reported *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related assay. The antioxidant potency was substantially lower than the reference standards. These findings constitute preliminary *in vitro* evidence only and do not establish therapeutic antihyperlipidemic efficacy. Validated enzyme assays with a statin positive control, replicated measurements with statistical analysis, quantitative phytochemical characterization, *in vivo* evaluation and toxicity studies are required.

Keywords: *3-hydroxy-3-methylglutaryl-coenzyme* A reductase, *Anisomeles malabarica*, Antihyperlipidemic activity, Antioxidant activity, Nitric oxide scavenging, Phytochemicals.

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INTRODUCTION

Hyperlipidemia is a major modifiable risk factor for atherosclerosis and cardiovascular disease.[1–6] Elevated total cholesterol, triglycerides and low-density lipoprotein cholesterol, together with reduced high-density lipoprotein cholesterol, contribute to vascular lipid deposition and endothelial dysfunction.[7–14] Oxidative stress further accelerates lipid peroxidation and oxidative modification of lipoproteins, thereby increasing the risk of atherogenesis.[15–21]

Synthetic lipid-lowering drugs are effective; however, long-term therapy may be associated with adverse effects, variable patient adherence and cost-related limitations.[5,6,22–29] Experimental and clinical studies of plant extracts and phytochemicals, including *Malus toringoides, Mesona chinensis, Moringa species*, curcumin and *Terminalia arjuna*, have reported antioxidant or lipid-modulating effects.[30–35] These observations support continued investigation of plant-derived compounds as potential sources of antioxidant and lipid-lowering agents.[36–44]

Anisomeles malabarica (L.) is a medicinal plant belonging to the family Lamiaceae. In traditional systems of medicine, its leaves

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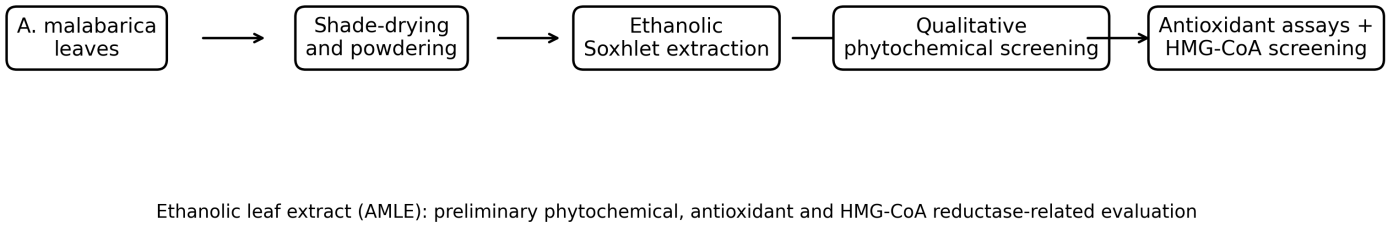


Figure 1: AMLE preparation and the qualitative phytochemical, antioxidant and *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related screening workflow.

and other parts have been used for fever, inflammation, convulsions, wounds and gastrointestinal disorders.[45–47] Previous studies have reported antioxidant, anti-inflammatory, antibacterial and related pharmacological activities of the plant.[45–48]

Despite its traditional relevance, the specific effect of *A. malabarica* leaf extract on *3-hydroxy-3-methylglutaryl-coenzyme A* reductase has received little attention in the plant-specific literature cited here.[47,48] Previous reports have primarily described antioxidant, anti-inflammatory, antibacterial, anticonvulsant and other pharmacological activities.[45–48] The novelty of the present study is therefore the extension of *in vitro* screening to an *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related endpoint, a cholesterol-biosynthesis target, while simultaneously comparing antioxidant activity in the same ethanolic leaf extract. Because the enzyme-assay validation details and statin positive-control data were not available in the supplied study records, the *3-hydroxy-3-methylglutaryl-coenzyme A* finding is interpreted as exploratory rather than as proof of a therapeutic lipid-lowering effect.

MATERIALS AND METHODS

Plant material and authentication

Fresh, disease-free leaves of *Anisomeles malabarica* (L.) were collected from Veppampalayam, Erode District, Tamil Nadu, India. The plant material was authenticated by Dr. M. U. Sharief, Scientist F and Head of Office, Botanical Survey of India, Southern Regional Centre, T.N.A.U. Campus, Coimbatore, Tamil Nadu. The authentication reference number was BSI/SRC/5/23/2024-25/TECH./550.

Preparation of ethanolic leaf extract

The collected leaves were washed, shade-dried at room temperature (25-27°C) and mechanically powdered. A total of 120 g of dried leaf powder was extracted with 1000 mL ethanol in a Soxhlet apparatus at 40-45°C for approximately 70 hr. The extract was concentrated by distillation and stored in an airtight container until analysis. Percentage yield was calculated as: percentage yield = (weight of dried extract / weight of dried plant material) × 100.

Preliminary phytochemical screening

The ethanolic leaf extract was screened qualitatively for alkaloids, flavonoids, tannins, saponins and glycosides using standard phytochemical tests.[42,50–53] Wagner's test was used for alkaloids, the ferric chloride test for flavonoids and tannins, the froth test for saponins and Legal's test for glycosides. No quantitative Total Flavonoid Content (TFC), Total Phenolic Content (TPC), or other quantitative phytochemical measurement was available in the supplied experimental dataset; therefore, no numerical TFC/TPC claim is made in this revision.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was determined using sodium nitroprusside and Griess reagent according to the established assay principle.[54,55] AMLE was evaluated at 125, 250, 500, 1000 and 2000 µg/mL. The same concentration series was used for the laboratory reference standard. After incubation with sodium nitroprusside, the reaction mixture was treated with Griess reagent, absorbance was read at 546 nm, and percentage inhibition was calculated relative to the assay control.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was evaluated using a standard spectrophotometric method with gallic acid as the reference standard.[56,57] AMLE and gallic acid were tested at 125, 250, 500, 1000 and 2000 µg/mL. Percentage inhibition was calculated relative to the control.

3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitory assay

The supplied study records describe a *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related inhibitory assay in which AMLE was tested at concentrations of 25, 50 and 100 µg/mL, and absorbance was recorded at 540 nm. However, the records do not specify whether the procedure was a direct enzyme-activity assay, a *3-hydroxy-3-methylglutaryl-coenzyme A*/mevalonate ratio method, or a commercial kit-based assay, and they do not document the enzyme source, manufacturer/catalogue number, reagent concentrations, incubation conditions, or validated percentage-inhibition formula. These details must be verified from the laboratory record before resubmission.

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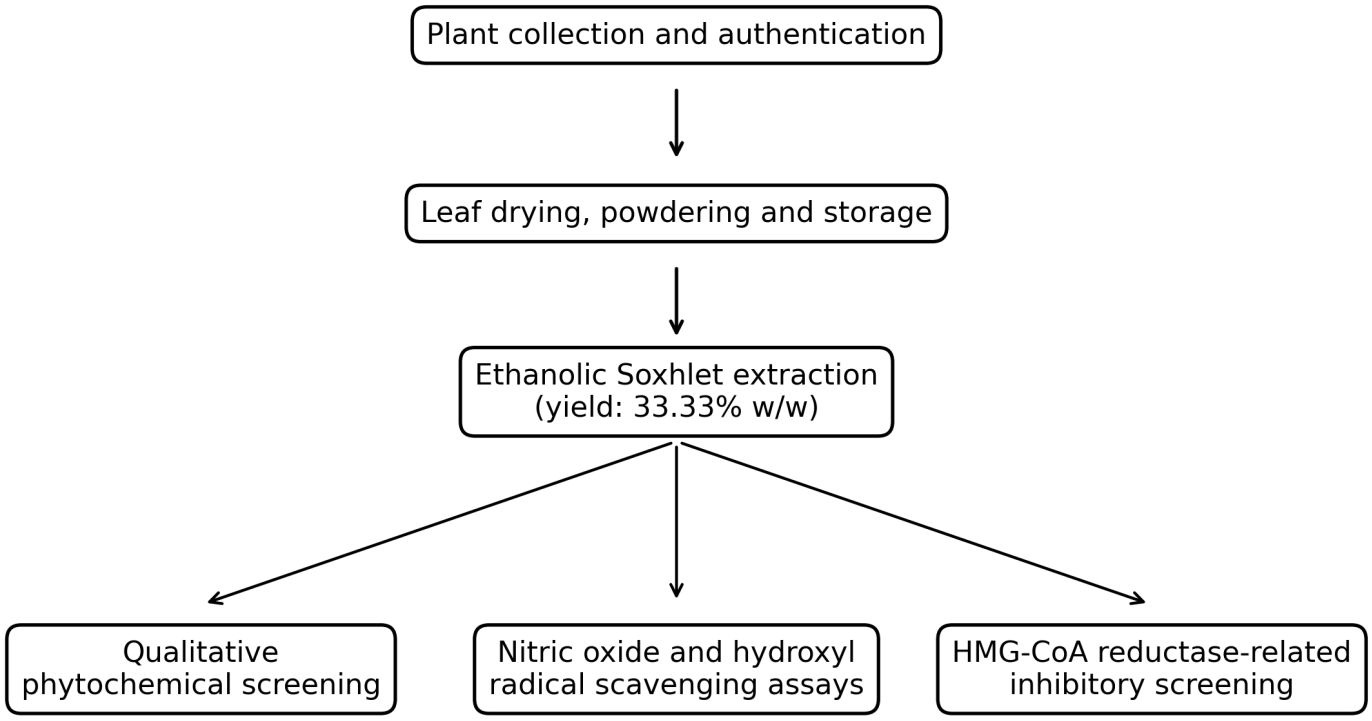


Figure 2: Experimental workflow from authenticated plant material through ethanolic extraction, antioxidant assays and exploratory *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related evaluation.

Statistical analysis

Replicate-level raw observations were not included in the supplied manuscript or supporting material, so the number of independent replicates, SD/SEM values and inferential statistics cannot be reconstructed without fabricating data. Before resubmission, the authors should insert the verified replicate number (n), report each result as mean±SD (or mean±SEM, if that was prospectively used), and analyze concentration-dependent comparisons using an appropriate model (for example, one-way ANOVA with a prespecified multiple-comparison procedure, or two-way ANOVA where treatment and concentration are both factors), with $p<0.05$ as the significance threshold. IC_{50} values in this revision were estimated from the reported concentration-response values by linear interpolation between the two concentrations bracketing 50% inhibition: $IC_{50}=C1 + [(50 - I1)/ (I2 - I1)] \times (C2 - C1)$, where C1 and C2 are the bracketing concentrations and I1 and I2 are their corresponding percentage inhibitions.

Ethics statement

Ethics approval was not applicable because the study involved authenticated plant material and *in vitro* assays only; no human participants, human samples or experimental animals were used.

The experimental workflow is summarized in Figure 2.

Table 1: Extractive yield of the ethanolic leaf extract of *A. malabarica*.

Parameter	Value
Dried leaf powder used	120 g
Solvent	Ethanol
Solvent volume	1000 mL
Extraction method	Soxhlet extraction
Dried extract obtained	40 g
Percentage yield	33.33% w/w

RESULTS

Extractive yield

Extraction of 120 g dried leaf powder with ethanol yielded 40 g dried extract, corresponding to 33.33% w/w. The extraction details are summarized in Table 1.

Phytochemical screening

Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, glycosides, saponins and tannins in AMLE (Table 2). These constituents are consistent with the reported pharmacological importance of plant secondary metabolites and flavonoid-rich extracts.[42,52,53]

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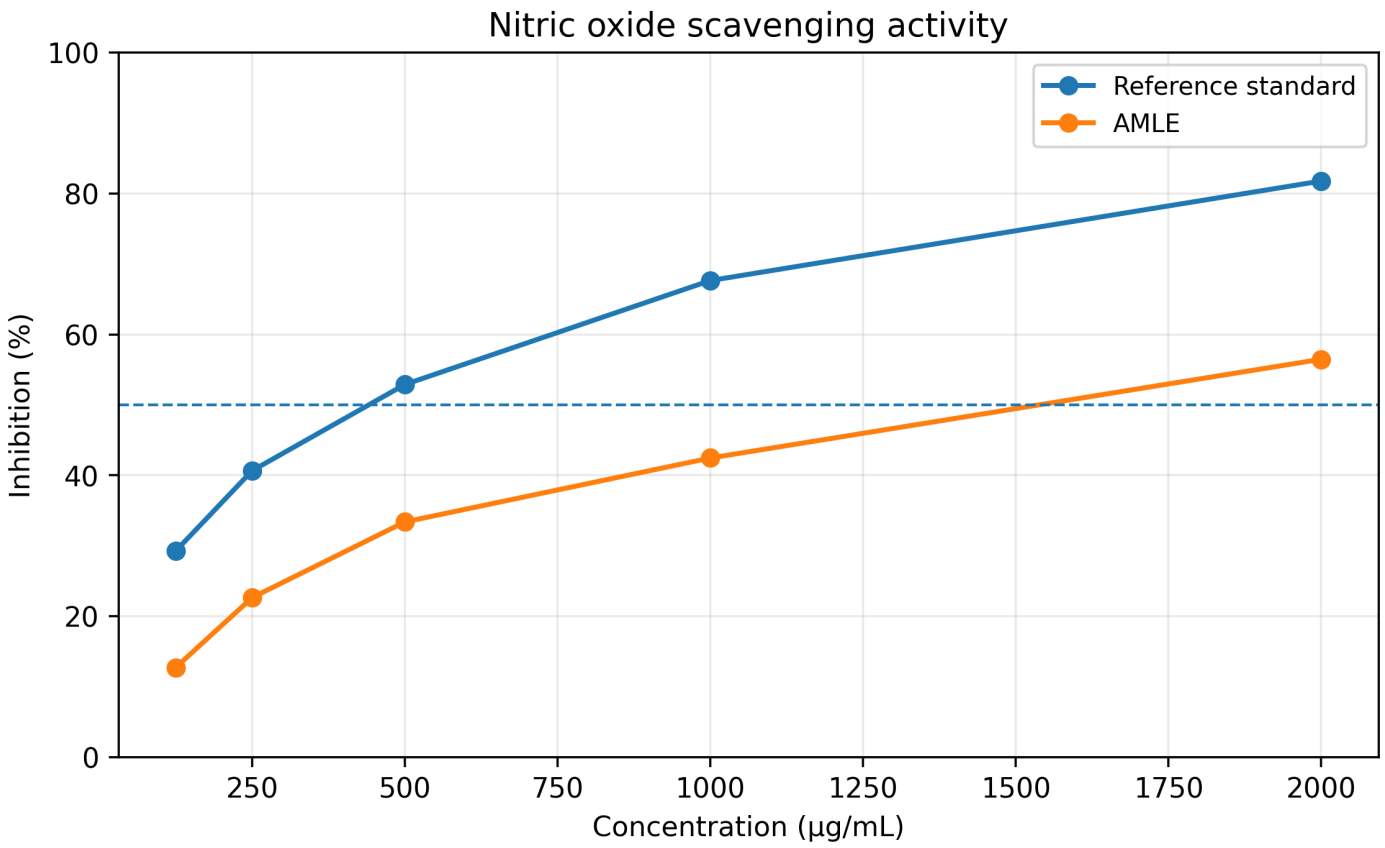


Figure 3: Nitric oxide scavenging concentration-response curves for AMLE and the laboratory reference standard. The horizontal dashed line indicates 50% inhibition.

Table 2: Qualitative phytochemical screening of AMLE.

Sl. No.	Phytochemical constituent	Result
1.	Alkaloids	Present
2.	Flavonoids	Present
3.	Glycosides	Present
4.	Saponins	Present
5.	Tannins	Present

Note: This is a qualitative presence/absence screen; no quantitative TFC/TPC result was available in the supplied dataset.

Table 3: Nitric oxide scavenging activity of AMLE and the reference standard.

Concentration (µg/mL)	Reference standard inhibition (%)	AMLE inhibition (%)
125	29.29	12.75
250	40.67	22.66
500	52.90	33.40
1000	67.65	42.47
2000	81.77	56.48

Nitric oxide scavenging activity

AMLE showed concentration-dependent nitric oxide scavenging activity (Table 3 and Figure 3). At 2000 µg/mL, AMLE produced 56.48% inhibition, whereas the reference standard produced 81.77% inhibition. Using linear interpolation between the concentrations bracketing 50% inhibition, the estimated IC_{50} was 1537.47 µg/mL for AMLE and 440.72 µg/mL for the reference standard. Thus, the AMLE IC_{50} was approximately 3.49-fold higher, indicating clearly lower potency than the reference standard.

Hydroxyl radical scavenging activity

AMLE demonstrated concentration-dependent hydroxyl radical scavenging activity (Table 4 and Figure 4). At 2000 µg/mL, AMLE produced 57.47% inhibition, while gallic acid produced 88.41% inhibition. Linear interpolation gave IC_{50} estimates of 1303.17 µg/mL for AMLE and 274.22 µg/mL for gallic acid. The AMLE IC_{50} was therefore approximately 4.75-fold higher than that of gallic acid, demonstrating weaker radical-scavenging potency.

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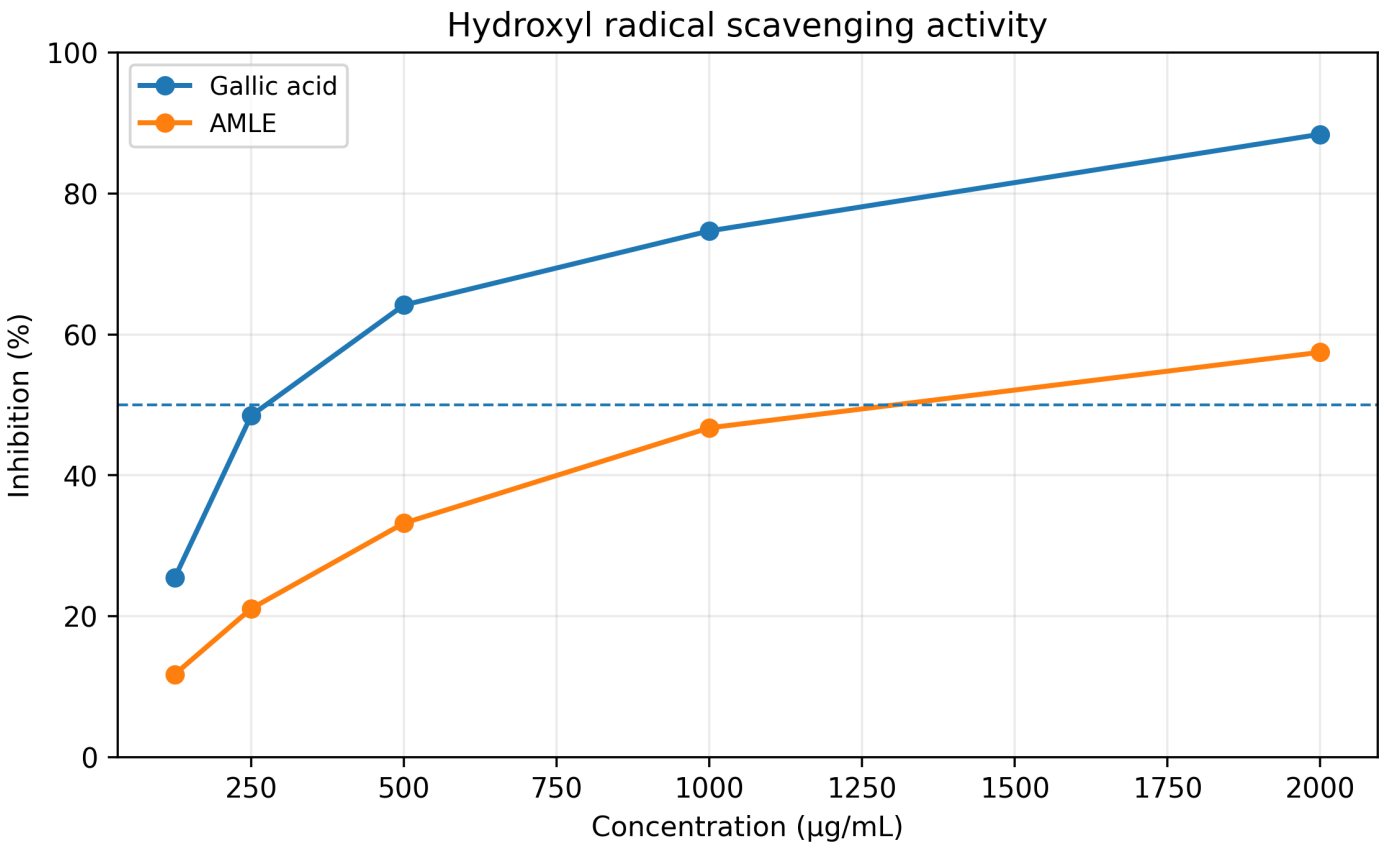


Figure 4: Hydroxyl radical scavenging concentration-response curves for AMLE and gallic acid. The horizontal dashed line indicates 50% inhibition.

Table 4: Hydroxyl radical scavenging activity of AMLE and gallic acid.

Concentration (µg/mL)	Gallic acid inhibition (%)	AMLE inhibition (%)
125	25.49	11.78
250	48.48	21.09
500	64.17	33.22
1000	74.69	46.75
2000	88.41	57.47

3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitory activity

AMLE showed a concentration-related response in the reported *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related assay (Table 5 and Figure 5). The reported percentage inhibition increased from 55.95% at 25 µg/mL to 83.29% at 100 µg/mL. Because the calculation formula, replicate-level observations and a statin positive control were not supplied, these values are retained as reported and are interpreted as exploratory rather than as validated enzyme-inhibition estimates.

Table 5: *3-hydroxy-3-methylglutaryl-coenzyme A* reductase inhibitory activity of AMLE.

Concentration of AMLE (µg/mL)	Absorbance at 540 nm	Reported inhibition (%)
Blank (None; values noted as reported-only pending validation)	0.5182	0.00
25	0.3725	55.95
50	0.3019	74.78
100	0.2606	83.29

Note: Values are retained exactly as reported in the supplied manuscript. The validated inhibition formula, replicate variability and statin positive-control data require author/laboratory confirmation.

DISCUSSION

The present study indicates that the ethanolic leaf extract of *Anisomeles malabarica* contains alkaloids, flavonoids, glycosides, saponins and tannins.[45–48] These constituents are frequently associated with antioxidant and lipid-modulating effects.[42,52,53,58–60] Flavonoids and tannins may contribute to free-radical scavenging through hydrogen donation, metal chelation and interruption of oxidative chain reactions, whereas saponins may influence lipid

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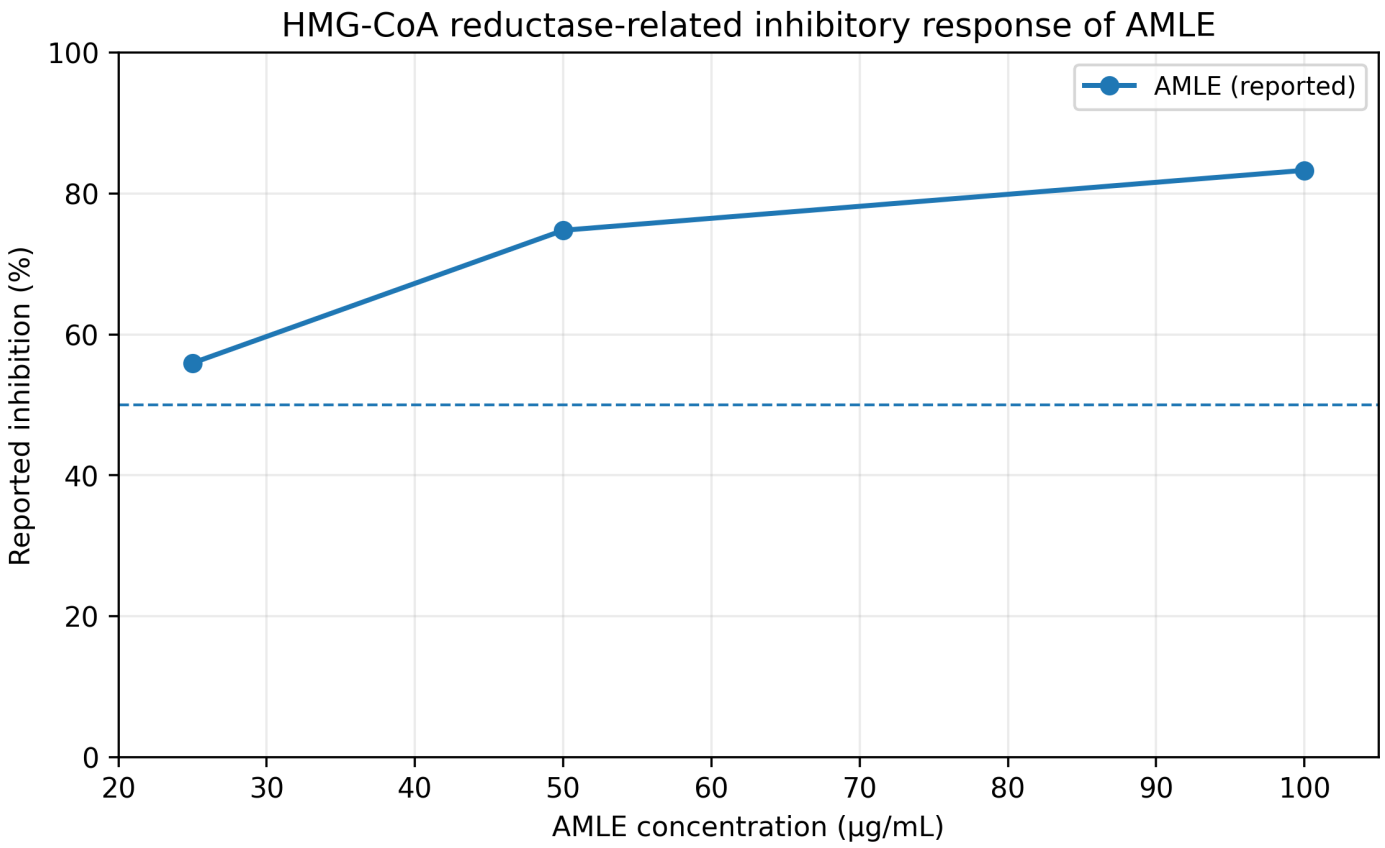


Figure 5: Concentration-related *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related response reported for AMLE. A statin positive-control curve must be added when the corresponding laboratory data are provided.

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metabolism through interactions with bile acids and intestinal lipid absorption.[52,53,58–60]

The nitric oxide and hydroxyl radical assays showed concentration-dependent activity, but the extract was substantially less potent than the corresponding reference standards.[54–57] At the highest tested concentration (2000 µg/mL), AMLE inhibited nitric oxide and hydroxyl radical readouts by only 56.48% and 57.47%, respectively, compared with 81.77% for the nitric oxide reference standard and 88.41% for gallic acid. The interpolated IC_{50} values for AMLE were approximately 3.49-fold and 4.75-fold higher than the respective standards. These differences should be viewed as a limitation rather than evidence of strong antioxidant potency. The data support only modest *in vitro* radical-scavenging activity over the tested range.

The *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related result is the most potentially novel aspect of the study because prior plant-specific reports cited here focused largely on other pharmacological effects.[45–48] *3-hydroxy-3-methylglutaryl-coenzyme A* reductase is a central regulatory enzyme in cholesterol biosynthesis and a pharmacological target of statins.[5–8,22–25] Nevertheless, the present dataset does not establish validated enzyme inhibition: a statin positive control was not documented, the assay principle and percentage-inhibition calculation require confirmation, and no replicate-level variability was supplied. Accordingly, the apparent concentration-related response should be treated as a hypothesis-generating observation that requires confirmation in a validated *3-hydroxy-3-methylglutaryl-coenzyme A* reductase enzyme-activity assay.

The study has several limitations. First, the phytochemical analysis was qualitative; quantitative TFC/TPC measurements and chromatographic characterization of the extract were not available. Second, replicate-level observations, variability estimates and inferential statistical results were not supplied. Third, the identity of the nitric oxide assay reference standard was not documented in the provided records. Fourth, the *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related assay lacks a documented statin positive control and complete validation information. Finally, all experiments were *in vitro*. Therefore, the present findings should be regarded as preliminary biochemical screening and should not be interpreted as evidence of clinical or therapeutic antihyperlipidemic efficacy.

CONCLUSION

The ethanolic leaf extract of *Anisomeles malabarica* contained alkaloids, flavonoids, glycosides, saponins and tannins on qualitative screening and showed concentration-dependent activity in nitric oxide and hydroxyl radical scavenging assays.[45–48,52–57] However, its antioxidant potency was lower than the reference standards. The extract also showed a concentration-related response in the reported *3-hydroxy-3-methylglutaryl-coenzyme A* reductase-related assay, but the absence of documented assay-validation details, replicate statistics and a statin positive control precludes a therapeutic inference. The findings therefore provide preliminary *in vitro* evidence that warrants confirmation through validated enzyme assays, quantitative phytochemical characterization, replicated statistical analysis, *in vivo* antihyperlipidemic models and toxicity assessment.

ACKNOWLEDGEMENT

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ABBREVIATIONS

AMLE: Ethanolic leaf extract of *A. malabarica*; **ELISA:** Enzyme-linked immunosorbent assay; **HMG-CoA:** 3-Hydroxy-3-methylglutaryl-coenzyme A; **IC_{50}:** Half-maximal inhibitory concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Nil.

AUTHORS CONTRIBUTION

All authors contributed to the study conception, experimental work, data analysis, manuscript preparation, review, and approval of the final manuscript.

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