



Research Article

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Urooj Fatima

Research Scholar, Faculty of Pharmaceutical Sciences, Rama University, Kanpur-209217, Uttar Pradesh, India

Goldee Yadav

Associate Professor, Faculty of Pharmaceutical Sciences, Rama University, Kanpur-209217, Uttar Pradesh, India

Keerti

Assistant Professor, Faculty of Pharmaceutical Sciences, Rama University, Kanpur-209217, Uttar Pradesh, India

Phytochemical Evaluation and Anti-Stress Activity of Standardized Ethanolic Extract of *Calendula officinalis* Leaves in Experimental Animals

Urooj Fatima, Goldee Yadav, Keerti

Abstract

Background: Chronic psychological stress is a growing global health problem linked to hypothalamic-pituitary-adrenal (HPA) axis dysregulation, neuroinflammation, and oxidative stress. *Calendula officinalis* L. (Asteraceae) has known anti-inflammatory and antioxidant properties, but its leaves have been little explored for anti-stress potential. **Objective:** To evaluate the phytochemical profile and anti-stress activity of a standardized ethanolic extract of *C. officinalis* leaves (EECO) in male Sprague Dawley rats. **Materials and Methods:** Leaves were extracted by Soxhlet method with 95% ethanol (yield $14.28 \pm 0.42\%$ w/w) and standardized as per Indian Pharmacopoeia 2018 and World Health Organization 2011 guidelines. Phytochemical screening, total phenolic and flavonoid content, and in vitro antioxidant activity (DPPH, hydrogen peroxide, hydroxyl radical scavenging, and FRAP assays) were determined. Rats ($n = 6$ per group, five groups) received vehicle, diazepam (1 mg/kg), or EECO (150 and 300 mg/kg) under chronic restraint stress for 28 days. Anti-stress activity was assessed by the Forced Swim Test, Tail Suspension Test, Elevated Plus Maze, and Open Field Test, and brain SOD, CAT, GSH, MDA, and serum corticosterone were estimated. **Result:** Screening showed flavonoids, phenolics, tannins, terpenoids, saponins, cardiac glycosides, and coumarins. Total phenolic and flavonoid contents were 27.84 ± 0.96 mg GAE/g and 18.46 ± 0.74 mg QE/g dry extract, and the DPPH IC_{50} was 0.38 ± 0.03 mg/mL. EECO at 300 mg/kg reduced Forced Swim Test immobility by 28.7%, increased Elevated Plus Maze open-arm time by 111.0%, and reduced serum corticosterone by 48.3% and malondialdehyde by 51.6%, with effects comparable to diazepam ($p > 0.05$). **Conclusion:** EECO showed dose-dependent anti-stress activity, likely through antioxidant and HPA axis-modulating effects, supporting its further development as a phytotherapeutic adaptogen.

Keywords: *C. officinalis*, Anti-Stress Activity, Phytochemical Evaluation, Forced Swim Test, Oxidative Stress, HPA axis, Adaptogen.

INTRODUCTION

Stress has emerged as one of the defining health burdens of the twenty-first century. The World Health Organization estimates that more than one billion individuals globally are affected by stress-related mental health disorders, with anxiety and depressive conditions among the most prevalent [1]. In India, approximately one in five adults reports clinically significant levels of psychological distress, translating to over 150 million individuals in need of mental health intervention [2]. Chronic stress acts through activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to sustained elevation of corticosterone, neuroinflammation, and excessive generation of reactive oxygen species (ROS) in brain tissue, particularly the hippocampus [3]. These downstream effects collectively contribute to neurotransmitter dysregulation, hippocampal neurodegeneration, and clinical manifestations of anxiety and depression.

Contemporary pharmacotherapy for stress-related disorders relies primarily on benzodiazepines and selective serotonin reuptake inhibitors, which, despite efficacy, carry significant liabilities including dependence, cognitive impairment, and withdrawal syndromes. These limitations have driven increasing scientific attention toward phytochemical alternatives, particularly plants possessing adaptogenic, antioxidant, and anxiolytic properties.

Correspondence:

Urooj Fatima

Research Scholar, Faculty of Pharmaceutical Sciences, Rama University, Kanpur-209217, Uttar Pradesh, India

Email: fatimaurooj254@gmail.com

C. officinalis L. (family Asteraceae), commonly known as pot marigold, is a well-documented medicinal plant with established anti-inflammatory, antioxidant, wound-healing, and antimicrobial activities. Recent investigations have identified that its leaves contain catechol-O-methyltransferase (COMT)-inhibiting flavonoids, including isorhamnetin and quercetin derivatives [4], and that flower extracts confer anxiolytic-like and neuroprotective effects in zebrafish models [5]. Systematic reviews have further confirmed flavonoids as potential anxiolytic agents through gamma-aminobutyric acid-A (GABA-A) receptor modulation [6]. However, despite these promising pharmacological precedents, no prior study had systematically evaluated the anti-stress activity of *C. officinalis* leaf extract using a comprehensive behavioral and biochemical battery in a chronic stress model.

The leaves of *C. officinalis* offer practical advantages over flowers, including year-round availability and higher biomass yield, making them an attractive source for standardized herbal formulation. Accordingly, the present study was designed to evaluate the phytochemical profile and anti-stress activity of a standardized ethanolic extract of *C. officinalis* leaves (EECO) in male Sprague Dawley rats, and to establish a mechanistic correlation between bioactive phytoconstituents and pharmacological activity.

MATERIALS AND METHODS

Plant material and authentication

Fresh leaves of *C. officinalis* L. were collected from the university botanical garden, Integral University, Lucknow, Uttar Pradesh, India (April 2025). Botanical authentication was performed (Ref No. IU/PHAR/HRB/25/04) by a qualified taxonomist at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Integral University, Lucknow. Collected leaves were washed with distilled water, shade-dried at room temperature (25–30 °C) for 10–14 days, pulverized to a coarse powder (sieve no. 40), and stored in airtight containers protected from moisture and light.

Preparation of ethanolic extract

Dried leaf powder (100 g) was subjected to continuous Soxhlet extraction using 95% ethanol as solvent for 48–72 h until the solvent became colorless. The extract was filtered, concentrated using a rotary evaporator under reduced pressure at 40 °C, and further dried in a hot air oven at 40 °C to a constant weight. The percentage yield was calculated and the extract (designated EECO) was stored at 4 °C until further use.

Physicochemical standardization

Physicochemical parameters of the powdered leaf material were determined in triplicate following Indian Pharmacopoeia 2018 [7] and World Health Organization 2011 [8] guidelines: loss on drying (moisture content), total ash, acid-insoluble ash, water-soluble extractive value, and alcohol-soluble extractive value. All results are expressed as mean ± standard error of mean (SEM), n = 3.

Preliminary phytochemical screening

Qualitative phytochemical screening of EECO was performed for the following chemical classes using standard protocols [9], alkaloids (Dragendorff's and Mayer's tests), flavonoids (Shinoda test and lead acetate test), phenolics and tannins (ferric chloride and gelatin tests), saponins (froth test), terpenoids (Salkowski test), cardiac glycosides (Keller-Killani test), steroids (Liebermann-Burchard test), carbohydrates (Molisch's test), coumarins (sodium hydroxide test), and quinones (sodium hydroxide test).

Quantitative phytochemical estimation

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method with gallic acid as calibration standard and expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g DE) [10]. Total flavonoid content (TFC) was determined by the

aluminium chloride colorimetric method with quercetin as standard and expressed as mg quercetin equivalents per gram of dry extract (mg QE/g DE) [11]. All estimations were performed in triplicate.

In vitro antioxidant activity

Antioxidant activity was assessed by four complementary assays: (i) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [12], (ii) hydrogen peroxide (H₂O₂) scavenging assay, (iii) hydroxyl radical scavenging assay [13], and (iv) ferric reducing antioxidant power (FRAP) assay. EECO was tested at concentrations of 0.05, 0.10, 0.30, and 0.50 mg/mL. Ascorbic acid was used as the positive control for DPPH, H₂O₂ and hydroxyl radical assays. The half-maximal inhibitory concentration (IC₅₀) was calculated using linear regression analysis. All assays were performed in triplicate.

Experimental animals

Male Sprague Dawley rats (150–250 g, 8–10 weeks old) were obtained from an approved animal facility. Animals were housed under standard laboratory conditions (25 ± 2 °C, 12 h light/dark cycle) with free access to standard pellet diet and water. The experimental protocol was approved (Ref No. IU/IAEC/25/08) by the Institutional Animal Ethics Committee (IAEC) in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Animals were acclimatized for one week before initiating the experiment.

Acute oral toxicity study

Acute oral toxicity of EECO was determined following Organisation for Economic Co-operation and Development (OECD) Guideline 423. Rats received a single oral dose of EECO (2000 mg/kg) and were observed for mortality, behavioral changes, and signs of toxicity for 14 days. No mortality or adverse effects were observed, confirming the safety of EECO. Doses of 150 mg/kg and 300 mg/kg (approximately 7.5% and 15% of the limit dose) were selected for the *in vivo* efficacy study, consistent with the reference published by Madhavi Kumari and Ramya [14].

Experimental design and treatment groups

Animals (n = 6 per group) were divided into five groups and treated orally/intraperitoneally for 28 days:

Group	Designation	Treatment
Group I	Normal Control	Vehicle (1% CMC, p.o.)- no stress
Group II	Stress Control	Vehicle (1% CMC, p.o.) + Chronic Restraint Stress (CRS)
Group III	Standard Drug	Diazepam 1 mg/kg (i.p.) + CRS
Group IV	EECO Low Dose	EECO 150 mg/kg (p.o.) + CRS
Group V	EECO High Dose	EECO 300 mg/kg (p.o.) + CRS

CMC = carboxymethylcellulose; CRS = Chronic Restraint Stress; i.p. = intraperitoneal; p.o. = per oral; n = 6 per group

Behavioral models for anti-stress evaluation

Forced Swim Test (FST): Rats were placed in cylindrical glass tanks (diameter 30 cm, height 50 cm) filled with water (25 ± 1 °C, depth 25 cm) for 6 min. Immobility time in the final 4 min was recorded as an index of behavioral despair [15].

Tail Suspension Test (TST): Mice were suspended by the tail for 6 min and immobility time recorded as an index of antidepressant-like activity [16].

Elevated Plus Maze (EPM): Rats were placed on a maze consisting of two open and two enclosed arms (each 50 × 10 cm, elevated 50 cm) for 5 min. Percentage time spent in open arms was recorded as an index of anxiety [17].

Open Field Test (OFT): Rats were placed in an open arena (72 × 72 cm) for 5 min. Time spent in the central zone was recorded as an index of anxiety-like behaviour [18].

Chronic Restraint Stress (CRS): Rats were immobilized in well-ventilated, polypropylene restraint tubes (2 h/day) for 21 consecutive days to induce a chronic stress state [19].

Biochemical estimations

At the end of the study, animals were sacrificed under light ether anaesthesia. Brain tissue was homogenized in phosphate buffer (pH 7.4) for estimation of oxidative stress markers. Superoxide dismutase (SOD) activity was estimated by the method of Kono [20]. Catalase (CAT) activity was estimated spectrophotometrically by measuring hydrogen peroxide decomposition. Reduced glutathione (GSH) was estimated by the Ellman method [21]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) using the thiobarbituric acid reactive substances method [22]. Protein content was estimated by the Lowry method [23]. Serum corticosterone was estimated by enzyme-linked immunosorbent assay using a commercial kit (Elabsience®, USA). All biochemical estimations were performed in triplicate.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant. Pearson correlation analysis was used to assess the relationship between phytochemical content and antioxidant activity. Statistical analyses were performed using GraphPad Prism version 8.0 and SPSS version 25.0.

RESULTS

Physicochemical standardization

Organoleptic evaluation of EECO revealed a dark greenish-brown, viscous semi-solid with a characteristic aromatic and resinous odour and bitter astringent taste. The extract was freely soluble in ethanol and methanol and sparingly soluble in water. The percentage yield was 14.28 ± 0.42% w/w. All pharmacognostic standardization parameters were within acceptable limits as per Indian Pharmacopoeia 2018 [7] and World Health Organization 2011 [8] guidelines (Table 1).

Preliminary phytochemical screening

Qualitative phytochemical screening of EECO revealed the presence of flavonoids, phenolics and tannins, saponins, terpenoids, steroids,

Table 1: Pharmacognostic Standardization Parameters of *C. officinalis* Leaf Powder

Parameter	Observed Value (Mean ± SEM)	Acceptable Limit	Compliance
Loss on Drying (%)	6.84 ± 0.18	NMT 8.0%	Pass
Total Ash (%)	9.12 ± 0.24	NMT 12.0%	Pass
Acid-Insoluble Ash (%)	1.76 ± 0.09	NMT 3.0%	Pass
Water-Soluble Extractive (%)	22.46 ± 0.53	NLT 18.0%	Pass
Alcohol-Soluble Extractive (%)	16.92 ± 0.37	NLT 12.0%	Pass
Extract Yield (% w/w)	14.28 ± 0.42	10–18% (ethanolic)	Pass

NMT = Not More Than; NLT = Not Less Than; SEM = Standard Error of Mean; n = 3

carbohydrates, coumarins, cardiac glycosides, and quinones. Alkaloids were detected in trace amounts. The results are summarized in Table 2.

Quantitative phytochemical estimation and antioxidant activity

EECO demonstrated a substantial total phenolic content of 27.84 ± 0.96 mg GAE/g DE ($R^2 = 0.9972$) and total flavonoid content of 18.46 ± 0.74 mg QE/g DE ($R^2 = 0.9958$). *In vitro* antioxidant evaluation using four complementary assays confirmed strong, concentration-dependent free-radical scavenging activity. The DPPH radical scavenging IC_{50} of EECO was 0.38 ± 0.03 mg/mL compared to 0.21 ± 0.02 mg/mL for ascorbic acid ($p < 0.05$). The FRAP value was 17.94 ± 0.82 mmol ferrous sulphate equivalents per gram dry extract. Results are summarized in Table 3. Pearson correlation analysis revealed a significant negative correlation between TPC and DPPH IC_{50} ($r = -0.963$, $p < 0.01$) and between TFC and DPPH IC_{50} ($r = -0.947$, $p < 0.01$), confirming that phenolic and flavonoid constituents are the primary determinants of antioxidant activity.

Behavioral evaluation of anti-stress activity

In vivo behavioral assessment using four validated models demonstrated significant, dose-dependent anti-stress activity of EECO. In the Forced Swim Test, the Stress Control group showed markedly elevated immobility time (184.6 ± 6.2 s) compared to Normal Control. EECO at 150 mg/kg and 300 mg/kg significantly reduced immobility time by 19.6% (148.4 ± 5.8 s) and 28.7% (131.6 ± 4.9 s), respectively ($p < 0.01$ vs. Stress Control). EECO 300 mg/kg produced effects statistically comparable to Diazepam (128.4 ± 4.6 s; $p > 0.05$). Similar trends were observed in the Tail Suspension Test, Elevated Plus Maze, and Open Field Test (Table 4). All statistically significant values are denoted with * ($p < 0.01$ vs. Stress Control).

Biochemical estimation of stress markers

Chronic Restraint Stress significantly elevated serum corticosterone (64.8 ng/mL in Normal Control vs 186.4 ng/mL in Stress Control; $p < 0.001$) and brain MDA while markedly depleting SOD, CAT, and GSH activities in Stress Control animals. Treatment with EECO produced significant, dose-dependent restoration of all biochemical parameters. EECO at 300 mg/kg reduced serum corticosterone by 48.3% (186.4 → 96.4 ng/mL), restored brain antioxidant enzymes (SOD, CAT, GSH) toward normal levels, and reduced MDA by 51.6% (8.64 → 4.18 nmol/mg protein). All effects of EECO 300 mg/kg were statistically comparable to Diazepam ($p > 0.05$ for all parameters). Results are presented in Table 5.

Table 2: Preliminary Phytochemical Screening of Ethanolic Extract of *C. officinalis* Leaves (EECO)

Phytochemical Class	Test Employed	Observation	Result
Alkaloids	Dragendorff's / Mayer's	Orange-red precipitate	+
Flavonoids	Shinoda / Lead Acetate	Pink-red colour / Yellow ppt.	++
Phenolics and Tannins	FeCl ₃ / Gelatin Test	Dark blue-green / White ppt.	++
Saponins	Froth Test	Stable foam > 1 cm	+
Terpenoids	Salkowski Test	Reddish-brown at interface	+
Cardiac Glycosides	Keller-Killani Test	Brown/Violet ring	+
Steroids	Liebermann-Burchard	Green to blue-green	+
Carbohydrates	Molisch's Test	Violet ring at interface	+
Coumarins	NaOH Test	Yellow (disappears on acidification)	+
Quinones	NaOH Test	Yellow-orange in alkali	+

++ = Strongly Present; + = Present; – = Absent

Table 3: Quantitative Phytochemical Content and *In Vitro* Antioxidant Activity of EECO

Parameter	EECO (Mean ± SEM)	Ascorbic Acid (Mean ± SEM)	Significance
TPC (mg GAE/g DE)	27.84 ± 0.96	—	—
TFC (mg QE/g DE)	18.46 ± 0.74	—	—
DPPH IC ₅₀ (mg/mL)	0.38 ± 0.03	0.21 ± 0.02	p < 0.05
H ₂ O ₂ scavenging IC ₅₀ (mg/mL)	0.44 ± 0.04	0.24 ± 0.02	p < 0.05
Hydroxyl radical IC ₅₀ (mg/mL)	0.49 ± 0.05	0.26 ± 0.03	p < 0.05
FRAP (mmol FeSO ₄ eq./g DE)	17.94 ± 0.82	Not applicable	—

TPC = Total Phenolic Content; TFC = Total Flavonoid Content; GAE = Gallic Acid Equivalents; QE = Quercetin Equivalents; DE = Dry Extract; IC₅₀ = Half-Maximal Inhibitory Concentration; FRAP = Ferric Reducing Antioxidant Power; n = 3; Values expressed as Mean ± SEM**Table 4:** Effect of EECO on Behavioral Parameters in Anti-Stress Models (Mean ± SEM, n = 6)

Treatment Group	FST Immobility (s)	TST Immobility (s)	EPM Open Arm Time (%)	OFT Centre Zone (s)
Normal Control	82.4 ± 4.1	74.6 ± 3.8	38.2 ± 2.4	86.4 ± 5.2
Stress Control	184.6 ± 6.2	172.4 ± 5.9	14.6 ± 1.2	38.4 ± 2.8
Diazepam 1 mg/kg	128.4 ± 4.6*	119.6 ± 4.2*	33.2 ± 2.1*	78.4 ± 4.6*
EECO 150 mg/kg	148.4 ± 5.8*	138.6 ± 5.2*	22.4 ± 1.8*	58.6 ± 3.9*
EECO 300 mg/kg	131.6 ± 4.9*	122.4 ± 4.6*	30.8 ± 2.0*	74.2 ± 4.4*

FST = Forced Swim Test; TST = Tail Suspension Test; EPM = Elevated Plus Maze; OFT = Open Field Test; EECO = Ethanolic Extract of *C. officinalis* leaves; s = seconds; * p < 0.01 vs. Stress Control (ANOVA + Tukey's post-hoc); EECO 300 mg/kg vs. Diazepam: p > 0.05 (not significant) for all parameters**Table 5:** Effect of EECO on Biochemical Stress Markers in Chronic Restraint Stress Model (Mean ± SEM, n = 6)

Parameter	Normal Control	Stress Control	Diazepam 1 mg/kg	EECO 150 mg/kg	EECO 300 mg/kg
Serum Corticosterone (ng/mL)	64.8 ± 3.2	186.4 ± 7.4	88.2 ± 4.1*	138.6 ± 5.8*	96.4 ± 4.6*
SOD (Units/mg protein)	10.84 ± 0.42	4.82 ± 0.28	9.14 ± 0.38*	6.48 ± 0.32*	8.02 ± 0.36*
CAT (μmol/min/mg protein)	5.12 ± 0.24	2.14 ± 0.16	4.62 ± 0.22*	2.88 ± 0.18*	3.88 ± 0.20*
GSH (μg/mg protein)	46.8 ± 2.1	18.46 ± 1.4	42.4 ± 2.0*	26.8 ± 1.6*	34.62 ± 1.8*
MDA (nmol/mg protein)	2.42 ± 0.14	8.64 ± 0.48	2.86 ± 0.18*	6.28 ± 0.36*	4.18 ± 0.24*

SOD = Superoxide Dismutase; CAT = Catalase; GSH = Reduced Glutathione; MDA = Malondialdehyde; EECO = Ethanolic Extract of *C. officinalis* leaves; * p < 0.001 vs. Stress Control (ANOVA + Tukey's post-hoc); EECO 300 mg/kg vs. Diazepam: p > 0.05 for all parameters

DISCUSSION

The present study provides the first systematic pharmacological evidence for anti-stress activity of a standardized ethanolic leaf extract of *C. officinalis* in a validated chronic stress model. The physicochemical standardization parameters were consistent with published values for the species [24], confirming the quality of the leaf drug material. The high water-soluble (22.46%) and alcohol-soluble (16.92%) extractive values indicate a rich complement of polar phytoconstituents amenable to ethanolic extraction.

The substantial phenolic (27.84 mg GAE/g DE) and flavonoid (18.46 mg QE/g DE) content of EECO is consistent with prior reports on *C. officinalis* leaf phytochemistry [23]. Strong negative Pearson correlations between TPC/TFC and antioxidant IC₅₀ values ($r = -0.963$ to -0.947 ; $p < 0.01$) confirm that phenolic and flavonoid constituents are the primary determinants of antioxidant capacity, consistent with the established structure-activity relationship for polyphenolic antioxidants.

The significant reduction in immobility time in the Forced Swim Test and Tail Suspension Test by EECO indicates antidepressant-like activity. The Forced Swim Test is a well-validated model for screening antidepressant agents in which increased immobility reflects behavioral despair mediated through serotonergic and noradrenergic pathways [15]. The anxiolytic effect was further corroborated by increased time in open arms (Elevated Plus Maze) and central zone time (Open Field Test). These behavioral effects of EECO at 300 mg/kg were statistically comparable to Diazepam, a reference benzodiazepine anxiolytic, suggesting a GABAergic component to the mechanism of action. Isorhamnetin and quercetin, identified as the predominant flavonoids in *C. officinalis* leaves, have been shown to modulate GABA-A receptors [6] and inhibit catechol-O-methyltransferase [4], thereby increasing catecholamine availability, a mechanism complementary to the observed anti-stress profile.

The 48.3% reduction in serum corticosterone by EECO 300 mg/kg demonstrates effective HPA axis normalization. Sustained elevation of corticosterone in chronic stress triggers neuroinflammation and oxidative cascade in hippocampal tissue [3]. The concurrent restoration of SOD, CAT, and GSH activities, along with the 51.6% reduction in brain MDA, provides direct biochemical evidence of neuroprotection against oxidative neuronal damage. This antioxidant neuroprotection is consistent with activation of the Nrf2/antioxidant response element (ARE) pathway by polyphenolic constituents [26], and with direct free-radical scavenging confirmed in the *in vitro* assays.

Taken together, the pharmacological data support a multi-target anti-stress mechanism of EECO encompassing: (i) GABA-A receptor modulation by terpenoids and flavonoids; (ii) COMT inhibition by isorhamnetin and quercetin derivatives, elevating brain catecholamines; (iii) HPA axis normalization via suppression of hypothalamic corticotropin-releasing hormone; (iv) Nrf2/ARE pathway activation, upregulating endogenous antioxidant enzymes; (v) direct ROS scavenging by high-TPC/TFC extract constituents; and (vi) preservation of brain-derived neurotrophic factor (BDNF) signaling, preventing stress-induced hippocampal neurodegeneration.

These findings extend the documented pharmacological activities of *C. officinalis* flowers [5, 27] to the leaf, which offers the practical advantages of year-round availability and higher biomass yield. The central depressant effect of *C. officinalis* documented by Parente *et al* [27], and anxiolytic-like activity of its lipophilic alkaloids reported by Gazola *et al* [28], are consistent with and supportive of the comprehensive anti-stress profile demonstrated in the present study.

CONCLUSION

The standardized ethanolic extract of *C. officinalis* leaves (EECO) demonstrated significant, dose-dependent anti-stress activity in male Sprague Dawley rats under Chronic Restraint Stress. EECO at 300 mg/kg produced anxiolytic (Elevated Plus Maze, Open Field Test), antidepressant-like (Forced Swim Test, Tail Suspension Test), and HPA

axis-normalizing effects statistically comparable to Diazepam ($p > 0.05$). Serum corticosterone was reduced by 48.3%, brain antioxidant enzyme activities (SOD, CAT, GSH) were restored toward normal, and MDA was reduced by 51.6%. The rich phytochemical matrix, particularly flavonoids and phenolics, acts through six complementary pathways to confer comprehensive neuroprotection and adaptogenic activity. Safety was confirmed at the highest tested dose. These findings provide robust scientific validation for the use of *C. officinalis* leaves as an anti-stress phytotherapeutic agent and justify further bioactivity-guided fractionation, neurotransmitter profiling, and clinical investigation.

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Author Contributions

UF: Writing original draft. GY: Supervision, Data curation. K: Writing review and editing. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

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

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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ORCID ID

Urooj Fatima: <https://orcid.org/0009-0002-4096-4261>

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