ORIGINAL ARTICLE

Exploring the *in vitro* Anti-Inflammatory, Antioxidant, and Neuroprotective Potentials of *Corchorus olitorius* (L) leaves: Insights from ADME and *in silico* studies

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Abstract

Purpose: Alzheimer's is a progressive, irreversible, and devastating neurodegenerative disease, clinically characterized by the impairment of cognitive skills. This study investigates the pharmacological potentials of *Corchorus olitorius*, focusing on its anti-inflammatory, antioxidant, and neuroprotective activities through *in silico* and *in vitro* analyses. **Materials and Methods:** The dried leaves of *C. olitorius* were subjected to sequential solvent extraction followed by qualitative phytochemical analyses and gas chromatography-mass spectrometry (GC-MS) of aqueous extract. Protein denaturation, Cyclooxygenase (COX-II) inhibition, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to evaluate *in vitro* anti-inflammatory and antioxidant potentials. Cytotoxicity, neuroprotective, and acetylcholinesterase (AChE) activities were assessed using L929 and SK-N-SH cell line, respectively. Absorption, distribution, metabolism, and excretion (ADME) and *in silico* studies including molecular docking against targeted proteins were performed. **Results:** Sterols, terpenoids, flavonoids, and phenols were found in the aqueous fraction. GC-MS analysis depicted the presence of various compounds. The dose-dependent inhibition of protein denaturation, COX-II, and cytotoxicity on normal L929 cell lines was observed. DPPH assay displayed significant antioxidant potential. Notable neuroprotective effect was found against trimethyltin chloride-induced neurotoxicity in SK-N-SH cell lines. The decreased expression of AChE activity further validates the neuroprotective potential. The ADME study predicted better gastrointestinal (GI) absorption, adherence to Lipinski rule, and drug likeness properties. The docking studies revealed favorable binding interactions with proteins of bovine serum albumin, COX-II protein, gamma-aminobutyric acid (GABA)-A receptor, and alpha-synuclein. **Conclusion:** Overall, the findings suggest that phytochemicals from *C. olitorius* leaves hold a significant potential to manage neurodegenerative diseases. Further research is warranted to validate these *in silico* findings through *in vivo* experimental studies.

Key words: Anti-inflammatory, antioxidant, *Corchorus olitorius,* gas chromatography-mass spectrometry, *in silico*, neuroprotective

INTRODUCTION

Alzheimer's disease (AD) is a complex, multifactorial, progressive, irreversible, and devastating neurodegenerative disease, affecting 55 million people globally. AD is clinically characterized by the impairment of cognitive skills and memory. Senile plaques and neurofibrillary tangles are their pathological markers. Multiple factors are involved in the development of AD such as age, genetics, family

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history, lifestyle, and environment.[1] AD possesses significant medical and healthcare challenges, with the incidence expected to rise as life expectancy continues to increase globally.[2] Traditional pharmacological therapies have shown limited effectiveness in treating neurodegenerative diseases; thus, increasing attention is being directed toward plant-based compounds.[3,4] As per the WHO, 80% of the world's population uses phytochemicals for diseases management.[5] Plants such as *Withania somnifera* and *Panax ginseng* have demonstrated encouraging outcomes in AD.[6] The therapeutic potential of plant extracts and phytochemicals in treating neurodegenerative disorders can be attributed to their ability to modulate various biochemical and cellular processes, such as reducing oxidative stress, inhibiting neuroinflammation, and promoting neuronal regeneration.

Corchorus olitorius, commonly known as jute mallow or molokhia (ملوخية), belongs to the family Malvaceae, which is widely cultivated as a leafy vegetable and used in traditional medicine for centuries. The leaves are used to prepare thick, sticky soup or added to stews, and they are rich in essential nutrients, vitamins, and minerals, offering a range of medicinal benefits.[7] Worldwide, *C. olitorius* is cultivated as a green leafy vegetable among including Arab, Bangladesh, Brazil, Caribbean, China, India, Latin America, Mediterranean, Turkey, and many African countries. Tropical and subtropical climates help *C. olitorius* growth, reaching 2–4 m in height.[8] Besides its food uses, *C. olitorius* is used as a herbal remedy to manage numerous ailments such as fevers, enteritis, dysentery, chronic cystitis, anemia, female infertility, and pains in various parts of the world. Several studies have explored its biological activities of including the protective effect against gamma irradiation-induced hepatic damage,[7] radical scavenging and antioxidant,[8] and pro-estrogenic and anti-inflammatory effects against dimethylbenz[a]anthracene (DMBA)-induced breast cancer.[9]

The objectives of this study are to perform successive solvent extraction of *C. olitorius*, preliminary phytochemical investigations, gas chromatography-mass spectrometry (GC-MS) analyses, *in vitro* anti-inflammatory, antioxidant, and neuroprotective evaluation, ADME, and *in silico* studies. By integrating these methodologies, this study is designed to understand the pharmacological activities of *C. olitorius* phytochemicals, paving the way for the discovery of natural resources-driven novel therapeutic compounds.

MATERIALS AND METHODS

Reagents and chemicals

All the chemicals and reagents used in this research were of analytical grade. The chemicals used are hexane, ethyl acetate, ethanol, sodium nitrite, aluminum chloride, sodium hydroxide, ascorbic acid, diclofenac sodium, bovine serum albumin (BSA), dimethyl sulfoxide, and 3-(4,5-dimethylthiazol- 2-yl)-2,5-dimethyl tetrazolium bromide. These reagents were purchased from Hi-media, India.

Collection of plant material and solvent extraction

Fresh plants of *C. olitorius* were obtained from an authorized hypermarket and authenticated by a faculty of the natural product department. A voucher specimen (*C. olitorius*-035) was saved in the herbarium for reference. The leaves were washed in running tap water, dried under shade, and ground into a coarse powder. The sequential extraction was done using four solvents in ascending polarity order: hexane, ethyl acetate, ethanol, and water, in 1:10 ratio that is 250 mL of solvent and 25 g of powdered leaves. The leaves were macerated with hexane for 24 h under dark condition in an Erlenmeyer flask followed by filtration. The remaining biomass was dried at room temperature for 24 h, before macerating with a second solvent (ethyl acetate). These steps were repeated for all four solvents. The filtrates of hexane, ethyl acetate, and ethanol were concentrated using a rotary evaporator at 45°C to obtain a semisolid mass. The dried extracts were stored at −20°C for future use. The leaves macerated with water formed a thick gel-like consistency, where filtration was not possible. Therefore, the mixture was centrifuged at 6000 rpm (Hettich, D-78532, Germany) for 20 min. A thick viscous gel-like supernatant (presence of polysaccharides) was collected. Equal volume of ethanol was added with continuous stirring at 0°C in ice. The precipitated polysaccharides were removed by centrifugation at 6000 rpm for 20 min.[10] The supernatant was collected carefully, followed by filtration and evaporation, using a rotavapor. Then, an evaporated aqueous extract of *C. olitorius* (Aq-CO) was stored at −20°C for further use.

Preliminary phytochemical analysis

All four extracts of *C. olitorius* were qualitatively tested for the presence of phytoconstituents including alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, sterols, and tannins, using the procedure mentioned in earlier studies.[11,12]

GC-MS profiling of Aq-CO

The phytochemicals present in Aq-CO were identified using the GC/MS-QP2010S model. Helium was used as a carrier gas at a steady flow of 1 mL/min to separate the components after the extract was given in 1 µL aliquots in the sample injector. The injector was initially set at 250°C, the column temperature was set at 100°C, and the flow temperature was set to rise at a rate of 10°C per minute during the procedure. After adjusting the ultimate temperature to 280°C and running it for 5 min, the true separation was noticed at the 24th min. The in-built GC-MS spectral library (NIST) was used in the identification of compounds.[13]

In vitro studies

Anti-inflammatory activity

Protein denaturation method

With a few minor modifications, the protein denaturation procedure was used to assess the anti-inflammatory activity of Aq-CO using diclofenac as standard drug.[14] Five concentrations of sample and standard (50, 100, 150, 200, and 250 µg/mL) were prepared in phosphate-buffered saline (pH 6.4). A 50 µL of BSA was combined with 500 µL of sample or standard solution, and the mixture was incubated at 37°C for 20 min before being heated to 70°C for 5 min to cause protein denaturation. Following cooling, absorbances were recorded using distilled water as a blank at 660 nm. The percentage of protein denaturation inhibition was computed.

In vitro cyclooxygenase inhibition assay

The cyclooxygenase inhibitory capability of the produced Ca (II) meloxicam complex was assessed using the COX inhibitor screening assay kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA). In addition to heme, the COX enzyme was combined with several quantities of sample and standard (50, 100, 150, 200, and 250 µg/mL) and incubated for 10 min at 37°C. After adding arachidonic acid to each tube, they were incubated at the same temperature for an additional 2 minutes. The efficacy of compound was determined by calculating the IC_{50} value.[15] The percentage of inhibition was calculated as per the formula given below:

$$Percentage\,inhibition = \frac{[Activity\,of\,control - Activity\,of\,Test]}{Activity\,of\,control} \times 100$$

DPPH free radical scavenging ability assay

An antioxidant activity of Aq-CO was assessed using the DPPH assay. A 100 µL of sample and standard (ascorbic acid) in different concentrations (50, 100, 150, 200, and 250 µg/mL) were mixed with 1 mL of 0.2 mM DPPH solution. After shaking, they were incubated for 60 min in the dark at room temperature. Thereafter, absorbance was measured spectrophotometrically at 517 nm. The efficacy of the compound was determined by calculating IC_{50}.[16]

In vitro toxicity of aqueous extract using MTT assay

The standard colorimetric 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to assess the impact of Aq-CO on the viability of normal (non-cancerous) fibroblast L929 cell lines. Three cell line groups were used in the experiment: A positive control group that received cisplatin treatment, a negative control group that was left untreated, and test groups (Aq-CO). The assay was conducted following a previously published protocol. Cell mortality was calculated based on optical density readings, and IC_{50} values were calculated.[17]

In vitro neuroprotective activity

The SK-S-NH neuroblastoma cell lines were used to assess the neuroprotective efficacy of Aq-CO utilizing the "MTT pretreatment method." After being seeded on 96-well plates at a density of 10^4 cells/well, these cells were cultured for 24 h at 37°C and 5% CO_2 in a CO_2 incubator. Cell lines were treated with different concentrations of Aq-CO (50, 100, and 150 µg/mL) and incubated for 24 h under identical conditions. After aspirating out the extract-containing medium, cells were challenged with 200 µL of 1 mM trimethyltin chloride (TMT) and incubated for 3 h. Following incubation, MTT was applied, and a microplate reader was used to check for formazan production.[18,19]

In vitro acetylcholine esterase (AChE) activity assay

Acetylcholine catalyzes the hydrolysis of acetylcholine to form choline, which further reacts with dithio p-nitrobenzoic acid to form 5-mercapto-nitrobenzoic acid (TNB). TNB has an absorption peak at 412 nm. Moreover, the activity of AchE is calculated by measuring the increasing rate of absorbance at 412 nm. The AChE activity assay kit (catalog No: 250202) was used to assess the enzyme activity on SK-N-SH cell lines using 96-well microplates. The expression of AChE activity was analyzed using methods described in earlier studies.[20,21]

In silico molecular docking studies

In this study, the SwissADME tool was used to predict the pharmacokinetics and drug likeliness of major active phytoconstituents identified in Aq-CO using GC-MS (http://www.swissadme.ch/). The SMILES (simplified molecular input line entry system) were imported into the SwissADME tool from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Details about pharmacokinetics, physicochemical properties, Lipinski violations, and drug likeness were retrieved. The structures of phytocompounds and the standard drugs were drawn using ChemDraw Ultra 18.0 and energy-minimized with ChemBio3D Ultra 18.0. These ligands were then used in docking simulations with AutoDock Vina (https://www.swissdock.ch/). Target protein structures including BSA (PDB: 4F5S), COX-2 (PDB:6COX), GABAA (PDB: 6X3X), and Alpha18synuclein (PDB: 1XQ8) were obtained from the protein data bank, with water molecules removed and polar hydrogens and Kollman charges added. Grid boxes were defined using the BMGL tools interface, and docking was performed on Windows 10. Ligand–protein interactions were visualized using Discovery Studio 2020.

Statistical analysis

The results were described as mean ± standard deviation. An independent student's *t*-test was used to perform statistical analysis, by considering a $P < 0.05$ as significant.

RESULTS

Phytochemical analysis of solvent extracts of the *C. olitorius*

The presence of fatty acids, sterols, and oils was noted among the hexane and ethyl acetate fractions. The ethanolic fraction depicted the presence of flavonoids, phenols, terpenoids, sterols, fatty acids, and oils, whereas an aqueous extract of *C. olitorius* (Aq-CO) showed the presence of alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and sterols. Based on the presence of rich phytoconstituents, Aq-CO was selected for the identification of active constituents and *in vitro* pharmacological evaluations.

GC-MS spectral analysis of Aq-CO

The GC-MS analysis of aqueous extract of *C. olitorius* demonstrated 19 peaks indicating the presence of numerous phytoactive compounds [Figure 1]. The major compounds identified were Solasonine, dl-Alanyl-l-alanine, Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)- and 1-(3,4-Methylenedioxybenzylidene) semicarbazide [Table 1].

In vitro anti-inflammatory activity

For initial concentration 50 µg/mL of standard and Aq-CO, the percentage of inhibition of protein denaturation was found to be 43.98 ± 1.3784 and 8.57 ± 0.7508, respectively. Further, for higher concentrations (250 µg/mL), the percentage inhibition increased to 80.49 ± 0.8331 and 61.98 ± 0.9676, respectively. The IC_{50} values of standard and Aq-CO were 78.31 and 199.81 µg/mL, respectively. Likewise, the COX-II assay demonstrated concentration-dependent inhibition of cyclooxygenase-II. The 50 µg/mL concentration of standard and Aq-CO revealed 44.99 ± 1.2279 and 9.85 ± 2.1903 percentage inhibition of COX-II, respectively, whereas 250 µg/mL indicated 88.34 ± 1.1633 and 67.53 ± 0.9920 percentage COX-II inhibition, respectively. The IC_{50} values for mefenamic acid and Aq-CO were 76.40 and 176.21 µg/mL [Table 2]. The unpaired Student's t-test did not show significant variations ($P > 0.05$) among standard drug and Aq-CO.

In vitro antioxidant activity

Although the standard drug has outperformed the Aq-CO at all concentrations, comparatively significant antioxidant activity was observed in Aq-CO, indicating its valuable role in radical scavenging. The IC_{50} for standard and Aq-CO was observed to be 61.35 and 190.59 µg/mL, respectively [Table 2]. The unpaired Student's t-test demonstrated moderate significant variations ($P = 0.0364$) among standard and Aq-CO.

In vitro cytotoxicity of Aq-CO using MTT assay

The results reveal the concentration-related cytotoxicity of Aq-CO with IC_{50} value of 182.75 µg/mL. The first three concentrations of 50, 100, and 150 µg/mL demonstrated a cell viability of more than 50% whereas highest concentrations (250 µg/mL) showed the least cell viability of 28.4 3 ± 0.5488. Nevertheless, these cell viability percentages were better compared to standard drug cisplatin, which showed the cell viability of 11.19 ± 0.4390 at dose of 15 µg/mL [Table 3]. The microscopic morphological observation indicated a greater number of dead cells (black circles), shrunken, irregular, and dispersed cells in cisplatin and high dose Aq-CO (250 µg) treated cell lines compared untreated and low doses Aq-CO treated cell lines where regular, elongated, uniform, even configurated and high number viable cells (white circles) were observed [Figure 2].

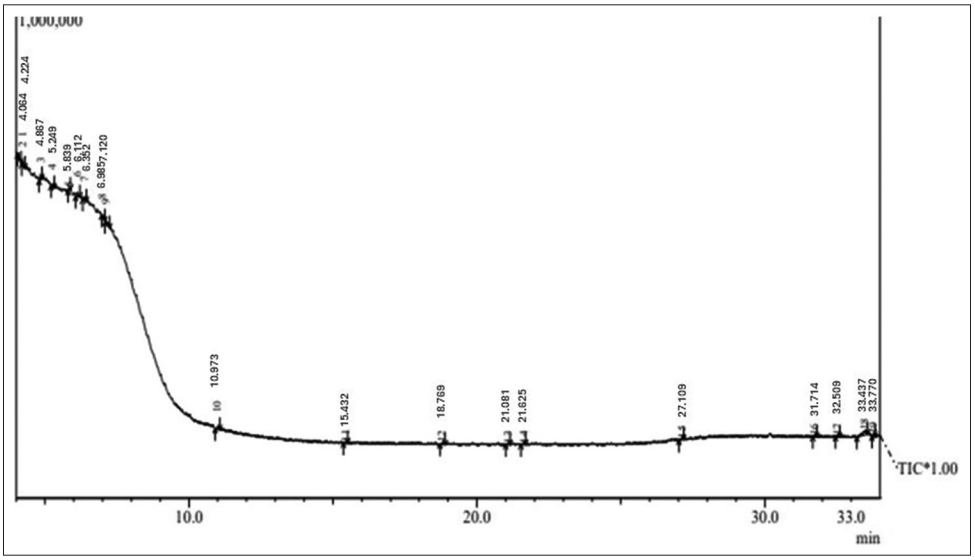

Figure 1: Gas chromatography-mass spectrometry spectral peaks of aqueous extract of *C. olitorius* leaves

Table 1: Compounds identified in the aqueous extract of *C. olitorius* using GC-MS

Peak no.	Retention time	Compound name	m/z	Molecular Formula	Mol wt g/mol
1	4.064	Formic acid, ethenyl ester	39.95	$C_3H_4O_2$	72.06
2	4.224	3-Butyn-1-ol	39.95	C_4H_6O	70.09
3	4.867	Borane carbonyl	39.95	CH_3BO	41.85
4	5.249	cis-Aconitic anhydride	39.95	$C_6H_4O_5$	156.09
5	5.839	Cyclopropene	40.00	C_3H_4	40.06
6	6.112	Propyne	39.95	C_3H_4	40.06
7	6.352	Cyclopropene	39.95	C_3H_4	40.06
8	6.985	dl-Alanyl-l-alanine	40.00	$C_6H_{12}N_2O_3$	160.17
9	7.120	3-Butyn-1-ol	39.95	C_4H_6O	70.09
10	10.973	Phenol, o-(propylsulfanyl)-	40.00		
11	15.432	(2-Benzyl-5-nitro-2H-pyrazol-3-yl)-(2,3-dihydro-indol-1-yl)-methanone	40.00	$C_{19}H_{16}N_4O_3$	348.4
12	18.769	Solasonine	277.00	$C_{45}H_{73}NO_{16}$	884.1
13	21.081	2-Pyrrolidinethione	40.00	C_4H_7NS	101.17
14	21.625	Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)-	69.00	$C_{16}H_{19}N_3O_4$	317.34
15	27.109	Pyrazole, 5-cyclohexylamino-3-methyl-4-nitro-	41.10	$C_{10}H_{16}N_4O_2$	224.26
16	31.714	1-(3,4-Methylenedioxybenzylidene) semicarbazide	44.05	$C_9H_9N_3O_3$	207.19
17	32.509	N-[(Benzoyl)(p-toluidine) methyl] acetamide	44.05	$C_{17}H_{18}N_2O_2$	282.34
18	33.437	Malonic acid di-tert-butyl ester, 2-(3,6-dichloropyridazin-4-yl)-	57.00	$C_{15}H_{20}Cl_2N_2O_4$	363.2
19	33.770	Alpha.-d-Glucopyranosyl benzenesulfonate	73.05	$C_{12}H_{16}O_8S$	320.32

m/z- mass-to-charge ratio. Retention time in minutes

Table 2: Anti-inflammatory and antioxidant activities of aqueous extract of *Corchorus olitorius* (Aq-CO)

Concentration in µg/mL	Anti-inflammatory activity				Antioxidant activity	
	Protein denaturation assay		COX-II inhibitory assay		DPPH assay	
	Aspirin (STD)	Aq-CO	Mefenamic acid (STD)	Aq-CO	Ascorbic acid (STD)	Aq-CO
50	43.98±1.3784	8.57±0.7508	44.99±1.2279	9.85±2.1903	43.84±0.7187	16.30±0.4660
100	55.59±1.2152	25.05±0.3264	53.75±1.3012	29.02±0.7986	61.16±0.5986	28.31±1.6330
150	62.81±0.6621	37.41±1.1307	66.21±1.1517	47.52±1.0473	72.89±0.9435	37.21±0.8532
200	70.86±0.8933	50.86±1.1902	79.25±1.1394	58.15±0.7019	82.20±0.5797	50.68±1.0164
250	80.49±0.8331	61.98±0.9676	88.34±1.1633	67.53±0.9920	89.52±0.5587	67.14±1.5010
IC_{50}	78.31	199.81	76.40	176.21	61.35	190.59

The results are expressed as Mean±standard error. IC50: 50% inhibitory concentration.

In vitro neuroprotective activity of Aq-CO on SK-N-SH cell lines

The results showed excellent protective activity (72.40 ± 1.6815) at an initial concentration of 50 µg/mL. Further, as the concentrations of Aq-CO increased, the protective nature decreased. For 100 and 150 µg/mL, the percentage cell viability was 45.20 ± 1.7804 and 27.10 ± 1.2858, respectively. For positive control (TMT alone treated cells), the percentage of cell viability was 9.69 ± 1.00880 [Table 4]. Microscopic cell morphology reveals normal and elongated-shaped cells (white arrows) in control and low-dose Aq-CO-treated cell lines. Conversely, only TMT-treated cells demonstrated a greater number of disfigured and dead cells (black arrows) with distorted morphology [Figure 3].

In vitro AChE activity assay

The Aq-CO demonstrated moderate AChE activity levels, ranging from 0.021648 to 0.043521 U/10^4 cells as the

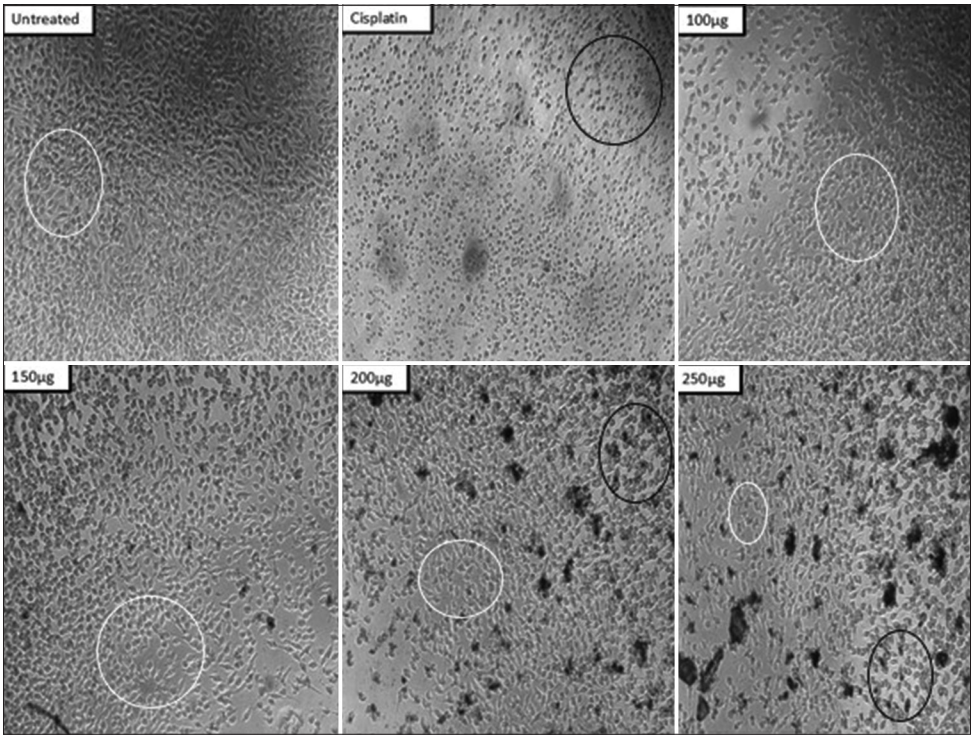

Figure 2: MTT assay of Aq-CO in non-cancerous L929 fibroblast cell lines showing cytotoxicity and viability (white circles indicate high number of viable cells and black circles indicate low number of viable cells)

Table 3: MTT assay depicting cytotoxicity of Aq-CO on L929 cell line

Treatments	Concentration in µg	Percentage of cell viability
Aq-CO	50	86.38±1.8660
	100	73.87±1.4270
	150	60.48±1.6465
	200	47.42±0.8781
	250	28.43±0.5488
Cisplatin	15	11.19±0.4390

The results are expressed as mean±standard error

Table 4: Neuro-protective activity of Aq-CO on SK-N-SH cell lines

Treatments	Concentration in µg	Percentage of cell viability
Aq-CO	50	72.40±1.6815
	100	45.20±1.7804
	150	27.10±1.2858
TMT	15	9.69±1.00880

The results are expressed as mean±standard error

Table 5: AChE enzyme activity in SK-N-SH neuro cell line treated with Aq-*C. olitorius*

Concentration of Aq-*C. olitorius* extract	AChE activity (U/10^4 cells)
Control (untreated)	0.098356
20 µg/mL	0.021648
40 µg/mL	0.025932
60 µg/mL	0.031119
80 µg/mL	0.039688
100 µg/mL	0.043521

concentration increased. At 20 µg/mL, the AChE enzyme activity in SK-N-SH neuroblastoma cell lines was found to be 0.021648 U/10^4 cells. As the concentration of Aq-CO increased, the activity of AChE enzyme also increased, yet enzyme overactivation is less and cholinergic function is still preserved [Table 5] compared to untreated cell lines where AChE activity was 0.098356.

Predicted ADME and drug likeness of the phytocompounds found in Aq-CO

The four major active compounds identified through GC-MS were selected for ADME and *in silico* models [Figure 4]. All of these showed higher GI absorption with no permeation into the blood–brain barrier (BBB). These compounds adhered to Lipinski's rule except solasonine which violated this rule in three aspects, namely mol. Wt of 884.1 (> 500), number of hydrogen bond acceptors 17 (>10), and donors 10 (>5). The results of ADME are depicted in Table 6.

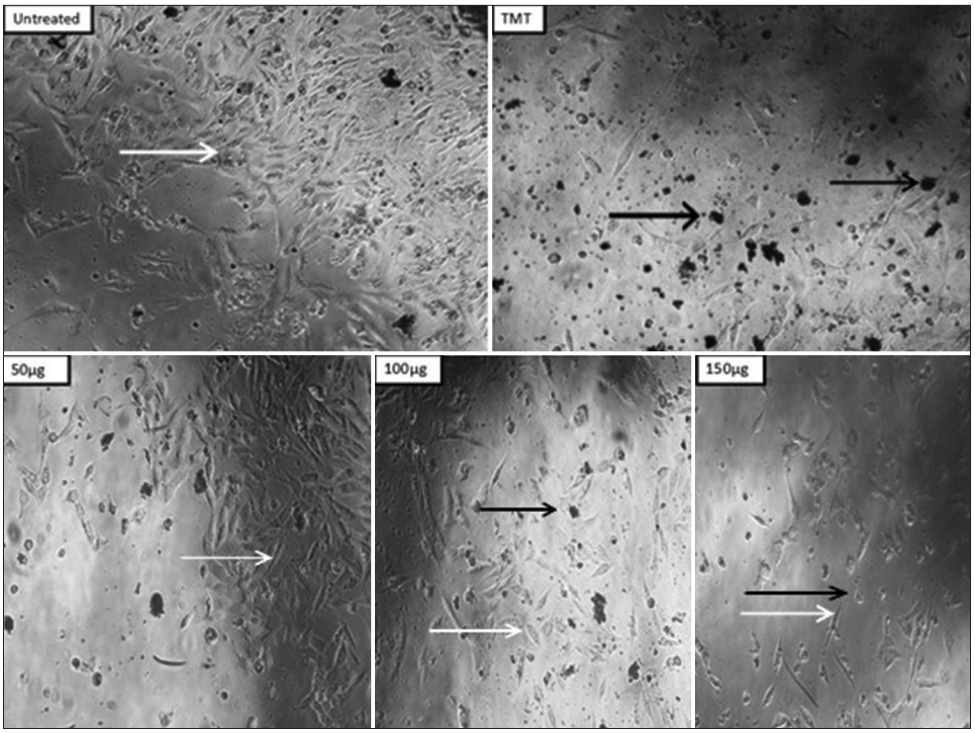

Figure 3: Neuroprotective activity of Aq-CO in SK-N-SH neuroblastoma cell lines showing morphological variations (high number of viable cells-indicated by white arrows and low number of viable cells indicated by black arrows)

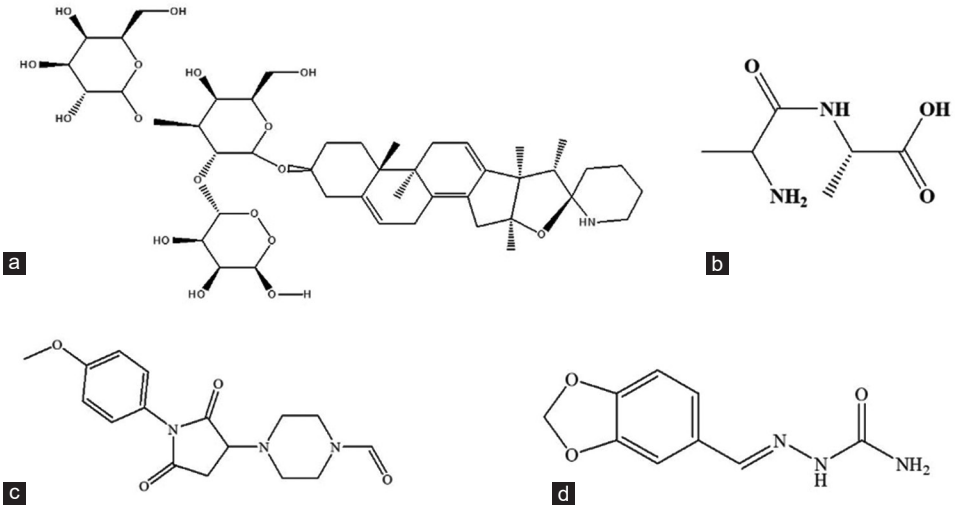

Figure 4: Structure of major compounds identified in GC-MS. (a) Solasonine, (b) dl-Alanyl-l-alanine, (c) Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)-, (d) 1-(3,4-Methylenedioxybenzylidene)semicarbazide

Table 6: Predicted ADME and drug likeness of the phytocompounds found in Aq-CO

Compound name	Molecular formula	Molecular weight	Lipinski violation	no. H bond acceptor\donors	GI absorption	BBB permeant
dl-Alanyl-l-alanine	$C_6H_{12}N_2O_3$	160.17	0	4/3	HIGH	NO
Solasonine	$C_{45}H_{73}NO_{16}$	884.1	3	17/10	HIGH	NO
Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)-	$C_{16}H_{19}N_3O_4$	317.34	0	5/0	HIGH	NO
1-(3,4-Methylenedioxybenzylidene) semicarbazide	$C_9H_9N_3O_3$	207.19	0	4/2	HIGH	NO

In silico studies of phytochemicals of Aq-CO with inflammatory and neuronal proteins

Two inflammatory (4F5S and 6COX) and neuronal (6X3X and 1XQ8) related target proteins were used to carry our molecular docking studies. Solasonine has demonstrated strong binding affinity toward BSA and GABA-A proteins with binding energies of −8.9 and −7.4 kcal/mol, respectively. For COX-II, the compound pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl) proven to be potent with the binding energy −7.9 kcal/mol [Table 7]. However, solasonine showed moderate binding affinity toward alpha-synuclein with a binding energy of −5.3 kcal/mol.

DISCUSSION

Neurodegeneration poses a major public health challenge, impacting millions of people globally through various neurodegenerative diseases. Developing effective treatments for these conditions is critically important. One promising strategy involves investigating plant-derived natural compounds, which are known for their diverse therapeutic properties, including neuroprotective benefits.[22,23]

The plant extract's neuroprotective effects are likely linked to its abundant phytochemical content, which is known to contain numerous bioactive compounds including flavonoids, polyphenols, alkaloids, and saponins which possess significant antioxidant, anti-inflammatory, and anti-apoptotic activities. Numerous studies have demonstrated the potential therapeutic benefits of plant extracts in the management of neurodegenerative disorders.[22-25]

In the present study, the GCMS analysis of Aq-CO has shown the presence of numerous compounds. The Aq-CO has shown a notable anti-inflammatory activity, especially the higher concentration (250 µg/mL) produced results (61.98 ± 0.9676) comparable with standard aspirin (80.49 ± 0.8331). Earlier research emphasized the crucial role of COX-II in several diseases, such as AD, colorectal cancer, and tumor-induced angiogenesis.[26] The present findings indicate potential COX-II inhibitory activity of Aq-CO. Recent studies have demonstrated the application of DPPH assay in evaluating the antioxidant properties of numerous plant extracts.[27] This study highlights concentration-dependent antioxidant potential of Aq-CO comparable with standard ascorbic acid. The first three concentrations of Aq-CO (50, 100, and 150 µg/mL) demonstrated a cell viability of more than 50%, indicating its safety and biocompatibility. Morphological observation of cell lines has supported these findings. A SK-N-SH neuroblastoma cell lines were used to assess the neuroprotective activity of Aq-CO against TMT-induced neurotoxicity. The initial concentration of Aq-CO (50µg/mL) depicted excellent neuroprotective activity (72.40 ± 1.6815). Our previous study demonstrated the neuroprotective ability of green-synthesized gold nanoparticles using *C. olitorius* leaves. However, in the present study, compound identification is performed using GC-MS and investigated for *in vitro*, ADME, and *in silico* studies to ascertain the compound's ability and pharmacotherapeutic effects.[28] At present, for the treatment of neurodegenerative disease, AChE inhibitors are one of the main pivotal classes of drug applied and many preclinical and clinical studies indicate the prime importance of AChE.[29] The increased activity of AChE adversely affects neurons. In our study, the Aq-CO has demonstrated a beneficial effect in reducing the expression of AChE activity. Lower concentrations of extract (20 µg/mL) were found to be most effective; however, *in vivo* studies would reflect actual AChE activity.

The ADME characteristic of phytochemicals provides the predicted details about the bioavailability, tissue distribution, metabolic stability, and elimination pathways, all of which are essential in determining the potential clinical efficacy and safety of these natural compounds.[30] The four major phytochemicals identified through GC-MS were selected for ADME analysis, namely. Except for solasonine, all other compounds adhered to Lipinski's rule of five indicating drug-likeness properties. However, research indicated that solasonine exhibits neuroprotective properties. Solasonine suppress the TLR4/MyD88/NF-κB pathway and activating AMPK/Nrf2/HO-1 pathway in cerebral ischemia-reperfusion injury.[31] Another study stated that solasonine relieves sevoflurane-induced neurotoxicity through activating the AMP-activated protein kinase/FoxO3a pathway.[32] Molecular docking studies enable researchers to simulate how phytochemicals interact with target proteins such as BSA, COX-II, GABA-A receptor, and alpha-synuclein to uncover potential binding modes, binding affinities, and the molecular interactions responsible for their biological effects.[27] By

Table 7: Docking results of phytochemicals of Aq-CO with inflammatory and neuronal proteins

Compound name	Binding energy (kcal/mol)			
	BSA (4F5S)	COX-II (6COX)	GABA-A (6X3X)	alpha-synuclein (1XQ8)
dl-Alanyl-l-alanine	−5.0	−5.1	−4.3	−3.2
Solasonine	−8.9	4.4	−7.4	−5.3
Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)-	−7.3	−7.9	−6.0	−3.9
1-(3,4-Methylenedioxybenzylidene) semicarbazide	−6.1	−6.8	−6.2	−3.9

utilizing the knowledge obtained from ADME and molecular docking studies, researchers can enhance the drug-like characteristics of phytochemicals, pinpoint promising lead compounds, and accelerate the drug development process.

CONCLUSION

This research study highlights the notable pharmacological benefits of phytochemicals found in *C. olitorius*. GC-MS analysis showed the presence of numerous beneficial phytochemicals. The aqueous extract of *C. olitorius* leaves demonstrated potential *in vitro* antioxidant, anti-inflammatory, decreased AChE expression, and neuroprotective potentials. ADME analysis and molecular docking studies provided insights into the predicted pharmacokinetic, pharmacodynamic, drug likeness, and target protein interaction possibilities of phytochemicals present in Aq-CO.

LIMITATIONS

This study has few limitations. The present study evaluated the *in vitro* pharmacological activities of the plant extract. ADME and *in silico* studies are performed briefly on selected major phytochemicals. We recommend extension of this research including *in vivo* pharmacological experiments, behavioral models, anti-inflammatory, antioxidant activities, and detailed network pharmacology with molecular docking study of potential phytochemicals.

COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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