

ORIGINAL RESEARCH ARTICLE

Asiaticoside and Madecassoside from *Centella asiatica* Demethylate the Nrf2 Promoter and Enhance Neuroprotection *In Vitro*

Akmal Hasan^{1*}, Wisnu Wardhana Putra²¹ Department of Neurology, Phlox Institute, Palembang, Indonesia² Department of Neurology, CMHC Research Center, Palembang, Indonesia

ARTICLE INFO

Article history

Received: July 28, 2025

Revised: August 19, 2025

Accepted: September 20, 2025

Available Online: September 28, 2025

Published: September 30, 2025

Keywords

Centella asiatica; DNA demethylation; Epigenetic modulation; Neuroprotection; Nrf2.

*Corresponding author

Akmal Hasan

E-mail: akmal.hasan@phlox.or.id

ORCID: 0009-0005-0324-8946

DOI: 10.37275/ehi.v6i2.141

ABSTRACT

Background: *Centella asiatica* (L.) Urban (Apiaceae), known as *pegagan* in Indonesian *Jamu* and as *Mandukaparni* (regionally *Brahmi*) in Ayurveda, contains triterpene saponins with reported neuroprotective properties, yet the epigenetic mechanisms underlying these effects remain poorly understood.

Objective: This study investigated whether a triterpene-enriched fraction (TEF) standardized to asiaticoside (28.4%) and madecassoside (22.7%) modulates activation of the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) pathway through promoter demethylation and histone acetylation in oxidatively stressed SH-SY5Y neuroblastoma cells.

Methods: Cells were pre-treated with TEF (25, 50, and 100 µg/mL) for 24 h before H₂O₂ (200 µM) challenge. Cell viability, reactive oxygen species (ROS), Nrf2 and HO-1 expression, Nrf2 promoter methylation (bisulfite sequencing), DNA methyltransferase 1 (DNMT1) protein, and histone H3 lysine-27 acetylation (H3K27ac) at the Nrf2 promoter were quantified.

Results: TEF at 100 µg/mL restored cell viability to 82.4 ± 4.3% versus 47.8 ± 4.1% in H₂O₂-only cells ($p < 0.001$, Cohen's $d = 8.22$), reduced ROS to 1.52 ± 0.15-fold ($p < 0.001$), and upregulated Nrf2 mRNA 2.83 ± 0.21-fold and HO-1 protein 3.14 ± 0.24-fold. Bisulfite sequencing revealed dose-dependent Nrf2 promoter demethylation from 67.8 ± 4.2% to 31.2 ± 2.6% ($p < 0.001$), paralleled by DNMT1 suppression (2.74 to 1.12-fold) and H3K27ac enrichment (0.34 to 1.43-fold) at the Nrf2 locus. A strong inverse correlation between methylation and Nrf2 expression ($r = -0.912$) and closely aligned IC₅₀ values for ROS suppression (62.4 µg/mL) and demethylation (58.7 µg/mL) support a coordinated epigenetic-transcriptional mechanism.

Conclusion: These findings provide the first evidence that *Centella asiatica* triterpenes activate neuroprotective pathways through dual epigenetic remodeling, offering a molecular rationale for the traditional cognitive-enhancing applications of this ethnopharmacologically significant herb.

All authors have reviewed and approved the final version of the manuscript.

Copyright: © 2025 The Author(s). Published by HM Publisher in collaboration with CMHC Research Center. The authors retain the copyright of their work.

Access licence: This is an open-access article distributed under the terms of the Creative Commons Attribution-ShareAlike 4.0 International License (CC BY-SA 4.0), which permits use, sharing, adaptation and reproduction in any medium, provided the original author and source are credited and any derivative work is distributed under the same licence.

1. Introduction

Neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, represent a growing global health burden affecting tens of millions of individuals worldwide, a figure projected to rise sharply with continued population aging.^{1,2} Oxidative stress, characterized by excessive accumulation of reactive oxygen species (ROS) and depletion of antioxidant defenses, serves as a central pathomechanism underlying neuronal dysfunction and death.^{3,4} Mitochondrial dysfunction,

protein aggregation, and neuroinflammation cascade from the initial oxidative insult, yet conventional pharmacotherapies targeting single pathways demonstrate limited efficacy.² This therapeutic gap has motivated renewed interest in phytochemical approaches that modulate multiple neuroprotective pathways simultaneously.

Centella asiatica (L.) Urban, a perennial creeping herb of the Apiaceae family, has been employed for generations across two ethnopharmacological traditions. In Indonesian *Jamu*, *pegagan* functions as a

key ingredient in cognitive tonics and wound-healing preparations,⁵ documented in formulations spanning centuries of use.⁶ In Ayurvedic medicine, the herb is recognized as *Mandukaparni* (also called *Brahmi* regionally) and *Gotu Kola*, and it is classified within the *Medhya Rasayana* category of cognitive-enhancing herbs.^{5,7} These empirical claims have increasingly attracted modern scientific validation.^{7,8}

The phytochemistry of *Centella asiatica* is dominated by pentacyclic triterpene saponins, principally asiaticoside and madecassoside, which together with their aglycones constitute a substantial fraction of dried leaf material.⁶ Hydrolysis yields the aglycones asiatic acid and madecassic acid, which penetrate cellular membranes more readily.⁹ These triterpenes promote neuronal differentiation, suppress neuroinflammatory cytokines, and attenuate apoptosis in cultured neurons,^{9,10} yet the epigenetic modulation of master antioxidant regulators remains incompletely characterized.

The nuclear factor erythroid 2-related factor 2 (Nrf2) is the principal transcriptional regulator of cellular antioxidant defense.¹¹ Under basal conditions, Nrf2 is sequestered by Kelch-like ECH-associated protein 1 (KEAP1) and directed toward proteasomal degradation.¹¹ Upon oxidative stress, Nrf2 accumulates in the nucleus and binds the antioxidant response element (ARE) of target genes, including heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase 1 (NQO1), and superoxide dismutase (SOD).^{12,13} Nrf2 activation also attenuates neuroinflammatory nuclear factor kappa B (NF- κ B) signaling and promotes mitochondrial resilience.^{14,15}

Nrf2 expression is subject to tight epigenetic control. The Nrf2 promoter contains a CpG island whose methylation silences expression and renders cells vulnerable to oxidative assault.^{16,17} DNA methyltransferase 1 (DNMT1) catalyzes this repressive methylation and is upregulated in stress and disease models,¹⁸ whereas histone H3 lysine-27 acetylation (H3K27ac) marks active chromatin and promotes Nrf2 accessibility.¹⁹ Nrf2 promoter hypermethylation contributes to impaired antioxidant responses,^{16,20} and DNMT inhibitors and dietary epigenetic modulators can reverse this dysregulation.^{16,19} However, synthetic inhibitors often display poor selectivity, prompting investigation of botanical dual-action epigenetic modulators.

To date, no study has examined whether triterpene saponins from *Centella asiatica* modulate Nrf2 through epigenetic remodeling; prior reports did not assess promoter methylation, DNMT activity, or histone

modifications.^{6,9} The present investigation therefore tests the hypothesis that a standardized triterpene-enriched fraction (TEF) activates the Nrf2/HO-1 pathway in H₂O₂-challenged SH-SY5Y cells through dual Nrf2 promoter demethylation (DNMT1 inhibition) and H3K27 acetylation, and that the magnitude of demethylation correlates with Nrf2 activation and ROS suppression.

2. Methods

2.1. Plant material and TEF isolation

Centella asiatica (L.) Urban was sourced from a cultivated medicinal-plant collection in South Sumatra, Indonesia, in May 2024 during the vegetative phase and authenticated by a qualified botanist, and a voucher specimen (CA-2024-0892) was deposited at the institutional herbarium. Dried aerial parts (500 g) were macerated in 70% ethanol (3 × 5 L), concentrated, and lyophilized (crude yield 12.4% w/w). The crude extract was fractionated by silica-gel column chromatography (chloroform:methanol gradient) with thin-layer chromatography (TLC) monitoring (R_f 0.35–0.55); triterpene-rich fractions were pooled and purified by preparative TLC to yield the TEF (≈8.2 g; 13.2% recovery).

2.2. HPLC standardization

The TEF was standardized for asiaticoside and madecassoside by validated reversed-phase high-performance liquid chromatography (HPLC; Shimadzu LC-20AT; Phenomenex Luna C18, 250 × 4.6 mm, 5 μ m; acetonitrile–water gradient; 1.0 mL/min; 206 nm). Calibration curves were linear (asiaticoside R^2 = 0.9987; madecassoside R^2 = 0.9991), and the TEF was analyzed in triplicate.

2.3. Cell culture and experimental design

SH-SY5Y human neuroblastoma cells (ATCC CRL-2266) were cultured in Dulbecco's modified Eagle's medium/Ham's F-12 (DMEM/F-12) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in 5% CO₂. Six groups (six replicates each, three independent experiments) were studied: Control; H₂O₂ 200 μ M; TEF 25, 50, and 100 μ g/mL + H₂O₂; and vitamin E (VitE) 50 μ M + H₂O₂ (positive control). Cells were seeded at 1 × 10⁴ cells/well (96-well plates) or 5 × 10⁵ cells/well (6-well plates), pre-treated with TEF or VitE for 24 h, and then challenged with H₂O₂ (200 μ M) for 24 h. Samples were coded to blind the analyst.

2.4. Assays and molecular analyses

Viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (570 nm) as $[\text{OD}_{\text{treatment}}/\text{OD}_{\text{control}}] \times 100$, where OD is

optical density, and ROS were measured by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) flow cytometry (BD FACSCalibur, 488 nm), expressed as $MFI_{treatment}/MFI_{control}$, where MFI is mean fluorescence intensity. Gene expression (Nrf2, HO-1, and NQO1; glyceraldehyde-3-phosphate dehydrogenase [GAPDH] as reference) was quantified by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) using the $2^{-\Delta\Delta Ct}$ method. HO-1, DNMT1, and β -actin proteins were assessed by Western blot with ImageJ densitometry. Nrf2 promoter methylation (–518 to –276 relative to the transcription start site) was quantified by bisulfite sequencing of ten cloned CpG sites, and H3K27ac enrichment at the Nrf2 promoter by chromatin immunoprecipitation-qPCR (ChIP-qPCR; percent-input method).

2.5. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD); analyses used IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA). Normality and variance homogeneity were tested by the Shapiro–Wilk and Levene tests; groups were compared by one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (HSD) test (or by the Kruskal–Wallis test with Mann–Whitney *U* tests and Bonferroni correction). Effect sizes were Cohen’s *d* and eta-squared (η^2); dose-response was assessed by linear regression with Pearson *r*, R^2 , and half-maximal inhibitory concentration (IC₅₀) estimation. A two-tailed $p < 0.05$ was considered significant.

2.6. Ethics

This study was reviewed and approved by the CMHC Ethics Committee (Ref. No. 2024/0175). The investigation used a commercially sourced human neuroblastoma cell line and authenticated plant material; no human participants or live animals were involved. All procedures were performed in a private university research laboratory in Palembang, Indonesia, in accordance with institutional biosafety guidelines.

3. Results

3.1. HPLC standardization of the TEF

HPLC profiling revealed a triterpene-saponin composition dominated by asiaticoside ($28.4 \pm 1.2\%$, w/w; 28.2 min) and madecassoside ($22.7 \pm 0.9\%$; 31.9 min), with minor asiatic acid ($14.3 \pm 0.6\%$) and madecassic acid ($11.8 \pm 0.5\%$). Combined triterpene content reached 77.2%, confirming successful enrichment.

3.2. Cell viability and dose-dependent cytoprotection

H₂O₂ challenge induced marked cell death, as detailed in Table 1 and illustrated in Figure 1A. Viability fell from 100% in controls to $47.8 \pm 4.1\%$ with H₂O₂ ($p < 0.001$, Cohen’s *d* = 15.93). TEF conferred dose-dependent cytoprotection, restoring viability to $61.3 \pm 3.8\%$ (25 $\mu\text{g/mL}$), $72.6 \pm 4.0\%$ (50 $\mu\text{g/mL}$), and $82.4 \pm 4.3\%$ (100 $\mu\text{g/mL}$; all $p < 0.001$ vs. H₂O₂), comparable to vitamin E ($78.9 \pm 3.9\%$; $p = 0.247$ vs. TEF 100). One-way ANOVA confirmed strong effects [$F(5,30) = 142.7$, $\eta^2 = 0.960$].

Table 1. Cell viability after H₂O₂ challenge and TEF pre-treatment.

Treatment group	Viability (%)	95% CI	vs. H ₂ O ₂	<i>p</i> -value	Cohen’s <i>d</i>
Control (untreated)	100.0 $\pm$ 3.2	96.2–103.8	—	—	—
H ₂ O ₂ 200 μM	47.8 $\pm$ 4.1	42.1–53.5	—	<0.001*	—
TEF 25 + H ₂ O ₂	61.3 $\pm$ 3.8	55.8–66.8	+13.5 pp	<0.001*	3.39
TEF 50 + H ₂ O ₂	72.6 $\pm$ 4.0	66.8–78.4	+24.8 pp	<0.001*	6.10
TEF 100 + H ₂ O ₂	82.4 $\pm$ 4.3	76.1–88.7	+34.6 pp	<0.001*	8.22
Vitamin E 50 + H ₂ O ₂	78.9 $\pm$ 3.9	72.6–85.2	+31.1 pp	<0.001*	7.64

Note: Data are mean $\pm$ SD ($n = 6$ per group, three independent experiments). Cohen’s *d* is relative to the H₂O₂ group. *Significant at $p < 0.05$ (two-tailed). CI, confidence interval; pp, percentage points (absolute difference from the H₂O₂ group); SD, standard deviation; TEF, triterpene-enriched fraction (25, 50, or 100 $\mu\text{g/mL}$); vitamin E, 50 μM .

3.3. Reactive oxygen species suppression

As shown in Figure 1B, H₂O₂ elevated ROS to 3.87 ± 0.34 -fold of control ($p < 0.001$, *d* = 11.61). TEF dose-dependently suppressed ROS to 2.54 ± 0.28 -fold (25 $\mu\text{g/mL}$), 1.93 ± 0.22 -fold (50 $\mu\text{g/mL}$), and 1.52 ± 0.15 -

fold (100 $\mu\text{g/mL}$; all $p < 0.001$ vs. H₂O₂), approaching baseline and comparable to vitamin E (1.68 ± 0.19 -fold). Regression confirmed strong dose-dependence ($r = 0.958$, $R^2 = 0.918$), with an IC₅₀ for ROS suppression of 62.4 $\mu\text{g/mL}$ (95% CI 54.1–71.8).

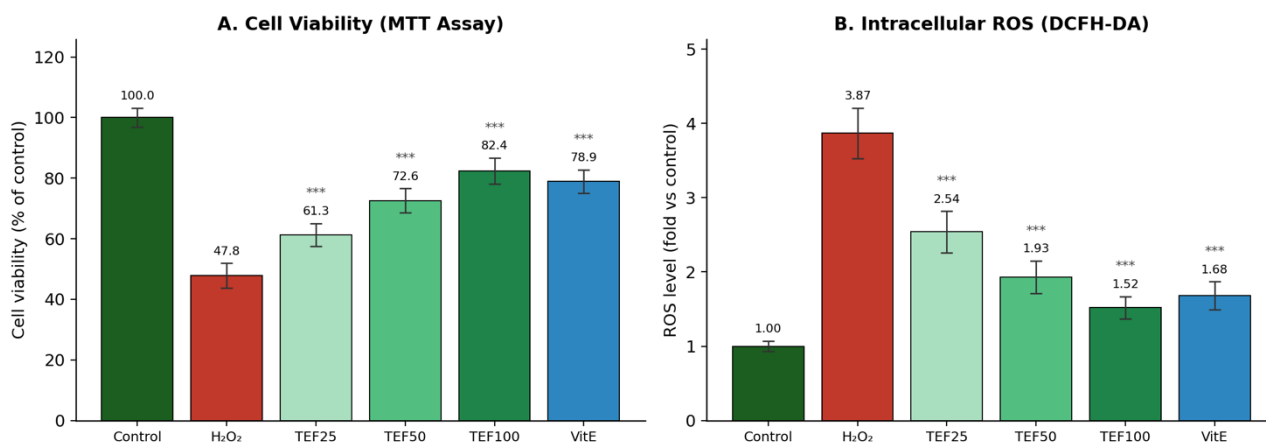


Figure 1. Cell viability and ROS suppression. (A) Viability (MTT) and (B) intracellular ROS (DCFH-DA) in SH-SY5Y cells pre-treated with TEF (25, 50, and 100 $\mu\text{g/mL}$) or vitamin E (50 μM) and then exposed to H_2O_2 (200 μM). Values are mean $\pm$ SD ($n = 6$). *** $p < 0.001$ versus H_2O_2 . DCFH-DA, 2',7'-dichlorodihydrofluorescein diacetate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; TEF, triterpene-enriched fraction; VitE, vitamin E.

3.4. Nrf2/HO-1 pathway activation

RT-qPCR and Western blotting confirmed Nrf2/HO-1 activation, as detailed in Table 2 and Figure 2A and 2B. H_2O_2 suppressed Nrf2 mRNA to 0.42 ± 0.07 -fold, whereas TEF restored it to 1.21 , 2.04 , and 2.83 ± 0.21 -

fold across ascending doses ($d = 14.30$ at 100 $\mu\text{g/mL}$; vitamin E 2.61 ± 0.18 -fold). HO-1 protein mirrored this pattern (0.38 -fold with H_2O_2 ; 1.47 , 2.34 , and 3.14 ± 0.24 -fold with TEF). ANOVA confirmed strong effects for Nrf2 mRNA [$F(5,30) = 127.3$] and HO-1 [$F(5,30) = 141.6$].

Table 2. Nrf2 mRNA (RT-qPCR, normalized to GAPDH) and HO-1 protein (Western blot, normalized to β -actin) expression.

Treatment group	Nrf2 mRNA (fold)	HO-1 protein (fold)	p (Nrf2)	p (HO-1)
Control	1.00 ± 0.08	1.00 ± 0.06	—	—
H_2O_2 200 μM	0.42 ± 0.07	0.38 ± 0.06	$<0.001^*$	$<0.001^*$
TEF 25 + H_2O_2	1.21 ± 0.16	1.47 ± 0.18	$<0.01^*$	$<0.001^*$
TEF 50 + H_2O_2	2.04 ± 0.19	2.34 ± 0.21	$<0.001^*$	$<0.001^*$
TEF 100 + H_2O_2	2.83 ± 0.21	3.14 ± 0.24	$<0.001^*$	$<0.001^*$
Vitamin E 50 + H_2O_2	2.61 ± 0.18	2.89 ± 0.22	0.332	0.408

Note: Data are mean $\pm$ SD fold-change versus control ($n = 6$). p -values are versus the H_2O_2 group. *Significant at $p < 0.05$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HO-1, heme oxygenase-1; Nrf2, nuclear factor erythroid 2-related factor 2; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; TEF, triterpene-enriched fraction.

3.5. Epigenetic remodeling: Nrf2 promoter demethylation

Bisulfite sequencing of ten CpG sites (Table 3; Figure 3A) showed control methylation of $22.4 \pm 2.8\%$, rising to $67.8 \pm 4.2\%$ with H_2O_2 ($p < 0.001$, $d = 12.48$). TEF reversed hypermethylation to $54.3 \pm 3.9\%$ (25 $\mu\text{g/mL}$;

a 19.9% reduction relative to the H_2O_2 group), $41.8 \pm 3.2\%$ (50 $\mu\text{g/mL}$; 38.3% reduction), and $31.2 \pm 2.6\%$ (100 $\mu\text{g/mL}$; 54.0% reduction; all $p < 0.001$). Regression revealed a strong negative dose-response ($r = -0.951$, $R^2 = 0.904$), with an IC_{50} for demethylation of 58.7 $\mu\text{g/mL}$ (95% CI 50.2–69.4), closely matching that for ROS suppression (62.4 $\mu\text{g/mL}$).

Table 3. Nrf2 promoter methylation (bisulfite sequencing) and DNMT1 protein (Western blot) expression.

Treatment group	Methylation (%)	Reduction vs. H_2O_2	DNMT1 (fold)	p (Meth)	p (DNMT1)
Control	22.4 ± 2.8	—	1.00 ± 0.08	—	—
H_2O_2 200 μM	67.8 ± 4.2	—	2.74 ± 0.23	$<0.001^*$	$<0.001^*$
TEF 25 + H_2O_2	54.3 ± 3.9	19.9%	1.87 ± 0.19	$<0.001^*$	$<0.001^*$
TEF 50 + H_2O_2	41.8 ± 3.2	38.3%	1.43 ± 0.15	$<0.001^*$	$<0.001^*$
TEF 100 + H_2O_2	31.2 ± 2.6	54.0%	1.12 ± 0.11	$<0.001^*$	$<0.001^*$
Vitamin E 50 + H_2O_2	35.7 ± 3.1	47.3%	1.19 ± 0.12	$<0.001^*$	0.697

Note: Data are mean $\pm$ SD ($n = 6$). Methylation reduction is relative to the H_2O_2 group. *Significant at $p < 0.05$. DNMT1, DNA methyltransferase 1; Meth, Nrf2 promoter methylation; TEF, triterpene-enriched fraction.

3.6. Epigenetic remodeling: DNMT1 suppression and H3K27ac enrichment

As summarized in Table 3 (DNMT1) and Figure 2C and 2D, H₂O₂ elevated DNMT1 to 2.74 ± 0.23 -fold, whereas

TEF suppressed DNMT1 to 1.87, 1.43, and 1.12 ± 0.11 -fold across doses. H₂O₂ did not significantly alter H3K27ac (0.31 vs. 0.34 in controls; $p = 0.621$). TEF enriched H3K27ac to 0.67, 1.08, and 1.43 ± 0.14 -fold ($r = 0.958$, $R^2 = 0.918$).

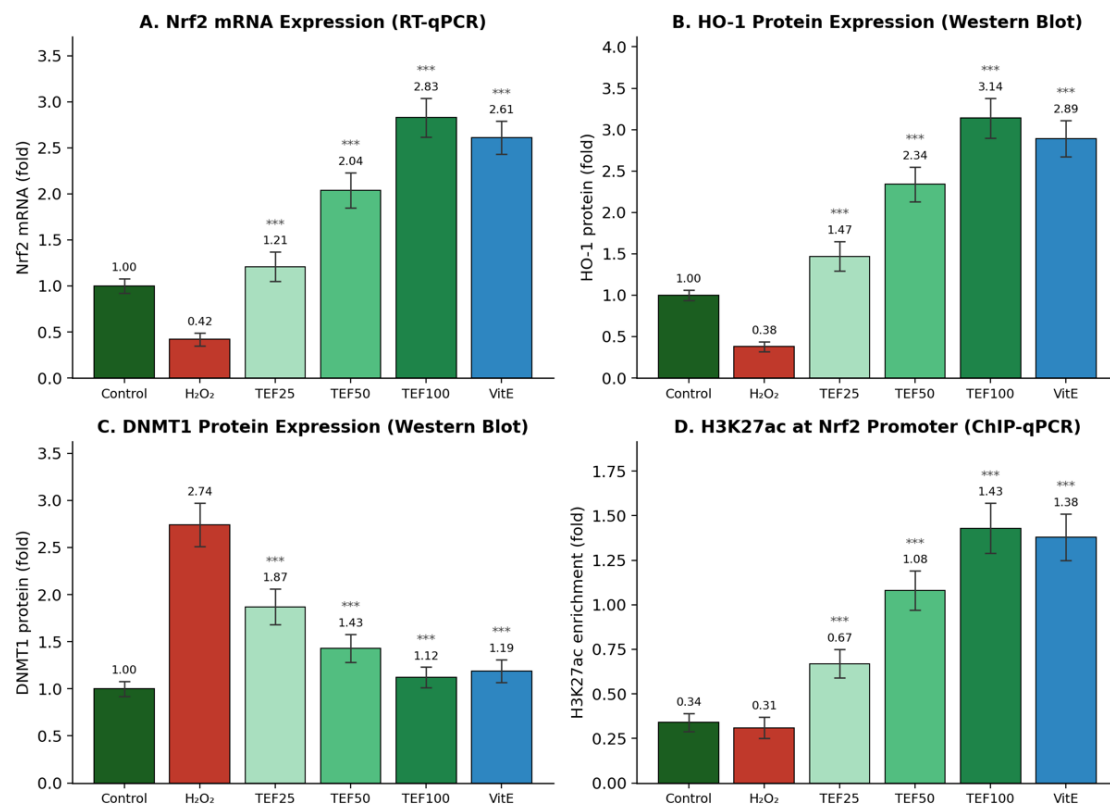


Figure 2. Nrf2/HO-1 activation and epigenetic remodeling. (A) Nrf2 mRNA (RT-qPCR); (B) HO-1 protein (Western blot); (C) DNMT1 protein; (D) H3K27ac enrichment at the Nrf2 promoter (ChIP-qPCR). Values are mean $\pm$ SD ($n = 6$). *** $p < 0.001$ versus H₂O₂. ChIP-qPCR, chromatin immunoprecipitation-quantitative polymerase chain reaction; DNMT1, DNA methyltransferase 1; H3K27ac, histone H3 lysine-27 acetylation; HO-1, heme oxygenase-1; TEF, triterpene-enriched fraction; VitE, vitamin E.

3.7. Integrated and secondary analyses

Across all groups ($n = 36$; Figure 3B; Table 4), Nrf2 promoter methylation and Nrf2 mRNA were strongly inversely correlated ($r = -0.912$, $p < 0.001$; $R^2 = 0.832$).

The IC₅₀ values for ROS suppression (62.4 μ g/mL) and demethylation (58.7 μ g/mL) overlapped considerably (Table 4; Figure 3A). H₂O₂ suppressed NQO1 to 0.51 ± 0.09 -fold, whereas TEF 100 restored it to 2.28 ± 0.25 -fold ($d = 7.08$).

Table 4. Dose-response analysis and epigenetic endpoints.

Parameter	Correlation (r)	R^2	IC ₅₀ (μ g/mL)	95% CI	p -value
ROS suppression	0.958	0.918	62.4	54.1–71.8	<0.001*
Nrf2 demethylation	–0.951	0.904	58.7	50.2–69.4	<0.001*
H3K27ac enrichment	0.958	0.918	61.2	52.6–72.1	<0.001*
Inverse correlation (all groups): methylation vs. Nrf2 mRNA — $r = -0.912$, $R^2 = 0.832$, $p < 0.001$ *					

Note: Linear regression of TEF dose (25–100 μ g/mL) versus ROS, methylation, and H3K27ac; the inverse correlation is computed across all groups ($n = 36$).

*Significant at $p < 0.05$ (two-tailed). CI, confidence interval; H3K27ac, histone H3 lysine-27 acetylation; IC₅₀, half-maximal inhibitory concentration; ROS, reactive oxygen species; TEF, triterpene-enriched fraction.

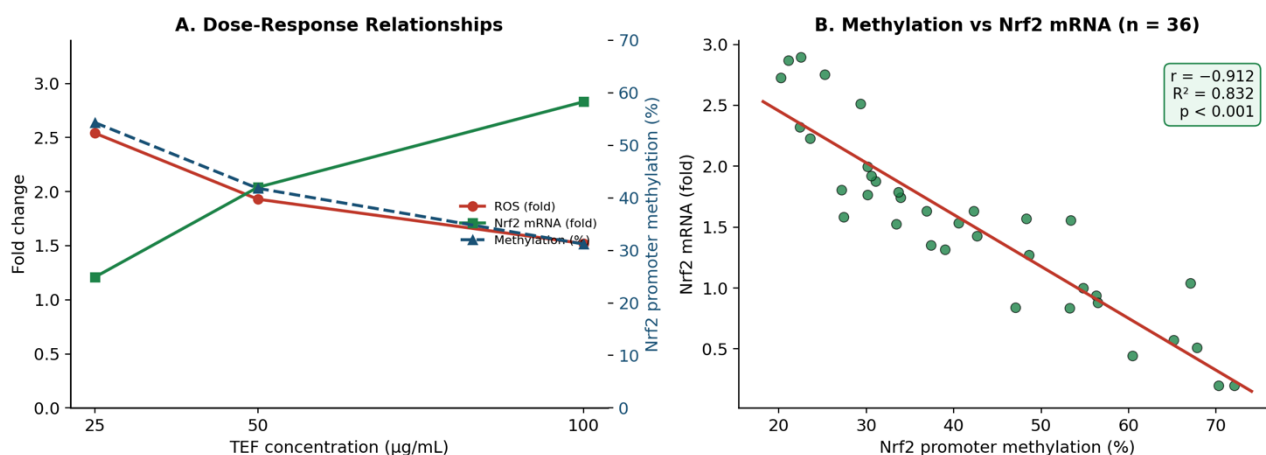


Figure 3. Dose-response and correlation analyses. (A) Dose-response of TEF versus ROS and Nrf2 mRNA (left axis) and Nrf2 promoter methylation (right axis). (B) Inverse correlation between methylation and Nrf2 mRNA across all groups ($n = 36$; $r = -0.912$, $p < 0.001$, $R^2 = 0.832$). ROS, reactive oxygen species; TEF, triterpene-enriched fraction.

4. Discussion

4.1. Triterpene enrichment and cytoprotection

HPLC profiling confirmed that the TEF was enriched in triterpene saponins, with a combined triterpene content of 77.2%. Beyond the two glycosides used for standardization, the membrane-permeant aglycones asiatic acid and madecassic acid may contribute to overall activity.⁹ The dose-dependent restoration of viability after H_2O_2 challenge is consistent with prior reports of *Centella asiatica* neuroprotection.^{5,9}

4.2. Activation of the Nrf2/HO-1 axis

The 2.83-fold Nrf2 and 3.14-fold HO-1 induction by TEF 100 exceed values reported for sulforaphane (2.1–2.5-fold),¹³ consistent with the activity of *Centella asiatica* triterpenes. The concurrent restoration of NQO1 confirms broader ARE-pathway activation and indicates a coordinated antioxidant transcriptional program.^{3,12}

4.3. Dual epigenetic remodeling of the Nrf2 promoter

The rise in Nrf2 promoter methylation under H_2O_2 explains the paradoxical Nrf2 suppression under oxidative stress, and its dose-dependent reversal by TEF is consistent with reports that DNMT inhibition reverses Nrf2 promoter hypermethylation.^{16,20} The H_2O_2 -induced elevation of DNMT1 provides a mechanism for this hypermethylation.¹⁸ Because H_2O_2 did not significantly alter H3K27ac, promoter methylation, not histone deacetylation, is the primary oxidative dysregulation. The combined demethylation and H3K27ac enrichment constitute a dual epigenetic remodeling mechanism that resets the Nrf2 promoter to an active state.¹⁹ The considerable overlap between

the IC₅₀ values for ROS suppression and demethylation further indicates mechanistically linked processes.¹⁷

4.4. Ethnopharmacological and mechanistic integration

These results provide the first molecular evidence linking traditional uses of *Centella asiatica* to epigenetic-transcriptional neuroprotection. TEF-mediated neuroprotection (82.4% viability at 100 µg/mL) equals or exceeds vitamin E, and the strong inverse methylation–mRNA correlation ($r = -0.912$) identifies epigenetic silencing as the dominant mechanism suppressing Nrf2 under oxidative stress. DNMT1 suppression is the proximal driver of demethylation, while H3K27ac enrichment installs active chromatin, together ensuring robust Nrf2 (2.83-fold) and HO-1 (3.14-fold) induction. Because Nrf2 hypermethylation characterizes Alzheimer's and Parkinson's disease,^{1,8} botanical restoration of Nrf2 through epigenetic remodeling is a promising, multi-pathway neuroprotective strategy.^{5,10,21–24}

4.5. Limitations

Limitations include the use of a single cell line (SH-SY5Y), a single oxidative stressor (H_2O_2), a 24-h timeframe, and a multicomponent TEF; future work should isolate individual triterpenes, assess apoptosis, and validate findings in animal models before clinical translation.

5. Conclusion

Triterpene saponins from *Centella asiatica*, standardized to asiaticoside and madecassoside, activate the neuroprotective Nrf2/HO-1 pathway in oxidatively stressed neuroblastoma cells through dual Nrf2 promoter demethylation and H3K27 acetylation. TEF at 100 µg/mL restored viability to 82.4% and reduced ROS to 1.52-fold—equivalent to vitamin E—

via DNMT1 suppression that lowered promoter methylation from 67.8% to 31.2% and H3K27ac enrichment from 0.34- to 1.43-fold. The strong inverse methylation–expression correlation ($r = -0.912$) and aligned IC₅₀ values reveal quantitative coupling of epigenetic remodeling and antioxidant activation, providing a molecular rationale for the traditional cognitive-enhancing use of *pegagan* and *Brahmi*.

Declarations

Use of Generative Artificial Intelligence. None. The authors did not employ generative artificial intelligence tools or large language models at any point in this work, whether for study design, laboratory experimentation, data analysis, interpretation of results, writing or editing of the manuscript, or preparation of tables and figures. The authors bear full responsibility for the accuracy and integrity of the content.

Author Contributions. A.H. conceived and designed the study, supervised the extraction, standardization, and cell-based experiments, analyzed and interpreted the data, and drafted the manuscript. W.W.P. performed the cell culture and molecular assays, contributed to data acquisition, curation, and statistical analysis, and critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Funding. None. This research did not receive any specific grant or financial support from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest. None. The authors declare that they have no financial, professional, or personal interests that could have influenced the work reported in this article.

Ethical Approval. The study protocol was reviewed and approved by CMHC Research Center Ethics Committee; Ref. No. 2024/0175). The study used a commercially sourced human cell line (SH-SY5Y, ATCC CRL-2266) and authenticated plant material; no human participants or live animals were involved.

Consent for Publication. Not applicable. This article contains no data from individual human participants.

Data Availability. The data supporting the findings of this study are available from the corresponding author on reasonable request.

Acknowledgements. The authors thank the technical and laboratory staff of the host institution for their support.

References

1. Li C, Zhao X, Xu Y, et al. NMN synbiotics: a multifaceted therapeutic approach for Alzheimer's disease. *Neurochem Res.* 2024;49(10):2888–2896. doi:10.1007/s11064-024-04210-z
2. May N, de Sousa Coelho M, Pansieri J, et al. Investigating the therapeutic potential of plants and plant-based medicines: relevance to antioxidant and neuroprotective effects. *Nutrients.* 2023;15(18):3912. doi:10.3390/nu15183912
3. Uy NP, He MT, Subedi L, et al. *Artemisia* extracts and phytochemicals inhibit glutamate-induced oxidative cell death and upregulate the Nrf2/HO-1 signaling pathway in HT22 neuronal cells. *J Ethnopharmacol.* 2025;349:119933. doi:10.1016/j.jep.2025.119933
4. Guo C, Wang Y, Piao Y, et al. Astilbin exerts a neuroprotective effect by upregulating the signaling of nuclear NF-E2-related factor 2. *Heliyon.* 2024;10(17):e37276. doi:10.1016/j.heliyon.2024.e37276
5. Jiang J, Han R, Wang Y, et al. A review of neuroprotective properties of *Centella asiatica* (L.) Urb. and its therapeutic effects. *Ann Med.* 2025;57(1):2559122. doi:10.1080/07853890.2025.2559122
6. Mando Z, Mando H, Rahimi K, et al. Biomarker triterpenoids of *Centella asiatica* as potential antidepressant agents: combining *in vivo* and *in silico* studies. *Behav Brain Res.* 2024;466:114976. doi:10.1016/j.bbr.2024.114976
7. Wright KM, Bollen M, David J, et al. Bioanalytical method validation and application to a phase 1, double-blind, randomized pharmacokinetic trial of a standardized *Centella asiatica* (L.) Urban water extract product in healthy older adults. *Front Pharmacol.* 2023;14:1228030. doi:10.3389/fphar.2023.1228030
8. Bhosale S, Ranganathan V, Sundaram S, et al. Neuroprotective and nootropic effects of a standardised Siddha polyherbal formulation against β -amyloid-induced neurodegeneration. *J Ethnopharmacol.* 2025;357:120850. doi:10.1016/j.jep.2025.120850
9. He Z, Hu Y, Niu Z, et al. Asiaticoside exerts neuroprotection through targeting NLRP3 inflammasome activation. *Phytomedicine.* 2024;127:155494. doi:10.1016/j.phymed.2024.155494
10. Jiang W, Feng XM, Zhang Y, et al. Asiaticoside alleviates migraine-induced cognitive impairment via TLR4-mediated apoptosis regulation. *Eur J Pharmacol.* 2025;1007:178204. doi:10.1016/j.ejphar.2025.178204
11. Ulasov AV, Rosenkranz AA, Georgiev GP, et al. Nrf2/Keap1/ARE signaling: towards specific regulation. *Life Sci.* 2021;291:120111. doi:10.1016/j.lfs.2021.120111
12. Zhang Q, Yao M, Qi J, et al. Puerarin inhibited oxidative stress and alleviated cerebral ischemia-reperfusion injury through PI3K/Akt/Nrf2 signaling pathway. *Front Pharmacol.* 2023;14:1134380. doi:10.3389/fphar.2023.1134380
13. Wang G, Wang L, Zhang X, et al. Neuroprotective effect of sulforaphane on hyperglycemia-induced cognitive dysfunction through the Nrf2/HO-1 pathway. *Int J Clin Exp Pathol.* 2024;17(12):469–476.

- doi:10.62347/CHBJ5517
14. Shen M, Zheng Y, Li G, et al. Dual antioxidant DH-217 mitigated cerebral ischemia-reperfusion injury by targeting IKK β /Nrf2/HO-1 signal axis. *Neurochem Res.* 2023;48(2):579–590. doi:10.1007/s11064-022-03783-x
 15. Nema M, Dutta BJ, Singh S. Alpha-lipoic acid alleviates imidacloprid-induced neuro-behavioral deficits in rats via Nrf2/HO-1 pathway. *Toxicol Mech Methods.* 2024;34(2):176–188. doi:10.1080/15376516.2023.2266027
 16. Gao Q, Chen F, Zhang L, et al. Inhibition of DNA methyltransferase aberrations reinstates antioxidant aging suppressors and ameliorates renal aging. *Aging Cell.* 2021;21(1):e13526. doi:10.1111/acer.13526
 17. Zheng J, Zhang Q, Zhao Z, et al. Epigenetically silenced lncRNA SNAI3-AS1 promotes ferroptosis in glioma via perturbing the m6A-dependent recognition of Nrf2 mRNA mediated by SND1. *J Exp Clin Cancer Res.* 2023;42(1):127. doi:10.1186/s13046-023-02684-3
 18. Wang J, Deng X, Yang Y, et al. DNA methyltransferase 1 modulates mitochondrial function through bridging m5C RNA methylation. *Mol Cell.* 2025;85(10):1999–2016.e11. doi:10.1016/j.molcel.2025.04.019
 19. Wang L, Shannar AAF, Wu R, et al. Butyrate drives metabolic rewiring and epigenetic reprogramming in human colon cancer cells. *Mol Nutr Food Res.* 2022;66(12):e2200028. doi:10.1002/mnfr.202200028
 20. Wu M, Jiang M, Dong T, et al. Nrf2 regulated lung DNA demethylation and CYP2E1 DNA methylation under PM2.5 exposure. *Front Genet.* 2023;14:1144903. doi:10.3389/fgene.2023.1144903
 21. Sharma H, Yang H, Sharma N, et al. Neuroprotection by *Anethum graveolens* (dill) seeds and its phytochemicals in SH-SY5Y neuroblastoma cell lines and acellular assays. *Int J Mol Sci.* 2024;25(13):7104. doi:10.3390/ijms25137104
 22. Hong Bui HT, Nguyen PH, Pham TN, et al. Quantum mechanics-based structural analysis of phenolic glycosides from *Cuscuta japonica* seeds with protective effects against H₂O₂-induced oxidative stress in SH-SY5Y cells. *Phytochemistry.* 2025;234:114420. doi:10.1016/j.phytochem.2025.114420
 23. Barak T, Miller O, Vaknin A, et al. Neuroprotective effects of *Pulicaria incisa* infusion on human neuroblastoma cells and hippocampal neurons. *Antioxidants (Basel).* 2022;12(1):32. doi:10.3390/antiox12010032
 24. Sharma H, Yang H, Sharma N, et al. Multivalent neuroprotective activity of cardamom and fennel in H₂O₂-induced oxidative stress in SH-SY5Y cells and acellular assays. *Pharmaceuticals (Basel).* 2024;18(1):2. doi:10.3390/ph18010002