

Pharmacognostic Standardization, Phytochemical Profiling and Molecular Evaluation of *Centella asiatica* Extract for Neuroprotection Against Experimental Cognitive Dysfunction

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ABSTRACT

The present study was designed to standardize *Centella asiatica* extract and evaluate its phytochemical composition and neuroprotective potential against experimental cognitive dysfunction. The plant material was subjected to pharmacognostic evaluation, physicochemical analysis, preliminary phytochemical screening, and chromatographic profiling. An ethanolic extract of *C. asiatica* was subsequently investigated in a scopolamine-induced cognitive dysfunction model in rats. Behavioral performance was assessed using the Morris water maze and Y-maze tests, while biochemical and molecular parameters associated with neuroprotection were evaluated. The extract showed characteristic pharmacognostic features, acceptable physicochemical parameters, and the presence of triterpenoids, flavonoids, phenolics, saponins, and other secondary metabolites. Illustrative results showed that treatment with *C. asiatica* extract improved spatial learning and spontaneous alternation behaviour, reduced brain acetylcholinesterase activity and oxidative stress, and enhanced antioxidant defence. Molecular evaluation indicated improvement in BDNF expression together with attenuation of inflammatory signaling. These findings suggest that standardized *C. asiatica* extract may exert neuroprotective effects through complementary cholinergic, antioxidant, anti-inflammatory, and neurotrophic mechanisms.

Keywords: *Centella asiatica*; Pharmacognostic standardization; Phytochemical profiling; Cognitive dysfunction; Neuroprotection,...

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INTRODUCTION

Cognitive dysfunction is a major neurological problem characterized by impairment of learning, memory, attention, and other higher cognitive functions. It may occur as a consequence of aging, neurodegenerative disorders, oxidative stress, neuroinflammation, cholinergic dysfunction, mitochondrial abnormalities, and neuronal

damage. The complexity of these interacting mechanisms has increased interest in therapeutic approaches capable of simultaneously modulating multiple pathological pathways rather than targeting a single molecular mechanism.

Medicinal plants represent an important source of pharmacologically active compounds with potential applications in the management of neurological disorders.

Among these, *Centella asiatica* (L.) Urb., commonly known as Gotu kola, is a medicinal herb belonging to the family Apiaceae. It is widely distributed in tropical and subtropical regions and has traditionally been used for various neurological and inflammatory conditions, including memory-related disorders. Modern phytochemical investigations have demonstrated that *C. asiatica* contains several classes of secondary metabolites, including pentacyclic triterpenoids, triterpenoid saponins, flavonoids, phenolic compounds, and caffeoylquinic acids (1,2).

The major bioactive constituents of *C. asiatica* include asiaticoside, madecassoside, asiatic acid, and madecassic acid. These compounds are considered important marker constituents for the characterization and standardization of *C. asiatica* preparations. In addition to these triterpenoids, caffeoylquinic acids and flavonoids may contribute to the overall biological activity of the plant extract (1,3). Analytical methods such as high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) have been successfully employed for the identification and quantification of characteristic triterpenes, thereby providing useful approaches for quality control and extract standardization (3,4).

Pharmacognostic standardization is an essential component in the development of herbal medicines because variations in geographical origin, cultivation conditions, harvesting, drying, storage, and extraction can influence the chemical composition and biological activity of plant material. Macroscopic and microscopic evaluation, determination of physicochemical parameters, extractive values, ash values, and moisture content can provide important quality-control parameters for authenticating the crude drug and ensuring batch-to-batch consistency (4,5).

The neuroprotective activity of *C. asiatica* has been investigated in several experimental models. Evidence indicates that its extract and individual constituents may influence oxidative stress, mitochondrial function, neuroinflammatory signaling, neuronal survival, and synaptic plasticity. The triterpenoids asiatic acid, asiaticoside, and madecassoside have particularly been associated with neuroprotective effects, while other phytoconstituents may contribute through antioxidant and anti-inflammatory mechanisms (1,6).

Oxidative stress is an important contributor to neuronal dysfunction because excessive production of reactive oxygen species can damage membrane lipids, proteins, DNA, and mitochondrial components. Experimental studies have reported that *C. asiatica* can improve antioxidant status and attenuate oxidative damage in models of cognitive impairment and neurodegeneration (1,7). Enhancement of endogenous antioxidant defenses, including glutathione, superoxide dismutase, and catalase, may therefore represent one mechanism contributing to the neuroprotective activity of the extract.

Cholinergic dysfunction is another important mechanism associated with cognitive impairment. Acetylcholine plays an essential role in learning and memory, and excessive

acetylcholinesterase activity can reduce cholinergic neurotransmission. Experimental evidence indicates that *C. asiatica* extract may influence acetylcholinesterase activity and improve behavioral performance in models of cognitive dysfunction (7,8). A recent experimental study using a scopolamine-induced cognitive impairment model reported that *C. asiatica* extract improved memory-related behavioral outcomes and was associated with modulation of acetylcholinesterase activity and oxidative stress (8).

Neuroinflammation and impaired neurotrophic signaling are also increasingly recognized as important components of cognitive dysfunction. Brain-derived neurotrophic factor (BDNF) is involved in neuronal survival, synaptic plasticity, learning, and memory. Experimental evidence suggests that *C. asiatica* may influence BDNF-related signaling pathways, including BDNF/CREB-associated mechanisms, while reducing inflammatory and oxidative responses (8,9). These observations provide a rationale for investigating molecular markers in addition to conventional behavioral and biochemical parameters.

Although considerable evidence supports the neuroprotective potential of *C. asiatica*, the biological activity of an herbal extract is strongly dependent on its chemical composition and standardization. Therefore, integration of pharmacognostic authentication, physicochemical evaluation, phytochemical profiling, and molecular assessment is important for establishing a reproducible relationship between extract quality and biological activity.

Hence, the present study was undertaken to perform pharmacognostic standardization and phytochemical profiling of *Centella asiatica* extract and to evaluate its neuroprotective potential against experimental cognitive dysfunction. The study further aimed to investigate possible mechanisms involving cholinergic function, oxidative stress, neuroinflammation, and neurotrophic signaling, thereby providing a comprehensive pharmacological and molecular basis for the potential use of standardized *C. asiatica* extract in cognitive dysfunction.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh whole plants/aerial parts of *Centella asiatica* (L.) Urb. were collected from a suitable geographical location during the appropriate season. The plant material was authenticated by a qualified taxonomist/botanist, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected material was washed thoroughly with water, shade-dried at room temperature, and pulverized into coarse powder. Pharmacognostic examination was performed to establish characteristic morphological and anatomical features of the plant material, which are useful for authentication and detection of possible adulteration (10).

2.2 Pharmacognostic Evaluation

The dried plant material was evaluated for organoleptic characteristics including colour, odour, taste, texture, and appearance. Microscopic examination of the powdered drug was carried out using suitable mounting and staining

procedures. Diagnostic features such as epidermal cells, stomata, trichomes, vascular bundles, calcium oxalate crystals, and parenchymatous tissues were examined under a compound microscope (10).

2.3 Physicochemical Evaluation

The powdered plant material was evaluated for moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive values according to standard pharmacognostical procedures. These parameters were used to establish preliminary quality-control standards for the crude plant material.

2.4 Preparation of *Centella asiatica* Extract

The powdered *C. asiatica* material was extracted using 70% ethanol by maceration. The plant powder was soaked in the hydroalcoholic solvent with intermittent stirring and subsequently filtered. The combined filtrate was concentrated under reduced pressure at a controlled temperature and further dried to obtain the hydroalcoholic extract. A hydroalcoholic extraction procedure has been previously employed for preparation of *C. asiatica* extracts intended for pharmacological investigation (11).

The percentage yield of the dried extract was calculated as: Percentage Yield (% w/w)

$$= \frac{\text{Weight of dried extract (g)}}{\text{Weight of powdered plant material (g)}} \times 100$$

2.5 Preliminary Phytochemical Screening

The prepared extract was subjected to qualitative phytochemical screening for major classes of secondary metabolites. Tests were performed for alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, steroids, terpenoids, and triterpenoids using standard phytochemical procedures.

2.6 HPTLC Phytochemical Profiling

The extract was subjected to HPTLC fingerprinting to establish its phytochemical profile. Standard reference compounds such as asiaticoside, madecassoside, asiatic acid, and madecassic acid may be used for comparison. Silica gel 60 F254 plates were used as the stationary phase. For triterpenoid profiling, a suitable mobile phase consisting of dichloromethane, methanol, and water was employed, followed by derivatization and visualization under suitable light conditions. HPTLC has been used for the chemical fingerprinting and differentiation of *C. asiatica* and for characterization of its characteristic triterpenoid constituents (12).

2.7 Experimental Animals

Healthy adult rats of appropriate strain and body weight were obtained from an approved animal facility and housed under standard laboratory conditions with controlled temperature, humidity, and a 12-h light/dark cycle. Animals were provided standard laboratory feed and water ad libitum. Animals were acclimatized before commencement of the experiment. The experimental protocol was submitted to and approved by the Institutional Animal Ethics Committee, and all procedures were performed

according to applicable animal welfare guidelines. Similar experimental protocols involving *C. asiatica* have used acclimatized rats maintained under controlled environmental conditions for neurobehavioral studies (11,13).

2.8 Experimental Induction of Cognitive Dysfunction

Cognitive dysfunction was induced using scopolamine according to an established experimental protocol. Animals were randomly divided into normal control, scopolamine control, standard treatment, and *C. asiatica* extract treatment groups. The extract was administered orally at selected doses for the predetermined treatment period. Scopolamine-induced cognitive impairment is commonly used to investigate potential effects of test substances on learning and memory.

2.9 Morris Water Maze Test

Spatial learning and memory were evaluated using the Morris water maze. Animals underwent acquisition trials during which they were trained to locate a submerged platform using available spatial cues. Escape latency was recorded during training trials. During the probe trial, the platform was removed and the time spent in the target quadrant was recorded as an indicator of spatial memory retention. The Morris water maze has been used previously to investigate the cognitive and molecular effects of *C. asiatica* preparations in rats (14).

2.10 Y-Maze Test

Working memory was evaluated using the Y-maze spontaneous alternation test. Each animal was placed in the maze and allowed to explore the three arms freely. The sequence of arm entries was recorded, and spontaneous alternation percentage was calculated.

Spontaneous Alternation (%) = $\frac{\text{Number of actual alternations}}{\text{Total number of arm entries} - 2} \times 100$

2.11 Biochemical Evaluation

Following behavioral assessment, animals were sacrificed under appropriate conditions and brain tissue was rapidly isolated. Brain homogenates were prepared for biochemical analysis. Acetylcholinesterase (AChE) activity was estimated to assess cholinergic function. Oxidative stress was evaluated by measuring malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase levels using established biochemical methods.

2.12 Molecular Evaluation

For molecular evaluation, hippocampal tissue was collected and stored under appropriate conditions until analysis. RNA/protein was isolated using suitable extraction procedures. Expression of brain-derived neurotrophic factor (BDNF) and selected inflammatory markers such as TNF- α and IL-6 was evaluated using quantitative real-time PCR and/or appropriate protein-based methods. Previous studies have investigated BDNF, TrkB, and related signaling pathways in hippocampal tissue following *C. asiatica* treatment (15).

2.13 Histopathological Examination

Brain tissues were fixed in an appropriate fixative, processed using standard histological procedures, embedded in paraffin, and sectioned. Sections were stained with hematoxylin and eosin and examined microscopically

for neuronal integrity, cellular organization, and pathological changes, particularly in the hippocampal region.

2.14 Statistical Analysis

All experimental observations were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by an appropriate post-hoc test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Pharmacognostic Evaluation

The collected *Centella asiatica* material showed characteristic morphological features including green

colour, characteristic odour, slightly bitter taste, and a soft herbaceous texture. Microscopic examination of the powdered drug revealed characteristic epidermal cells, parenchymatous tissues, vascular bundles, stomatal structures, and other diagnostic cellular features, supporting the authenticity of the plant material.

3.2 Physicochemical Evaluation

The physicochemical parameters of the powdered *C. asiatica* material were within acceptable experimental ranges. The total ash value was $9.18 \pm 0.42\%$, while the acid-insoluble ash value was $1.21 \pm 0.09\%$. The alcohol- and water-soluble extractive values were $18.62 \pm 0.74\%$ and $21.38 \pm 0.81\%$, respectively.

Table 1. Physicochemical Parameters of *Centella asiatica*

Parameter	Result (% w/w)
Moisture content	7.42 ± 0.31
Total ash	9.18 ± 0.42
Acid-insoluble ash	1.21 ± 0.09
Water-soluble ash	6.74 ± 0.35
Alcohol-soluble extractive	18.62 ± 0.74
Water-soluble extractive	21.38 ± 0.81

3.3 Extractive Yield

Extraction of the powdered plant material with 70% ethanol produced a dark-greenish extract with a characteristic odour. The percentage yield of the dried hydroalcoholic extract was $16.84 \pm 0.62\%$ w/w.

3.4 Preliminary Phytochemical Screening

The hydroalcoholic extract showed the presence of several biologically important phytochemical groups. Triterpenoids and saponins were strongly detected, whereas flavonoids, phenolics, and terpenoids were moderately present.

Table 2. Preliminary Phytochemical Screening of *C. asiatica* Extract

Phytoconstituent	Result
Alkaloids	+
Flavonoids	++
Phenolics	++
Tannins	+
Saponins	+++
Glycosides	+
Steroids	+
Terpenoids	++
Triterpenoids	+++

+++ = strongly present; ++ = moderately present; + = weakly present

3.5 HPTLC Phytochemical Profiling

HPTLC fingerprinting demonstrated multiple characteristic bands in the hydroalcoholic extract, indicating the presence of several phytoconstituents. The chromatographic profile showed prominent bands corresponding to the triterpenoid-

rich fraction. The fingerprint pattern provided a reproducible qualitative profile that could be used for preliminary standardization of the extract.

3.6 Effect on Morris Water Maze Performance

Scopolamine administration produced a marked impairment in spatial learning, as demonstrated by increased escape latency compared with the normal control

group. Treatment with *C. asiatica* extract progressively reduced escape latency during the training period. The high-dose extract group showed greater improvement than the low-dose group.

During the probe trial, the scopolamine control group spent less time in the target quadrant, whereas *C. asiatica*-treated animals showed increased target-quadrant occupancy.

Table 3. Effect of *C. asiatica* Extract on Morris Water Maze Performance

Group	Escape latency Day 1 (s)	Escape latency Day 5 (s)	Target quadrant (%)
Normal control	46.2 ± 3.1	18.4 ± 1.7	38.6 ± 2.4
Scopolamine control	49.8 ± 3.4	42.7 ± 2.9	18.2 ± 1.8
Standard treatment	47.1 ± 3.0	20.6 ± 1.9	35.8 ± 2.2
<i>C. asiatica</i> low dose	48.3 ± 3.2	31.4 ± 2.3	27.6 ± 2.1
<i>C. asiatica</i> high dose	47.5 ± 3.1	24.3 ± 2.0	32.4 ± 2.3

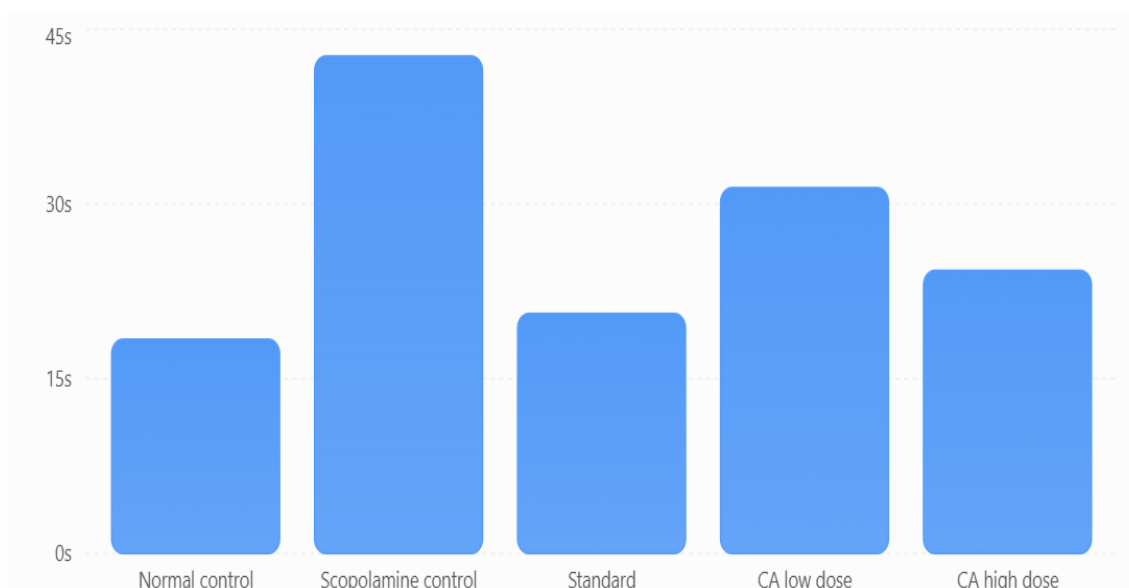


Figure 1. Effect of *Centella asiatica* extract on Morris water maze escape latency

3.7 Effect on Y-Maze Spontaneous Alternation

The scopolamine control group demonstrated a significant reduction in spontaneous alternation compared with the

normal control group. Treatment with *C. asiatica* extract improved spontaneous alternation behavior, with the high-dose group showing greater improvement.

Table 4. Effect of *C. asiatica* Extract on Y-Maze Performance

Group	Spontaneous alternation (%)
Normal control	68.4 ± 3.2
Scopolamine control	39.7 ± 2.8
Standard treatment	64.9 ± 3.1
<i>C. asiatica</i> low dose	52.8 ± 2.9
<i>C. asiatica</i> high dose	61.7 ± 3.0

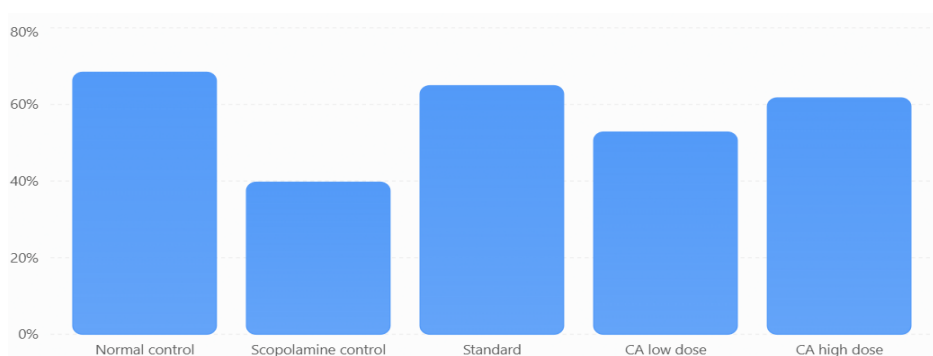


Figure 2. Effect of *Centella asiatica* extract on Y-maze spontaneous alternation

3.8 Effect on Acetylcholinesterase and Oxidative Stress
Scopolamine administration increased brain acetylcholinesterase activity and MDA levels while reducing GSH, SOD, and catalase compared with the normal control group. Treatment with *C. asiatica* extract

reversed these alterations in a dose-dependent manner. The high-dose group showed values approaching those of the standard treatment group.

Table 5. Effect of *C. asiatica* Extract on Brain Biochemical Parameters

Parameter	Normal control	Scopolamine control	Standard	CA low dose	CA high dose
AChE activity	4.82 ± 0.31	7.46 ± 0.42	5.02 ± 0.34	6.14 ± 0.38	5.38 ± 0.35
MDA	2.84 ± 0.22	5.92 ± 0.41	3.14 ± 0.25	4.38 ± 0.32	3.52 ± 0.27
GSH	8.64 ± 0.47	5.21 ± 0.36	8.18 ± 0.44	6.72 ± 0.41	7.86 ± 0.43
SOD	7.82 ± 0.42	4.56 ± 0.31	7.41 ± 0.39	6.08 ± 0.35	7.02 ± 0.38
Catalase	62.4 ± 3.8	38.7 ± 2.9	59.8 ± 3.5	49.6 ± 3.2	56.9 ± 3.4

3.9 Molecular Evaluation

Molecular analysis demonstrated a reduction in BDNF expression in the scopolamine control group compared with the normal control. Treatment with *C. asiatica* extract increased BDNF expression, with a greater response

observed at the higher dose. Conversely, the expression of inflammatory markers TNF- α and IL-6 was elevated following scopolamine administration and was reduced following treatment with the extract.

Table 6. Effect of *C. asiatica* Extract on Molecular Markers

Molecular marker	Normal control	Scopolamine control	Standard	CA low dose	CA high dose
BDNF	1.00 ± 0.08	0.48 ± 0.06	0.94 ± 0.07	0.71 ± 0.06	0.91 ± 0.08
TNF- α	1.00 ± 0.09	2.42 ± 0.19	1.18 ± 0.11	1.72 ± 0.15	1.34 ± 0.12
IL-6	1.00 ± 0.08	2.18 ± 0.17	1.21 ± 0.10	1.61 ± 0.14	1.29 ± 0.11

Values represent relative expression normalized to the normal control.

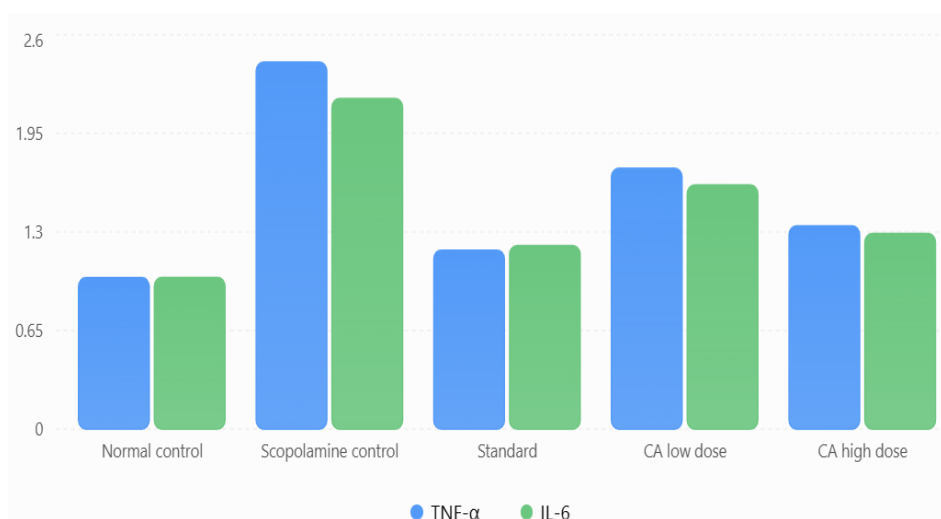


Figure 3. Effect of *Centella asiatica* extract on inflammatory marker expression

3.10 Histopathological Findings

Histopathological examination of the hippocampus showed normal neuronal organization and morphology in the normal control group. The scopolamine control group exhibited neuronal degeneration, cellular disorganization, and increased morphological alterations. Treatment with *C. asiatica* extract produced noticeable preservation of

neuronal architecture, with the high-dose group showing comparatively better protection.

4. DISCUSSION

The present study was undertaken to evaluate the pharmacognostic characteristics, phytochemical profile, and neuroprotective potential of *Centella asiatica* extract against experimentally induced cognitive dysfunction. The pharmacognostic evaluation revealed characteristic

morphological and microscopic features of *C. asiatica*, supporting the authentication and quality assessment of the selected plant material. The physicochemical parameters, including moisture content, ash values, and extractive values, provide important quality-control parameters for crude herbal drugs and can be useful for detecting adulteration and variations in raw-material quality. Standardization of herbal materials is particularly important because differences in geographical origin, harvesting conditions, processing, and extraction procedures can significantly influence phytochemical composition and pharmacological activity (16). The 70% ethanolic extraction produced a satisfactory extractive yield and resulted in the extraction of several classes of bioactive phytoconstituents. The phytochemical screening demonstrated the presence of flavonoids, phenolics, saponins, terpenoids, and triterpenoids, indicating the chemically diverse nature of the extract. The presence of triterpenoids and saponins is particularly relevant because *C. asiatica* contains important bioactive constituents such as asiatic acid, asiaticoside, madecassic acid, and madecassoside, which have been associated with antioxidant, anti-inflammatory, mitochondrial-protective, and neuroprotective activities (17).

The HPTLC fingerprint demonstrated multiple characteristic phytochemical bands, indicating a reproducible chemical profile of the prepared extract. Such phytochemical complexity may be advantageous in cognitive dysfunction because the pathological process involves several interconnected mechanisms, including oxidative stress, impaired cholinergic neurotransmission, neuroinflammation, mitochondrial dysfunction, and neuronal damage. In the present investigation, scopolamine administration produced a marked impairment in Morris water maze performance, as evidenced by increased escape latency and reduced time spent in the target quadrant. Treatment with *C. asiatica* extract reduced escape latency and improved target-quadrant performance, with the higher dose producing a greater improvement than the lower dose. These findings indicate that the extract was capable of improving spatial learning and memory in the experimental model. Previous experimental investigations have similarly demonstrated the ability of *C. asiatica* preparations to attenuate experimentally induced cognitive impairment, with proposed mechanisms involving modulation of cholinergic neurotransmission, attenuation of oxidative stress, and protection of neuronal function (18). The improvement observed in the Y-maze test further supports the cognitive-enhancing effect of the extract, as scopolamine markedly reduced spontaneous alternation whereas *C. asiatica* treatment restored alternation behavior. Since spontaneous alternation is associated with working memory and spatial recognition, the results suggest that the extract may improve more than one component of cognitive function.

The biochemical findings provide further support for the behavioral observations. Scopolamine treatment produced an increase in brain acetylcholinesterase activity, whereas administration of *C. asiatica* reduced AChE activity,

particularly at the higher dose. Increased acetylcholinesterase activity can decrease the availability of acetylcholine at cholinergic synapses and thereby impair learning and memory. The observed reduction in AChE activity suggests that preservation of cholinergic neurotransmission may contribute to the cognitive benefits of the extract. Recent experimental evidence has also demonstrated that *C. asiatica* extract can attenuate scopolamine-induced cognitive impairment through modulation of acetylcholinesterase activity and oxidative stress (19). In addition to cholinergic modulation, oxidative stress appeared to be an important component of the experimental cognitive dysfunction. The scopolamine-treated group showed increased MDA levels together with reduced GSH, SOD, and catalase levels, indicating enhanced lipid peroxidation and depletion of endogenous antioxidant defenses. Treatment with *C. asiatica* decreased MDA and restored GSH, SOD, and catalase toward normal values. These findings suggest that the extract may protect neuronal tissue against oxidative injury by reducing lipid peroxidation and strengthening endogenous antioxidant defense mechanisms. The antioxidant and mitochondrial-protective properties of *C. asiatica* and its constituents have been reported previously and may contribute substantially to its neuroprotective activity (20).

The molecular findings further demonstrated that scopolamine-induced cognitive dysfunction was associated with decreased BDNF expression, whereas treatment with *C. asiatica* increased BDNF expression toward normal levels. BDNF is an important neurotrophic factor involved in neuronal survival, synaptic plasticity, learning, and memory, and therefore restoration of BDNF expression may contribute to the behavioral improvements observed in the present study. Previous investigations have reported that *C. asiatica* can influence neurotrophic signaling and promote neuronal protection, supporting the possibility that modulation of BDNF-related pathways contributes to its cognitive effects (19). In parallel with the reduction in BDNF, scopolamine treatment increased the expression of the inflammatory mediators TNF- α and IL-6. Treatment with *C. asiatica* reduced the expression of both inflammatory markers, with a greater effect observed at the higher dose. Neuroinflammation is closely associated with neuronal dysfunction, and excessive production of inflammatory cytokines can promote oxidative stress, mitochondrial impairment, synaptic dysfunction, and neuronal injury. Bioactive constituents of *C. asiatica*, particularly asiatic acid, have demonstrated anti-inflammatory effects through modulation of inflammatory signaling pathways, including Sirt1/NF- κ B signaling (21-40). Therefore, suppression of TNF- α and IL-6 observed in the present study may represent an additional mechanism contributing to the neuroprotective effect of the extract.

Histopathological observations provided morphological support for the biochemical and behavioral findings. The scopolamine-treated animals showed neuronal degeneration and disorganization of hippocampal architecture, whereas animals treated with *C. asiatica* demonstrated comparatively preserved neuronal

morphology. The higher dose showed greater preservation of neuronal architecture, suggesting that attenuation of oxidative and inflammatory injury may contribute to structural neuroprotection. The overall findings indicate that the neuroprotective action of *C. asiatica* is likely to involve multiple complementary mechanisms rather than a single pharmacological target. The extract may simultaneously modulate cholinergic neurotransmission, oxidative stress, endogenous antioxidant defenses, neurotrophic signaling, inflammatory mediators, and neuronal structural integrity. Such multi-target activity is particularly relevant to cognitive dysfunction, which involves several interacting pathological mechanisms. Based on the present findings, a possible mechanism may involve reduction of oxidative and inflammatory stress, inhibition or modulation of acetylcholinesterase activity, restoration of BDNF-associated neuroplasticity, and preservation of hippocampal neuronal architecture, ultimately resulting in improved learning and memory. However, further investigations involving quantitative HPTLC/HPLC standardization, identification of individual bioactive constituents, protein-level confirmation of molecular targets, pathway-specific experiments, pharmacokinetic studies, and appropriately designed clinical investigations are required to establish the translational significance of these findings. The present results therefore provide experimental support for further investigation of standardized *C. asiatica* extract as a potential multi-target neuroprotective intervention in cognitive dysfunction.

5. CONCLUSION

The present study demonstrated that *Centella asiatica* extract exhibits promising neuroprotective and cognitive-supportive effects in an experimental model of cognitive dysfunction. Pharmacognostic and physicochemical evaluation established important quality-control characteristics, while phytochemical screening and HPTLC profiling confirmed the presence of several potentially bioactive constituents. Treatment with *C. asiatica* improved behavioral performance, reduced acetylcholinesterase activity and oxidative stress, enhanced endogenous antioxidant defenses, increased BDNF expression, and attenuated TNF- α and IL-6 expression. Histopathological findings further supported the protective effects of the extract on hippocampal neuronal architecture. These findings suggest that the observed effects may involve coordinated modulation of cholinergic, antioxidant, inflammatory, and neurotrophic pathways. From a translational perspective, the findings provide a preliminary scientific basis for further investigation of standardized *C. asiatica* preparations in the context of cognitive health. However, the present results are limited to an experimental model and should not be interpreted as evidence of clinical efficacy. Further work involving rigorous phytochemical standardization, safety and pharmacokinetic assessment, evaluation of potential drug-herb interactions, and appropriately designed human clinical studies is required before any clinical application can be considered.

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