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A comprehensive investigation of *Clerodendrum Infortunatum* Linn. using LC-QTOF-MS/MS metabolomics as a promising anti-alzheimer candidate

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Alzheimer's disease (AD) poses a global health challenge, demanding innovative approaches for effective treatments. *Clerodendrum infortunatum* Linn. (Lamiaceae) is a shrub traditionally used as a medicinal plant to treat inflammation, skin diseases, and bronchitis. This study aims to identify the main bioactive metabolites in *C. infortunatum* using LC-QTOF-MS/MS and investigate its potential in protecting against cognitive decline in rats with scopolamine-induced AD disease. Metabolite profiling was performed on the methanol extract of the plant's aerial parts using LC-QTOF-MS/MS. The inhibitory activity of the acetylcholinesterase enzyme was measured *in vitro*. To evaluate the cognitive effects, the methanol extract was orally administered at three doses (100, 200, and 400 mg/kg) to scopolamine-induced AD rats, and their cognitive functions were assessed using the novel object recognition test. Additionally, acetylcholinesterase enzyme activity, as well as the levels of acetylcholine, dopamine, noradrenaline, glutathione, malondialdehyde, tumor necrosis factor- α , interleukin-1 β , and amyloid- β in the rat hippocampus, were measured using ELISA, followed by histopathological evaluation. A total of 79 metabolites, spanning various chemical classes, such as organic acids, phenolic acids, phenylpropanoids and phenylethanoids, flavonoids, coumarins, other phenolics, and fatty acids and their derivatives, were identified. The results showed that the extract promoted enhanced cognitive functions in the novel object recognition test. Scopolamine administration significantly altered the acetylcholinesterase enzyme activity and biomarker levels in the rat's hippocampus. However, treatment with *C. infortunatum* at 200 and 400 mg/kg almost restored these neurotransmitter levels to normal, which was further confirmed by histopathological analysis. This study demonstrates the therapeutic potential of *C. infortunatum* in mitigating cognitive decline in AD, with its first metabolite profiling revealing a range of bioactive compounds. The extract improved cognitive function in scopolamine-induced AD rats, restored acetylcholinesterase activity, normalized neurotransmitter levels, and reduced oxidative stress and inflammation. These findings suggest that *C. infortunatum* is a promising candidate for the development of natural therapies targeting AD.

Keywords Alzheimer's disease, Anticholinesterase, *Clerodendrum*, LC-MS/MS, Metabolomics, Neurotransmitters

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Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
A β	Amyloid- β
Ag	Aglycon
<i>C. infortunatum</i>	<i>Clerodendrum infortunatum</i>
DA	Dopamine
DI	Discrimination index
DMSO	Dimethyl sulfoxide
DON	Donepezil
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
GSH	Glutathione
IP	Intraperitoneal injection
IC	Inhibition concentration
IL-1 β	Interleukin-1 β
LC-QTOF-MS/MS	Liquid chromatography-quadrupole time-of-flight tandem mass spectrometry
LD	Lethal dose
MDA	Malondialdehyde
NA	Noradrenaline
NOR	Novel object recognition
R _t	Retention time
ROS	Reactive oxygen species
SCOP	Scopolamine
SCOP-induced AD	Scopolamine-induced Alzheimer's disease
TNF- α	Tumor necrosis factor- α

Alzheimer's disease (AD) has become one of the most significant global health concerns of this century, affecting millions of individuals worldwide. According to recent predictions, the number of people with elderly dementia related to AD is expected to reach around 152 million by 2050¹. AD is a neurological disorder characterized by memory loss and deterioration of several mental faculties². Individuals suffering from AD commonly exhibit neurofibrillary tangles, amyloid plaques of amyloid- β (A β) protein, and a reduction in neuronal synapses within the brain². Moreover, age-related neurodegeneration and cognitive decline are believed to be significantly influenced by oxidative stress, which is defined as an imbalance in the generation of radical reactive oxygen species (ROS) and antioxidative defense. One of the early events in AD is neuro-inflammation, which likely precedes the manifestation of A β deposits³. The intricate interplay among these factors contributes to the observed deterioration in both cognitive and motor abilities⁴. The main trigger of AD is dysfunction in the cholinergic system, which includes malfunctioning neurotransmitters, receptors, and cholinergic neurons, resulting in diminished cognitive function⁵. Consequently, elevating acetylcholine (ACh) levels stands as a key objective in AD treatment. This is accomplished through the inhibition of the acetylcholine esterase (AChE) enzyme⁶. Presently, symptomatic treatments for AD, such as rivastigmine, galantamine, and donepezil (DON), predominantly target memory enhancement by inhibiting the AChE enzyme. However, these drugs are often associated with undesirable side effects⁴. Additionally, AD is a multifaceted condition with multiple contributing factors, rendering the “one change, one disease, one drug” approach ineffective⁷. Therefore, there exists a pressing need for novel, natural medications that can decelerate or halt AD progression while minimizing the side effects commonly associated with these synthetic drugs.

Plant-based remedies have shown promise in effectively slowing down the progression and alleviating symptoms of AD. Particularly interesting are plants such as *Curcuma longa*, *Centella asiatica*, *Ginkgo biloba*, *Zingiber officinale*, *Allium sativum*, and *Clerodendrum infortunatum* (*C. infortunatum*), which present potential antioxidant, anti-inflammatory, anticholinesterase, and anti-amyloidogenic properties^{7,8}. *C. infortunatum*, a shrub in the Lamiaceae family commonly found in India, possesses various medicinal properties. It has been reported to exhibit free radical scavenging and wound healing effects⁹. Since some research suggests a connection between A β protein toxicity and an increase in ROS, these antioxidant properties may be essential in combating oxidative stress, a critical trigger in AD¹⁰. However, there is limited research on *C. infortunatum* and its association with neurological deterioration, but some authors have demonstrated promising memory enhancing effects¹¹. Additionally, there is limited phytochemical data available regarding the complete profile of *C. infortunatum* constituents, including potential bioactive metabolites. Presently, mass spectrometers with quadrupole time-of-flight (QTOF) mass analyzers are widely utilized for the untargeted metabolite profiling of natural products as they can acquire MS and tandem MS (MS/MS) spectra with excellent mass accuracy and resolution. Accordingly, recent studies have shown the successful application of liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (LC-QTOF-MS/MS) for the global metabolite profiling of constituents in medicinal and edible plants¹².

In this study, untargeted LC-QTOF-MS/MS metabolomics was conducted to profile the methanol extract of the aerial parts of *C. infortunatum*. Subsequently, the anti-AD potential of the methanol extract was investigated in vitro on the AChE enzyme and in vivo in scopolamine (SCOP)-induced AD rats. SCOP was administered to induce AD in rats, as it is known to increase AChE enzyme activity and ROS, accumulate A β protein, and disrupt neurotransmitters¹⁰. The rats underwent evaluation with the novel object recognition (NOR) test to evaluate their memory and cognitive abilities. Additionally, AChE enzyme activity and various biomarker levels were measured

in the rat hippocampus, including neurotransmitters (ACh, noradrenaline (NA), and dopamine (DA)), antioxidant parameters (glutathione (GSH) and malondialdehyde (MDA)), anti-inflammatory parameters (tissue necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β)), and an AD marker, A β protein. Finally, the rat brains were subjected to histopathological assessment. To the best of our knowledge, this is the first comprehensive LC-QTOF-MS/MS analysis conducted on *C. infortunatum* aerial parts. The proposed integrated approach unveils the anti-AD potential of *C. infortunatum* and identifies the metabolites responsible for such functionality. These findings hold promise for the future development of novel dietary supplements, nutraceuticals, or drugs for the amelioration and prevention of AD.

Materials and methods

Plant material and preparation of plant extracts

The aerial parts of *C. infortunatum* were collected in March 2021 from Mazhar Botanical Garden, Giza, Egypt, and authenticated by Agriculture Engineer Therese Labib, a plant taxonomy consultant of the Ministry of Agriculture (Giza, Egypt). The plant material was collected with permission in compliance with national guidelines from the Agriculture Research Center, Giza, Egypt at “9 Cairo University Road, Giza District, Giza Governorate”. Samples of the plant material were deposited in the herbarium of the Faculty of Pharmacy, Cairo University, Cairo, Egypt (sample No.10.3.2021). Following air-drying, the aerial parts were ground into powder using a powder grinder. Dried powder (5 kg) was repetitively macerated with a methanol-water mixture (85:15, v/v) until exhaustion (25 L) at room temperature. All extracts were filtered using Whatman No. 1 filter paper after maceration. The solvent was evaporated in a rotary evaporator at 45 °C (BUCHI Rotavapor R-300, Cole-Parmer, Vernon Hills, USA), resulting in 500 g of total extract, which was stored at 4 °C. For *in vitro* analysis, 10 mg of the extract was dissolved in 1 mL of dimethyl sulfoxide (DMSO). The resulting DMSO stock solution was then diluted to the required concentrations, maintaining a final DMSO concentration of less than 0.1% throughout the experiment to minimize any potential solvent effects on the extract's bioactivity. The use of DMSO effectively dissolves plant extracts that may not completely dissolve in water or other solvents. Three biological replicates were prepared and extracted in parallel under the same conditions.

Drugs and chemicals

Methanol was purchased from Piochem Company for Pharmaceuticals and Chemicals (Cairo, Egypt). SCOP and DON were provided by Merck (Darmstadt, Germany) and Pfizer (Cairo, Egypt), respectively, and both were prepared in saline solution. Formalin, DMSO, paraffin, xylene, eosin, and hematoxylin stains were purchased from Sigma-Aldrich (Burlington, USA). Formic acid ($\geq 95.0\%$), acetonitrile, water, and methanol (LC-MS grade) were supplied by Merck (Darmstadt, Germany).

LC-QTOF-MS/MS metabolite profiling

For LC-QTOF-MS/MS analysis, the samples were prepared by dissolving 10 mg of the dried *C. infortunatum* methanol extract in 1 mL of methanol, assisted by sonication. The resulting solutions were centrifuged at 13,000 $\times$ g for 10 min, and the supernatants were filtered through a 0.22 μ m syringe nylon filter. The LC-QTOF-MS/MS analyses were carried out using a 1260 Infinity liquid chromatograph coupled to a 6546 LC/QTOF mass spectrometer with an orthogonal electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Metabolite profiling in both negative and positive ESI mode involved injecting 5 μ L of sample into a Zorbax SB-C18 (5 μ m, 150 mm $\times$ 2.1 mm) column using an optimized acetonitrile: water gradient (both with 0.1% v/v of formic acid). The chromatographic, MS, and MS/MS conditions, as well as data processing, were conducted as described in previous works¹². Detected compounds were annotated as metabolites considering their retention time (R_t), accurate molecular mass, predicted molecular formula, and MS/MS spectra, through comparison with specific literature and various free databases, such as the Human Metabolome Database (<http://www.hmdb.ca/>), PubChem (<https://www.pubchem.ncbi.nlm.nih.gov/>), ChemSpider (<https://www.chemspider.com/>), and the Phytochemical Dictionary of Natural Product Database (<https://dnp.chemnetbase.com/faces/chemical/ChemicalSearch.xhtml>). Consequently, the annotated metabolites were identified at a high confidence level (probable structure, level 2: MS, MS/MS, and bibliography/database¹³).

In vitro AChE enzyme activity

The AChE enzyme activity of the methanol extract was assessed colorimetrically using an AChE enzyme inhibitor screening kit (BioVision, K197-100, Waltham, USA). Following a previously described method¹⁴, the AChE enzyme and the colorimetric substrate (5,5'-dithiobis (2-nitrobenzoic acid) solutions provided with the kit were mixed with 10 μ L of the extract at different concentrations (0.1, 1.0, and 10.0 μ g/mL in Tris-HCl buffer (pH 8.0)) in a 96-well microplate. The mixture was then incubated for 10–15 min at room temperature without exposure to light. Additionally, an enzyme-free blank and a DON (10 mM) positive standard were prepared. Absorbance was measured in all cases at 412 nm. Each analysis was carried out in triplicate, and the results were recorded as IC₅₀ (the concentration inhibiting 50% of the target enzyme). Data were expressed as mean $\pm$ SD ($n = 3$).

In vivo anti-AD activity

Animals

All male adult Wistar albino rats (200–250 g) were supplied by the National Research Centre (Giza, Egypt). They were housed in polypropylene cages under controlled environmental conditions of 55% $\pm$ 5% humidity and 25 $\pm$ 2 °C temperature. Before starting the study, the animals were given *ad libitum* access to a commercially available rat regular pellet diet and water for 7 days. The experiments adhered to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No 85–23,

2011). Approval was granted by the Research Ethics Committee of the Faculty of Pharmacy of Cairo University (approval code: MP (3118)).

Acute toxicity study

The lethal dose (LD_{50}) of *C. infortunatum* extract (the concentration causing death to 50% of the tested group of animals) was determined in accordance with the guidelines outlined by the Organization for Economic Co-operation for Development (OECD, Test No 420, 2002). Thirty-six Rats, which had fasted overnight, were divided into six groups of six individuals. The extract, suspended in saline, was orally administered to the rats at doses of 125, 250, 500, 1000, and 2000 mg/kg body weight. The normal group was maintained under the same conditions and received a vehicle (saline). The animals were monitored over 48 h for signs of toxicity, and occurrences of death were recorded as mortality rates. Throughout the investigation, the rats had ad libitum access to food and water.

SCOP-induced AD study

The SCOP approach used in this study to induce AD was adapted from previous research¹⁰. Forty-eight male Wistar albino rats were divided into six groups of eight individuals, and the study extended over nine consecutive days. The healthy normal group (Group 1) received only saline. AD was induced in the remaining groups by intraperitoneal injection (IP) administration of SCOP at 5 mg/kg/day (daily for 9 days). Group 2 served as the SCOP-induced AD control group. The groups receiving the prophylactic treatments, after 30 min of SCOP, were orally administered the standard drug DON (2.5 mg/kg/day, daily for 9 days) or different doses of the methanol extract (100, 200, and 400 mg/kg/day, daily for 9 days). SCOP, DON, and the extracts were dissolved in saline using sonication to ensure complete dissolution. On day 8, the groups of rats were habituated and familiarized to the novel object (phases 1 and 2 of the NOR test). Memory was assessed on day 9 (phase 3) and rats were euthanized using thiopental (50 mg/kg; IP). The hippocampi of the eight rats from the group were isolated and homogenized in 10% phosphate-buffered saline for the enzyme-linked immunosorbent assay (ELISA) measurement of different biomarkers. The following biomarkers were measured: the memory function parameters (AChE enzyme activity and neurotransmitters ACh, DA, and NA), the oxidative stress parameters (GSH and MDA), the anti-inflammatory parameters (TNF- α and IL-1 β), and the AD marker, A β protein. Rat ELISA kits from different manufacturers were used for the determination of the biomarkers, including assay kits from Abcam (Cambridge, UK) for AChE enzyme activity and ACh; from MyBioSource (San Diego, USA) for DA, NA, TNF- α , IL-1 β , and A β ; and from Lifespan Biosciences (Shirley, USA) for GSH and MDA. Finally, the brains were histopathologically evaluated. They were dissected out and kept in 10% neutral buffered formalin. After fixation, tissues were processed in alcohol and xylene. They were embedded in paraffin 5 μ m thick, cut, and stained with hematoxylin and eosin¹⁵.

In the NOR test, a black open rectangular wooden box (45 $\times$ 65 $\times$ 45 cm) was used. The test was performed in a room with low noise levels and constant illumination. Two different opaque cubes served as familiar objects, and a pink pyramid was introduced as the novel object. These objects were 6 cm high and sufficiently heavy to prevent the rats from moving them. In addition, the objects were placed in opposite corners, positioned 10 cm away from the walls.

The NOR test involved three phases: Phase 1 (habituation phase): rats were individually allowed to explore the empty field for 5 min. Phase 2 (familiarization phase): rats were released into the open field and allowed to explore for 3 min two identical familiar objects (a_1 and a_2). Phase 3 (test phase): this phase was performed with the novel object (b) and the familiar object (a). The objects and open field were cleaned with 70% ethanol after each trial to minimize the presence of olfactory stimuli. Exploration was defined as rats directing their noses 2 cm from the object or touching it during the 3-minute test. After recording with a stopwatch, the time spent exploring the new object (t_b) and the familiar object (t_a), the discrimination index (DI) was determined ($t_b - t_a / t_b + t_a$) %.

Statistical analysis

GraphPad Prism software (version 8; GraphPad Software, Inc., San Diego, USA) was used for graphical presentations and statistical analyses. All data were presented as mean $\pm$ standard deviation (SD). One-way ANOVA, followed by Tukey's multiple comparison test, was conducted in the SCOP-induced AD study. A probability level of <0.05 was considered statistically significant for all tests conducted.

Results and discussion

LC-QTOF-MS/MS metabolite profiling

The chemical profile of the methanol extract of *C. infortunatum* aerial parts was analyzed using LC-QTOF-MS/MS in the negative and positive ESI modes. The analysis enabled the comprehensive elution based on the polarity of plant constituents within 24 min, obtaining the first comprehensive metabolite profile of the methanol extract of *C. infortunatum* aerial parts. The representative base peak chromatograms of the methanol extract are shown in Fig. 1, and the MS/MS spectra of some of the most relevant metabolites are displayed in Fig. S1–S25. A total of 79 bioactive metabolites, detailed in Table 1 and belonging to various metabolite classes, were confidently annotated. These include 9 organic acids, 17 phenolic acids and other phenolics, 19 phenylpropanoids and phenylethanoids, 15 flavonoids, 4 coumarins, and 15 fatty acids and their derivatives. The interpretation of the mass spectra of some major metabolites is discussed below.

Organic acids

It has been stated that organic acids, known for imparting sour or acidic taste to plants, significantly impact their organoleptic qualities³². Early elution time along with characteristic fragmentation patterns involving losses of

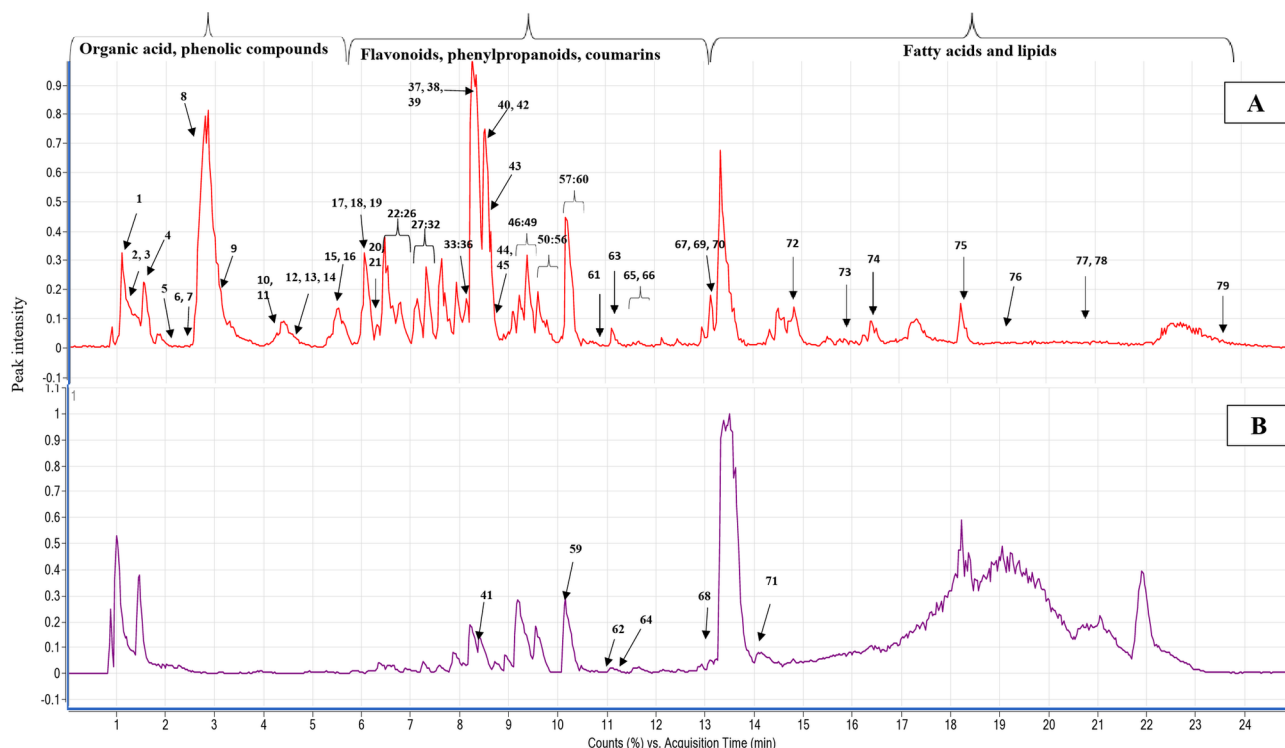


Fig. 1. Representative LC-QTOF-MS/MS base peak chromatograms in (A) Negative ESI mode and (B) Positive ESI mode of the methanol extract of *C. infortunatum* aerial parts. The peak numbers of the identified metabolites are listed in Table 1.

H₂O (-18) and CO₂ (-44), aided in their annotation. For example, Peaks 4 [m/z 191.0198, C₆H₇O₇⁻] and 8 [m/z 191.0563, C₇H₁₁O₆⁻] were annotated as citric acid and quinic acid, respectively. Both peaks exhibited fragment ions at m/z 129, resulting from the successive losses of CO₂ and H₂O (Table 1 and Fig. S1 & S2), in accordance with the literature¹⁸.

Phenolic acids

Phenolic acids constitute a major group of secondary metabolites in plants, primarily categorized into two subgroups: hydroxybenzoic and hydroxycinnamic acids²⁰. These compounds are typically identified by characteristic losses of CO₂ (-44), CO (-28), H₂O (-18), and CH₃ (-14)³³ (Table 1).

For instance, peaks 9 [m/z 169.0140, C₇H₅O₅⁻], 15 [m/z 153.0195, C₇H₅O₄⁻] and 26 [m/z 163.0397, C₉H₇O₃⁻] displayed the loss of CO₂, resulting in fragment ions at m/z 125, 109 and 119, respectively. They were identified as gallic acid, protocatechuic, and *p*-coumaric acid, respectively (Fig. S3-S5)²⁰.

Peaks 27 [m/z 457.1351, C₂₀H₂₅O₁₂⁻] and 29 [m/z 473.1301, C₂₀H₂₅O₁₃⁻] were annotated as phenolic acid glycosides. Their assignment was confirmed by the loss of the attached pentose-hexose unit [M-162-132]⁻, yielding fragment ions at m/z 163 and 179, respectively. Accordingly, metabolites 27 and 29 were identified as coumaric acid pentosyl hexoside and caffeic acid pentosyl hexoside, respectively (Fig. S6 & S7)¹⁹.

Phenylpropanoids and phenylethanoids

Phenylpropanoids represent the major components of *C. infortunatum* (Table 1). They display a variety of pharmacological properties such as anti-inflammatory, antimicrobial, and anti-skin-aging effects³⁴. The phenylpropanoid, primarily caffeic acid, can potentially bind with sugars forming ester in association with a phenylethanoid moiety. In detail, peaks 32 [m/z 693.1934, C₂₉H₃₅O₁₆⁻], 36 [m/z 755.2412, C₃₄H₄₃O₁₉⁻], and 37 [m/z 623.1990, C₂₉H₃₅O₁₅⁻] revealed their base peak fragment at m/z 161 for caffeoyl moiety, in addition to other ions corresponding to the loss of the attached sugar residues. Eventually, metabolites 32, 36, and 37 were annotated as β-hydroxy-verbascoside, forsythoside B, and verbascoside (Fig. S8-S10)^{18,19}. Following the same fragmentation pattern, metabolites 48 [m/z 783.2711, C₃₆H₄₇O₁₉⁻], 49 [m/z 651.2295, C₃₁H₃₉O₁₅⁻], and 57 [m/z 693.2399, C₃₃H₄₁O₁₆⁻] presented their base peak fragment at m/z 175 for feruloyl moiety, along with additional fragment ions corresponding to the loss of the attached sugar residues. They were identified as angoroside C, martynoside, and acetyl martynoside (Fig. S11-S13).

Flavonoids

Flavonoids are secondary metabolites that perform many functions, like regulating cell growth, attracting pollinators and insects, and protecting against biotic and abiotic stresses³⁵. Because of their bioactive characteristics, these substances have been linked to a wide range of health advantages in people, including

Peak no.	R_t (min)	Ion mode	m/z	Molecular formula	Error (ppm)	MS/MS	Identification	Reference
Organic acids								
1	1.16	[M-H] ⁻	133.0143	C ₄ H ₅ O ₅ ⁻	-0.4	115, 89, 71 , 73	Malic acid	12
2	1.4	[M-H] ⁻	115.0037	C ₄ H ₃ O ₄ ⁻	0.7	71	Fumaric acid	16
3	1.56	[M-H] ⁻	111.0088	C ₅ H ₃ O ₃ ⁻	-0.2	78 , 67	Furoic acid	PubChem CID 10,268
4	1.57	[M-H] ⁻	191.0198	C ₆ H ₇ O ₇ ⁻	0.1	129, 111, 87 , 85	Citric acid	17
5	2.10	[M-H] ⁻	117.0193	C ₄ H ₅ O ₄ ⁻	2.8	73	Succinic acid	18
8	2.77	[M-H] ⁻	191.0563	C ₇ H ₁₁ O ₆ ⁻	3.1	129, 115, 111, 101	Quinic acid	18
10	4.33	[M-H] ⁻	279.1086	C ₁₁ H ₁₉ O ₈ ⁻	-0.2	117, 99, 89, 59	2-Hydroxy-2-methyl butyric acid hexoside	19
21	6.43	[M-H] ⁻	175.0614	C ₇ H ₁₁ O ₅ ⁻	-1.1	131, 115 , 85	Isopropyl malic acid	12
24	6.83	[M-H] ⁻	225.0407	C ₁₀ H ₉ O ₆ ⁻	-1.0	207, 163, 119 , 107	Chorismic acid	HMDB0012199
Phenolic acids								
9	3.12	[M-H] ⁻	169.0140	C ₇ H ₅ O ₅ ⁻	1.4	147, 125 , 124, 115	Gallic acid	HMDB0005807
12	4.79	[M-H] ⁻	197.0449	C ₉ H ₉ O ₅ ⁻	1.2	135 , 123, 109, 72	Danshensu	HMDB0003503
13	4.82	[M-H] ⁻	315.0724	C ₁₃ H ₁₅ O ₉ ⁻	-0.7	153, 152, 109, 108	Protocatechuic acid hexoside	HMDB0303826
14	4.99	[M-H] ⁻	167.035	C ₈ H ₇ O ₄ ⁻	3.4	123 , 77, 55	Vanillic acid	18
15	5.48	[M-H] ⁻	153.0195	C ₇ H ₅ O ₄ ⁻	-1.0	109 , 91	Protocatechuic acid	HMDB0001856
17	6.11	[M-H] ⁻	395.0986	C ₁₅ H ₁₉ O ₁₀ ⁻	-0.6	197 , 153, 138, 96	Glucosyringic acid	HMDB0303364
20	6.361	[M-H] ⁻	285.0617	C ₁₂ H ₁₃ O ₈ ⁻	-0.3	152 , 109, 108	Catechol hexuronide	HMDB0240490
25	6.9	[M-H] ⁻	325.0932	C ₁₅ H ₁₇ O ₈ ⁻	-0.9	163, 119	Coumaric acid hexoside	20
26	6.92	[M-H] ⁻	163.0397	C ₉ H ₇ O ₃ ⁻	-1.4	119 , 101	<i>p</i> -Coumaric acid	20
27	7.21	[M-H] ⁻	457.1351	C ₂₀ H ₂₅ O ₁₂ ⁻	-0.1	163 , 119	Coumaric acid pentosyl hexoside	19
29	7.32	[M-H] ⁻	473.1301	C ₂₀ H ₂₅ O ₁₃ ⁻	-0.1	179 , 161, 135	Caffeic acid pentosyl hexoside	19
31	7.45	[M-H] ⁻	179.035	C ₉ H ₇ O ₄ ⁻	-1.7	135 , 117	Caffeic acid	20
52	9.64	[M-H] ⁻	193.0507	C ₁₀ H ₉ O ₄ ⁻	-0.3	161 , 134	Ferulic acid	12
Phenylpropanoids and phenylethanoids								
18	6.21	[M-H] ⁻	461.1664	C ₂₀ H ₂₉ O ₁₂ ⁻	0.1	315, 135, 113	Decaffeoyl-acteoside (phenylethanoid glycoside)	18
19	6.25	[M-H] ⁻	593.2079	C ₂₅ H ₃₆ O ₁₆ ⁻	1.3	461, 315 , 135	Pentosyl-decaffeoyl acteoside (phenylethanoid glycoside)	
22	6.5	[M-H] ⁻	487.1459	C ₂₁ H ₂₇ O ₁₃ ⁻	-0.3	179 , 161, 135	Cistanoside F (caffeoyl glycoside)	18
23	6.83	[M-H] ⁻	341.0879	C ₁₅ H ₁₇ O ₉ ⁻	-0.2	221, 179 , 161, 135	1-O-Caffeoyl glucose	21
28	7.22	[M-H] ⁻	501.1608	C ₂₂ H ₂₉ O ₁₃ ⁻	1.1	193 , 175, 161, 134	Clemomandshuricoside B (feruloyl glycoside)	22
32	7.74	[M-H] ⁻	639.1934	C ₂₉ H ₃₅ O ₁₆ ⁻	-0.5	621, 459, 179, 161 , 135	β-Hydroxy-verbascoside (caffeoyl phenylethanoid glycoside)	18
33	8.10	[M-H] ⁻	785.2493	C ₃₅ H ₄₅ O ₂₀ ⁻	-0.8	623, 461, 315, 161	Echinacoside (caffeoyl phenylethanoid glycoside)	23
35	8.15	[M-H] ⁻	653.2089	C ₃₀ H ₃₇ O ₁₆ ⁻	-0.0	179, 161 , 135	Campneoside I (caffeoyl phenylethanoid glycoside)	18
36	8.21	[M-H] ⁻	755.2412	C ₃₄ H ₄₃ O ₁₉ ⁻	-0.0	593, 461, 161 , 135, 89	Forsythoside B (caffeoyl phenylethanoid glycoside)	18
37	8.30	[M-H] ⁻	623.1990	C ₂₉ H ₃₅ O ₁₅ ⁻	-1.3	461, 315, 179, 161 , 135, 113	Verbascoside (caffeoyl phenylethanoid glycoside)	19
40	8.56	[M-H] ⁻	623.1990	C ₂₉ H ₃₅ O ₁₅ ⁻	-1.3	461, 315, 179, 161	Iso-verbascoside (caffeoyl phenylethanoid glycoside)	19
42	8.68	[M-H] ⁻	607.2034	C ₂₉ H ₃₅ O ₁₄ ⁻	-0.2	461, 315, 163, 161	Lipidoside A-I (coumaroyl phenylethanoid glycoside)	24
43	8.87	[M-H] ⁻	785.2291	C ₃₉ H ₄₁ O ₁₈ ⁻	0.9	623, 461 , 161, 89	Caffeoyl-verbascoside (caffeoyl phenylethanoid glycoside)	
44	8.91	[M-H] ⁻	637.2139	C ₃₀ H ₃₇ O ₁₅ ⁻	0.1	461, 315, 193, 175	Leucosceptoside A (feruloyl phenylethanoid glycoside)	25,26
48	9.26	[M-H] ⁻	783.2711	C ₃₆ H ₄₇ O ₁₉ ⁻	0.7	435, 355, 193, 175 , 160, 134	Angoroside C (feruloyl phenylethanoid glycoside)	25
49	9.38	[M-H] ⁻	651.2295	C ₃₁ H ₃₉ O ₁₅ ⁻	-0.1	329, 193, 175 , 160, 113	Martynoside (feruloyl phenylethanoid glycoside)	20
50	9.45	[M-H] ⁻	577.1923	C ₂₈ H ₃₃ O ₁₃ ⁻	0.6	179, 161 , 113	Salsaside A (caffeoyl phenylmethanoid glycoside)	27
56	9.98	[M-H] ⁻	591.2085	C ₂₉ H ₃₅ O ₁₃ ⁻	-0.3	179, 161	Jionoside C (caffeoyl phenylethanoid glycoside)	25
Continued								

Peak no.	R_t (min)	Ion mode	m/z	Molecular formula	Error (ppm)	MS/MS	Identification	Reference
57	10.06	[M-H] ⁻	693.2399	C ₃₃ H ₄₁ O ₁₆ ⁻	0.1	193, 175, 160, 134	Acetyl martynoside (feruloyl phenylethanoid glycoside)	25
Flavonoids								
34	8.12	[M-H] ⁻	637.1781	C ₂₉ H ₃₃ O ₁₆ ⁻	-1.0	475, 329, 193, 161, 110	Rhamnazin hexoside rhamnoside	19
38	8.45	[M-H] ⁻	923.1526	C ₄₂ H ₃₅ O ₂₄ ⁻	-0.2	285, 113	Scutellarin derivatives	28
39	8.45	[M-H] ⁻	461.0726	C ₂₁ H ₁₇ O ₁₂ ⁻	-0.1	285, 113, 57	Scutellarin	20
41	8.53	[M+H] ⁺	287.0552	C ₁₅ H ₁₁ O ₆ ⁺	-0.6	287, 269, 169	Scutellarein	18
45	8.95	[M-H] ⁻	431.0988	C ₂₁ H ₁₉ O ₁₀ ⁻	-0.9	311, 269, 268	Apigenin hexoside	HMDB0041591
46	9.09	[M-H] ⁻	445.0778	C ₂₁ H ₁₇ O ₁₁ ⁻	-0.3	269	Apigenin 7-glucuronide	25
47	9.20	[M-H] ⁻	609.1261	C ₃₀ H ₂₅ O ₁₄ ⁻	2.9	285, 161, 135, 119	Kaempferol caffeoyl-hexoside	HMDB0030239
53	9.65	[M-H] ⁻	475.0884	C ₂₂ H ₁₉ O ₁₂ ⁻	-0.4	299, 284, 113, 57	Hispidulin 7-glucuronide	16
54	9.67	[M-H] ⁻	593.1293	C ₃₀ H ₂₅ O ₁₃ ⁻	1.2	285	Kaempferol coumaroyl-hexoside	29
55	9.93	[M-H] ⁻	593.1293	C ₃₀ H ₂₅ O ₁₃ ⁻	1.2	269, 161, 113	Apigenin caffeoyl-hexoside	PubChem CID 44,257,827
58	10.30	[M-H] ⁻	459.0936	C ₂₂ H ₁₉ O ₁₁ ⁻	-0.6	283, 268, 85, 59	Acacetin 7-glucuronide	19,25
60	10.43	[M-H] ⁻	577.1353	C ₃₀ H ₂₅ O ₁₂ ⁻	-0.2	269	Apigenin coumaroyl-hexoside	PubChem CID 44,257,855
61	10.87	[M-H] ⁻	299.0552	C ₁₆ H ₁₁ O ₆ ⁻	0.3	284, 255, 227	4'-Methyl scutellarein	19
63	11.15	[M-H] ⁻	269.0458	C ₁₅ H ₉ O ₅ ⁻	-0.3	269, 225	Apigenin	19
67	13.00	[M-H] ⁻	283.0615	C ₁₆ H ₁₁ O ₅ ⁻	-1.0	268	Acacetin	16
Coumarins								
30	7.43	[M-H] ⁻	177.0194	C ₉ H ₅ O ₄ ⁻	-0.3	133, 105, 81	Esculetin	HMDB0030819
51	9.50	[M+H] ⁺	485.1645	C ₂₂ H ₂₉ O ₁₂ ⁺	1.7	177, 145, 71	4-Methyl-umbelliferyl rhamnosyl-hexoside	
59	10.29	[M+H] ⁺	339.1078	C ₁₆ H ₁₉ O ₈ ⁺	-1.0	277, 251, 177, 145, 93	4-Methyl-umbelliferyl hexoside	
62	11.14	[M+H] ⁺	177.0548	C ₁₀ H ₉ O ₃ ⁺	-1.0	162, 145, 133, 117	4-Methyl-umbelliferone	HMDB0059622
Other phenolics								
6	2.42	[M-H] ⁻	331.0707	C ₁₃ H ₁₅ O ₁₀ ⁻	-6.4	331, 169, 113, 96	Galloyl hexose	HMDB0301708
7	2.58	[M-H] ⁻	125.0249	C ₆ H ₅ O ₃ ⁻	-3.8	125, 79	Pyrogallol or phloroglucinol	HMDB0013674 HMDB0013675
11	4.42	[M-H] ⁻	153.0558	C ₈ H ₉ O ₃ ⁻	-0.5	123, 109	Syringol	HMDB0034158
16	5.54	[M-H] ⁻	109.0295	C ₆ H ₅ O ₂ ⁻	0.0	109, 91	Catechol	18
Fatty acids and their derivatives								
64	11.5	[M+H] ⁺	246.2430	C ₁₄ H ₃₂ NO ₂ ⁺	-0.0	246, 228	Tetradecasphinganine	PubChem CID 12,590,335
65	11.60	[M-H] ⁻	327.2177	C ₁₈ H ₃₁ O ₅ ⁻	-0.0	291, 239, 185	Trihydroxy-octadecadienoic acid	12
66	11.87	[M-H] ⁻	329.2328	C ₁₈ H ₃₃ O ₅ ⁻	0.1	249, 229	Trihydroxy-octadecenoic acid	HMDB0038555
68	13.02	[M+H] ⁺	333.2063	C ₂₀ H ₂₉ O ₄ ⁺	-0.8	333, 315, 107	Clerodermic acid	19
69	13.17	[M-H] ⁻	311.2242	C ₁₈ H ₃₁ O ₄ ⁻	-4.4	293	Dihydroxy-octadecadienoic acid	30
70	13.18	[M-H] ⁻	675.3604	C ₃₃ H ₅₅ O ₁₄ ⁻	-0.9	468, 415, 334, 277, 59	Dihexosyl monoacyl glycerol (18:3)) (Ginger glycolipid A)	HMDB0041093
71	14.2	[M+H] ⁺	272.2589	C ₁₆ H ₃₄ NO ₂ ⁺	-0.0	254, 100	Hexadecasphingosine	PubChem CID 14,767,871
72	14.81	[M-H] ⁻	275.2175	C ₁₈ H ₂₇ O ₂ ⁻	0.5	254, 231, 79, 70	Octadecatetraenoic acid	HMDB0032672
73	15.95	[M-H] ⁻	295.2277	C ₁₈ H ₃₁ O ₃ ⁻	0.5	277, 195, 171	Hydroxy-octadecadienoic acid	19
74	16.47	[M-H] ⁻	293.2123	C ₁₈ H ₂₉ O ₃ ⁻	-0.2	293, 249, 191	Hydroxy-octadecatrienoic	30
75	18.32	[M-H] ⁻	277.2175	C ₁₈ H ₂₉ O ₂ ⁻	-0.7	277, 233, 179	Octadecatrienoic acid (Linolenic acid)	HMDB0001388
76	19.29	[M-H] ⁻	279.2328	C ₁₈ H ₃₁ O ₂ ⁻	0.5	279, 86, 59	Octadecadienoic acid (Linoleic acid)	16
77	20.70	[M-H] ⁻	255.2323	C ₁₆ H ₃₁ O ₂ ⁻	-0.9	255, 211, 92	Hexadecanoic acid	31
78	20.9	[M-H] ⁻	281.2485	C ₁₈ H ₃₃ O ₂ ⁻	0.3	281, 240	Octadecenoic acid (Oleic acid)	16
79	22.72	[M-H] ⁻	819.5264	C ₄₆ H ₇₅ O ₁₂ ⁻	0.0	353, 277	Monohexosyl diacyl glycerol (18:3/18:3)	12

Table 1. Identified metabolites in the methanol extract of the *C. Infortunatum* aerial parts by LC-QTOF-MS/MS in the negative and positive ESI modes. Numbers in bold represent the base peak.

antidiabetic, cardio-protective, neuroprotective, antiviral, antibacterial, and anti-aging effects³⁶. The extract was found enriched in flavonols (mainly kaempferol and rhamnazin glycosides) and flavones (apigenin, hispidulin, and acacetin derivatives) (Table 1). Flavonoid glycosides commonly occur as O-glycosides where the sugar moieties are linked to the flavonoid aglycone (Ag) through an O-glycosidic bond. This can be readily identified

in tandem mass spectrometry, according to the neutral loss(es) of the attached sugar, whereby -162 , -146 , and -132 mass units indicate the loss of hexose, deoxyhexose (rhamnose), and pentose moieties, respectively³⁷. A flavonol glycosylated with two sugar moieties, peak **34** [m/z 637.1781, $C_{29}H_{33}O_{16}^-$], showed fragment ions 475 and 329 due to loss of hexose followed by rhamnose, respectively, and was annotated as rhamnazin hexoside-rhamnoside (Fig. S14)¹⁹. Flavonoids **39** [m/z 461.0726, $C_{21}H_{17}O_{12}^-$], **46** [m/z 445.0778, $C_{21}H_{17}O_{11}^-$], **53** [m/z 475.0884, $C_{22}H_{19}O_{12}^-$] and **58** [m/z 459.0936, $C_{22}H_{19}O_{11}^-$] revealed base peak fragments at m/z 285, 269, 299, and 283, respectively, due to loss of the attached sugar unit $[M-176]^-$. They were consequently identified as scutellarin, apigenin 7-glucuronide, hispidulin 7-glucuronide, and acacetin 7-glucuronide, respectively (Fig. S15–S18)^{16,25}. Among other flavonoids annotated, showing a conjugation between sugar and cinnamoyl moieties (Figs. S19–S22), there were kaempferol caffeoyl-hexoside, **47** [m/z 609.1261, $C_{30}H_{25}O_{14}^-$]; kaempferol coumaroyl-hexoside, **54** [m/z 593.1293, $C_{30}H_{25}O_{13}^-$]; apigenin caffeoyl-hexoside, **55** [m/z 593.1293, $C_{30}H_{25}O_{13}^-$]; and apigenin coumaroyl-hexoside, **60** [m/z 577.1353, $C_{30}H_{25}O_{12}^-$]³⁸.

Coumarins

Coumarins are an important and widely distributed group of natural phytochemicals, representing a class of lactones structurally constructed by a benzene ring fused to an α -pyrone ring³⁹. Three umbelliferone compounds were detected in the investigated samples, namely, 4-methyl-umbelliferoyl rhamnosyl-hexoside (**51**) [m/z 485.1645, $C_{22}H_{29}O_{12}^+$], 4-methyl-umbelliferoyl hexoside (**59**) [m/z 339.1078, $C_{16}H_{19}O_8^+$], and 4-methyl-umbelliferone (**62**) [m/z 177.0548, $C_{10}H_9O_3^+$]. Peaks **51** and **59** showed an intense ion at m/z 177 corresponding to the 4-methyl-umbelliferone moiety after the detachment of linked sugar(s) (Fig. S23 & S24 & S25).

Fatty acids and their derivatives

Fatty acids represent the primary major structural elements of lipids and the fundamental building block of cell membranes⁴⁰. A total of 15 fatty acids or their derivatives, were observed in the chromatogram (Fig. 1; Table 1). The primary process of fatty acid fragmentation involved neutral water loss(es) and decarboxylation³². Examples of identified hydroxylated fatty acids were peaks **65**, **66**, **73**, and **74**. Peak **65** [m/z 327.2177, $C_{18}H_{31}O_5^-$] can be distinguished from peak **66** [m/z 329.2328, $C_{18}H_{33}O_5^-$], by a difference of +2 mass units, indicating the existence of an extra double bond in the former. These two peaks were tentatively identified as trihydroxy-octadecadienoic acid and trihydroxy-octadecenoic acid. Similarly, peaks **73** [m/z 295.2277, $C_{18}H_{31}O_3^-$] and **74** [m/z 293.2123, $C_{18}H_{29}O_3^-$] were identified as hydroxy-octadecadienoic acid and hydroxy-octadecatrienoic, respectively (Table 1). Additionally, fatty acid **75** [m/z 277.2175, $C_{18}H_{29}O_2^-$] exhibits a difference of -16 mass units compared to **74** suggesting the presence of an additional hydroxyl group in the latter, and was annotated as octadecatrienoic acid (linolenic acid) (Table 1).

Lipid synthesis occurs through the esterification of fatty acids on the glycerol skeleton. Most lipids are insoluble in water and fall into a number of groups, including sulfolipids, glycolipids, and sphingolipids³². In the negative ESI mode, the presence of carboxylate ions $[RCOO]^-$ enables the identification of individual fatty acids esterified on the glycerol skeleton. For example, glycolipids **70** [m/z 675.3604, $C_{33}H_{55}O_{14}^-$] and **79** [m/z 819.5264, $C_{46}H_{75}O_{12}^-$] both show a fragment ion at m/z 277 for octadecatrienoic moiety. Consequently, they were annotated as dihexosyl monoacyl glycerol (18:3) and monohexosyl diacyl glycerol (18:3/18:3), respectively (Table 1).

In vitro AChE enzyme activity

The inhibitory effect on AChE enzyme activity was assessed colorimetrically for the extract, revealing a dose-dependent reduction in AChE enzyme activity. The extract and DON significantly inhibited the AChE enzyme activity compared to the control, with IC_{50} values of 0.18 ± 0.01 and 0.08 ± 0.00 $\mu\text{g/mL}$, respectively.

In vivo anti-AD activity

Acute toxicity study

The term “toxicity” refers to a substance’s ability to harm humans or animals, as well as a description of the effect and the concentration at which the effect occurs⁴¹. Toxicity is often estimated by LD_{50} at 24 h. In the present study, no mortality nor signs of toxicity were observed even at a dose as high as 2000 mg/kg of the extract over 48 h. Consequently, the selected dose for further investigation was set at 400 mg/kg.

SCOP-induced AD study

The NOR test was performed to assess the cognitive function and memory of rats, relying on the natural inclination of rodents to explore novel objects. As shown in Fig. 2, SCOP-induced AD rats took less time to identify the novel object, compared to the normal group, indicating deteriorated neurocognitive function⁴². Additionally, DON and the extract significantly increased the time rats spent examining the novel object compared to the SCOP-induced AD group. Specifically, the DI for the groups receiving DON, as well as 400, 200, and 100 mg/kg extract were 49, 42, 42, and 24 s, respectively. The most notable impact was observed in the DON group followed by the 400 mg/kg and 200 mg/kg extract groups. All these groups displayed significant differences when compared to the SCOP-induced group and minimal non-significant differences in comparison to the normal group.

In SCOP-induced AD rats, the brain level of ACh was significantly decreased by 82% compared to the normal group (Table 2), consistent with previous findings⁴³. Rats treated with DON, 200 mg/kg extract, and 400 mg/kg extract showed significantly higher levels of ACh, compared to the SCOP-induced AD group, with increases of 329%, 172%, and 287%, respectively. In contrast, the AChE enzyme activity and levels of DA and NA in SCOP-induced AD rats were elevated by 352%, 317%, and 375%, respectively, compared to the normal group, aligning with reported data⁴³. On the other hand, both DON and the extract at certain concentrations lowered

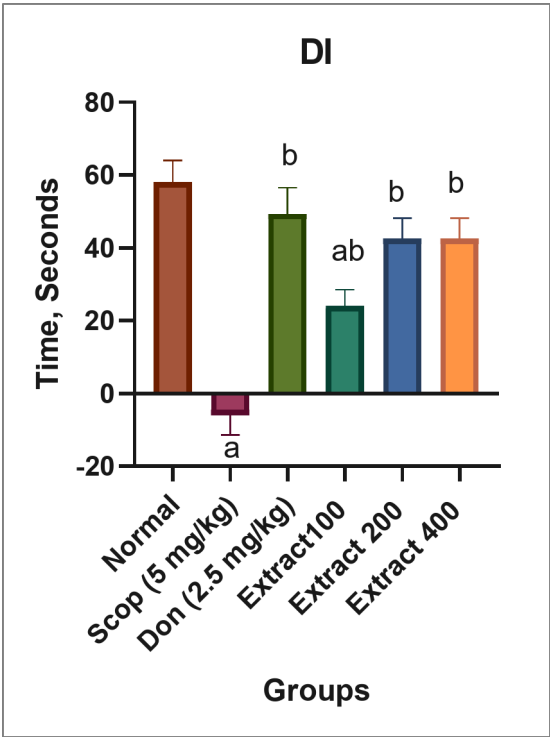


Fig. 2. NOR test discrimination index (DI) values in the studied groups of rats. The normal group was not treated with SCOP. The rest of the groups were treated with SCOP to induce AD, and in some cases also with DON (2.5 mg/kg) or extract (100, 200, and 400 mg/kg). Values are expressed as mean $\pm$ SD ($n = 8$). Different inset letters indicate statistically significant differences ($p < 0.05$) compared to the: (a) normal group, (b) SCOP-induced AD group, and ab. normal and SCOP-induced AD groups.

Parameter	Normal	SCOP (5 mg/kg)	DON (2.5 mg/kg)	Extract (100 mg/kg)	Extract (200 mg/kg)	Extract (400 mg/kg)
ACh (nmol/mg)	5.17 $\pm$ 0.5	0.93 ^a $\pm$ 0.2	3.98 ^b $\pm$ 0.3	1.28 ^a $\pm$ 0.2	2.52 ^{ab} $\pm$ 0.2	3.52 ^b $\pm$ 0.2
AChE enzyme activity (U/mg.tissue)	0.20 $\pm$ 0.0	0.90 ^a $\pm$ 0.0	0.17 ^b $\pm$ 0.02	0.81 ^a $\pm$ 0.1	0.40 ^{ab} $\pm$ 0.0	0.22 ^b $\pm$ 0.0
DA (ng/mg.tissue)	12.10 $\pm$ 1.4	50.50 ^a $\pm$ 2.3	13.41 ^b $\pm$ 2.3	36.63 ^{ab} $\pm$ 4.7	27.17 ^{ab} $\pm$ 4.3	18.17 ^{ab} $\pm$ 2.3
NA (pg/mg.tissue)	5.70 $\pm$ 0.9	27.07 ^a $\pm$ 4.3	7.91 ^b $\pm$ 0.8	21.25 ^a $\pm$ 3.1	14.27 ^{ab} $\pm$ 1.9	9.32 ^b $\pm$ 1.7

Table 2. Evaluation of the memory function parameters (AChE enzyme activity, ACh, DA, and NA levels) in the studied groups of rats. The normal group was not treated with SCOP. The rest of the groups were treated with SCOP to induce AD, and in some cases also with DON (5 mg/kg) or methanol extract (100, 200, and 400 mg/kg). Data are expressed as mean $\pm$ SD ($n = 8$). Different inset letters indicate statistically significant differences ($p < 0.05$) compared to the: ^aNormal group, ^bSCOP-induced AD group, and ^{ab}Normal and SCOP-induced AD groups.

brain AChE enzyme activity, as well as DA and NA levels. The most significant improvement, compared to the SCOP-induced AD group, was observed in the DON group, followed by the 400 and 200 mg/kg extract groups. Specifically, DON significantly reduced AChE enzyme activity, as well as DA and NA levels, with reductions of 81%, 73%, and 71%, respectively. Similarly, the 400 mg/kg extract group displayed significant reductions of 76%, 64%, and 66%, respectively, while the 200 mg/kg group showed slightly lower values at 55%, 46%, and 47%, respectively.

In terms of oxidative stress parameters, SCOP administration is expected to result in the elevation of hippocampal MDA content, the final product of lipid peroxidation and one of the most frequently used markers for damage caused by free radicals⁴⁴. This is accompanied by a subsequent reduction in the endogenous antioxidant GSH⁴⁴. Accordingly, the MDA level was significantly higher in the SCOP-induced AD group, showing a 113% increase compared to the normal group (Fig. 3). Concurrently, GSH levels in the SCOP-induced AD group were significantly lower, showing a 67% decrease compared to the normal group, as described in previous works⁴³. Administration of DON or various concentrations of extract to rats demonstrated potent antioxidant properties by lowering MDA levels and elevating GSH levels in brain tissues. Notably, the group treated with 400 mg/kg of extract exhibited the most significant improvements, with a reduction in MDA levels by 66% and an increase in GSH levels by 208% compared to the SCOP-induced AD rat group. Following closely

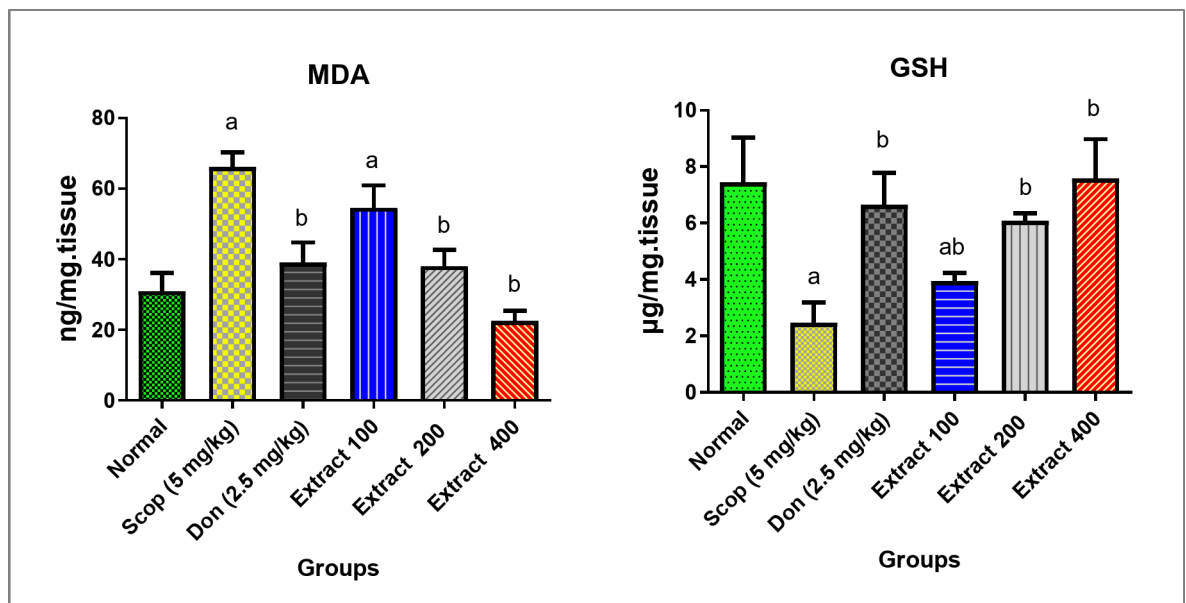


Fig. 3. Evaluation of the oxidative stress parameters (MDA and GSH levels) in the studied groups of rats. The normal group was not treated with SCOP. The rest of the groups were treated with SCOP to induce AD, and in some cases also with DON (2.5 mg/kg) or total extract (100, 200, and 400 mg/kg). Parameters are expressed as mean $\pm$ SD ($n=8$). Different inset letters indicate statistically significant differences ($p < 0.05$) compared to the: (a) normal group, (b) SCOP-induced AD group, and ab. normal and SCOP-induced AD groups.

were the DON and 200 mg/kg extract groups, which showed reductions in MDA levels by 41% and 42%, and increases in GSH levels by 170% and 147%, respectively. The 100 mg/kg group also showed a significant increase in GSH levels compared to the SCOP-induced AD group, demonstrated comparatively lesser effects. These results suggested that the anti-AD protective effect of *C. infortunatum* extract on SCOP-induced AD rats may be partly due to its antioxidant activity.

Additionally, the increase in ROS resulting from oxidative stress is widely recognized for triggering the activation of pro-inflammatory cytokines, such as TNF- α and IL-1 β ⁴⁵. In SCOP-induced AD rats, the levels of TNF- α and IL-1 β were significantly higher by 97% and 103%, respectively, compared to the normal group (Fig. 4). These elevated levels persisted with the treatment at a dose of 100 mg/kg of extract. Notably, the 200 mg/kg and 400 mg/kg extract, and DON groups exhibited significantly decreased levels of TNF- α , with reductions of 32%, 45%, and 37%, respectively, compared to the SCOP-induced AD group. Similarly, IL-1 β levels in these groups showed reductions of 26%, 48%, and 45%, respectively. This suggested that the extract ameliorated cognitive deficits by suppressing the inflammatory cascade, probably through its antioxidant effect. This finding regarding the anti-inflammatory activity of *C. infortunatum* was consistent with previously reported research⁴⁶.

A β protein, a β -sheet protein, is derived from the A β precursor protein through the activities of β and γ -secretase. The clinical manifestation of AD is associated with multiple accumulations of senile plaques composed of A β protein⁴⁷. In SCOP-induced AD rats, a significant increase in A β protein levels by 337% was observed compared to the normal group, consistent with the reported findings⁴⁸. Remarkably, A β protein levels in groups treated with 200 mg/kg extract, 400 mg/kg extract, and DON were significantly reduced by 54%, 72%, and 64%, respectively, compared to the SCOP-induced AD group (Fig. 5). Therefore, the extract showed an anti-AD protective effect by decreasing A β in the hippocampus.

As a complement to the molecular biomarker analyses, the results of the histopathological evaluation are displayed in Fig. 6. Microscopic examination of the hippocampi of the normal group (Fig. 6A), DON (Fig. 6C), 200 mg/kg (Fig. 6E), and 400 mg/kg extract (Fig. 6F) groups revealed the normal histological architecture of the hippocampus compared to the normal group. In contrast, hippocampal sections of SCOP-induced AD rats (Fig. 6B) and 100 mg/kg of extract (Fig. 6D) exhibited numerous histopathological changes due to neuronal degeneration (arrows in Fig. 6B), including diffuse mild gliosis (arrows in Fig. 6D).

Metabolite profiling of *C. infortunatum* extract revealed the presence of many phytochemicals, which may explain its observed anti-AD bioactivity. These phytochemicals include organic acids, phenolic acids, phenylpropanoids and phenylethanoids, flavonoids, coumarins, other phenolics, and fatty acids and their derivatives. Notably, flavonoids, phenylpropanoids, and fatty acids emerged as major metabolites, considering their peak intensities, all showing promising anti-AD properties^{49–51}. To the best of our knowledge, this is the first report highlighting the involvement of brain neurotransmitters, cholinergic activity, oxidative stress biomarkers (MDA and GSH), inflammatory biomarkers such as TNF- α and IL-1 β , as well as histopathological changes in the anti-AD effect of *C. infortunatum* in SCOP-induced AD rats. Additionally, cognitive function, including memory and recognition, was assessed using the NOR test.

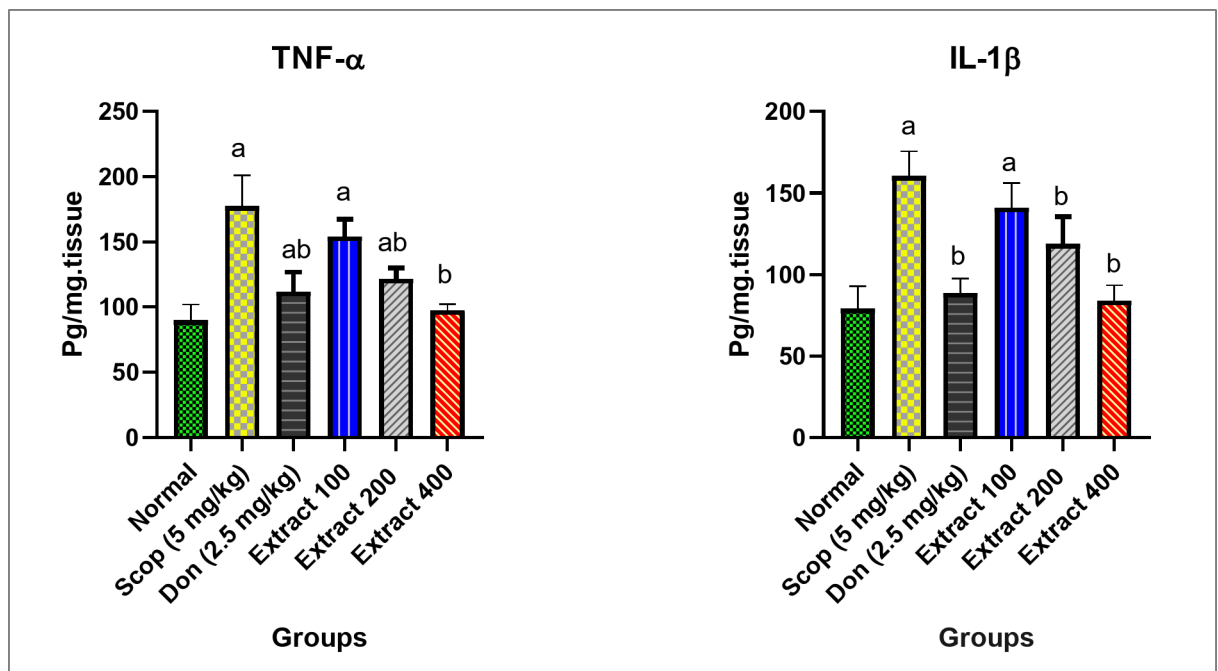


Fig. 4. Evaluation of the anti-inflammatory parameters (TNF- α and IL-1 β levels) in the studied groups of rats. The normal group was not treated with SCOP. The rest of the groups were treated with SCOP to induce AD, and in some cases also with DON (2.5 mg/kg) or total extract (100, 200, and 400 mg/kg). Parameters are expressed as mean $\pm$ SD ($n=8$). Different inset letters indicate statistically significant differences ($p < 0.05$) compared to the: (a) normal group, (b) SCOP-induced AD group, and ab. normal and SCOP-induced AD groups.

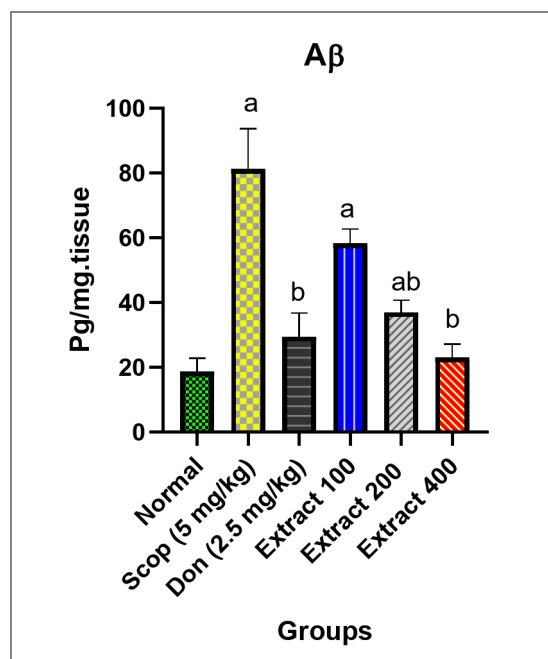


Fig. 5. Evaluation of the anti-A β protein level in the studied groups of rats. The normal group was not treated with SCOP. The rest of the groups were treated with SCOP to induce AD, and in some cases also with DON (2.5 mg/kg) or total extract (100, 200, and 400 mg/kg). Parameters are expressed as mean $\pm$ SD ($n=8$). Different inset letters indicate statistically significant differences ($p < 0.05$) compared to the: (a) normal group, (b) SCOP-induced AD group, and ab. normal and SCOP-induced AD groups.

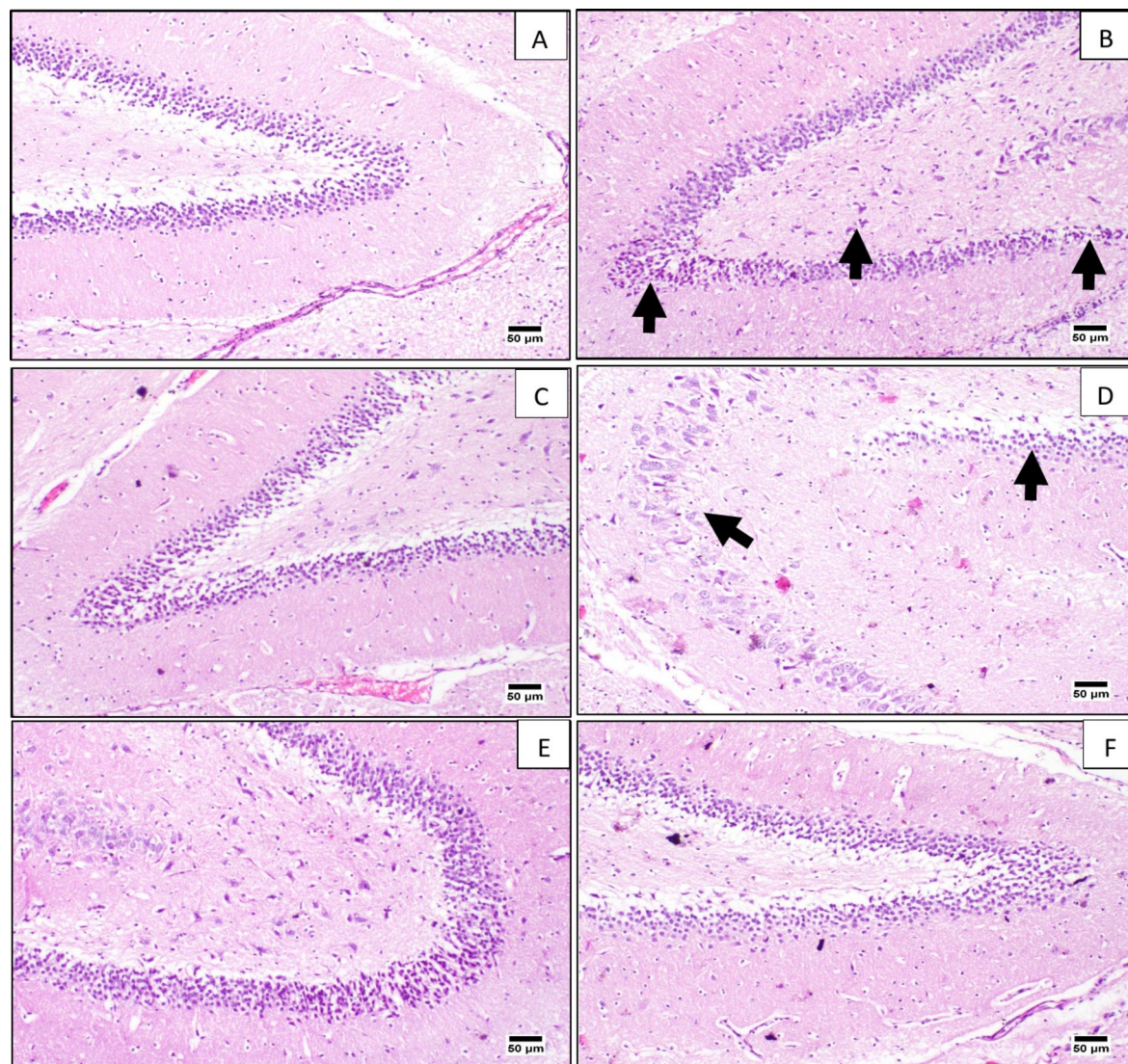


Fig. 6. Histopathological evaluation of the studied groups of rats ($n=8$). (A) Normal group was not treated with SCOP and showed the typical hippocampus region. The rest of the groups were treated with SCOP to induce AD. (B) SCOP group, with no preventive treatment, showed neuronal degeneration (arrows), (C) DON group showed almost normal neurons, (D) 100 mg/kg methanol extract showed diffuse mild neuronal gliosis, (E) 200 mg/kg methanol extract, and (F) 400 mg/kg methanol extract both showed apparently normal neurons.

In this study, SCOP-induced AD rats exhibited impaired recognition memory in the NOR test, as they explored both familiar and novel objects similarly and were unable to discriminate between the two. These results align with previous studies⁴³. Interestingly, *C. infortunatum*-treated rats reversed SCOP-induced AD in the NOR test as they were able to discriminate between the familiar and novel objects and spent more time identifying the novel object relative to the familiar one. These effects were comparable to those observed with the standard drug, DON. Moreover, SCOP administration resulted in cholinergic system dysfunction as evidenced by elevated AChE activity and a reduction in ACh, an essential neurotransmitter involved in learning and memory. This finding is consistent with prior studies⁴³. Preventive treatment with *C. infortunatum* and DON decreased hippocampal AChE activity and increased ACh levels, indicating that *C. infortunatum* might have ameliorated the cognitive deficits observed in the NOR test partly by enhancing cholinergic neurotransmission.

The role of organic and phenolic acids in memory enhancement has been well-documented. Quinic acid (8, Table 1), administered orally at doses of 200 mg/kg and 400 mg/kg, normalized AChE activity and mitigated the behavioral deficits in aluminum-induced AD rats⁵². Similarly, caffeic acid (31, Table 1), whether in its free form or combined with other groups like quinic acid and sugars, exhibits notable brain effects, including protection against AD-induced models⁵³.

Additionally, neurotransmitters such as DA and NA play significant roles in memory retrieval¹⁰. SCOP administration increased DA and NA levels in AD-induced rats, consistent with previous research¹⁰, while *C. infortunatum* administration restored these neurotransmitters to normal levels, similar to DON. These findings underscore the potential of *C. infortunatum* in modulating neurotransmitter activity to alleviate AD.

Oxidative stress has been implicated in the pathogenesis of AD. This was evident in the current investigation, as SCOP-induced AD resulted in elevated MDA content and a subsequent reduction in the endogenous antioxidant GSH, likely due to increased ROS levels. This finding aligns with a prior study⁴³ and suggests that oxidative stress associated with SCOP contributes to memory impairment. Interestingly, preventive treatment with *C. infortunatum* reversed SCOP-associated oxidative stress, bringing it to levels similar to DON by reducing MDA and enhancing the ROS-scavenging activity of GSH. Previous studies on *Clerodendrum speciosum* revealed its antioxidant effects³⁸, which may also apply to *C. infortunatum*. This reveals that the ameliorative effects of natural compounds like flavonoids as apigenin (63, Table 1) on SCOP-induced AD may partly be due to their ability to restore oxidative balance by increasing GSH and decreasing MDA⁵⁴.

Furthermore, SCOP increased the expression of inflammatory biomarkers such as TNF- α and IL-1 β , suggesting that elevated inflammation may contribute to cognitive deficits in SCOP-induced AD rats^{43,44}. Previous studies^{46,55} have demonstrated the anti-inflammatory effects of *C. infortunatum* in inflamed rat models. This suggests that *C. infortunatum* may ameliorate cognitive deficits through the suppression of inflammatory biomarkers, likely *via* its antioxidant properties.

Additionally, SCOP-induced AD resulted in a significant elevation of A β protein, a well-established biomarker of AD pathology. This finding aligns with previous studies⁴⁸ and suggests that A β accumulation exacerbates neuronal damage and cognitive decline by forming neurotoxic plaques and initiating inflammatory responses. Administration of *C. infortunatum* significantly reduced A β protein levels, similar to DON, indicating its neuroprotective effects.

The neuroprotective effects of *C. infortunatum* extract can be attributed primarily, to its abundant content of phenylpropanoids and flavonoids, which exhibit potent antioxidant and anti-inflammatory activities. For instance, echinacoside (33, Table 1) has been found to exhibit protective activity in neurodegenerative disease by reducing ROS production, glia cell activation, A β deposition, and pro-inflammatory cytokine IL-1 β , and TNF- α release⁵⁶. Forsythoside B (36, Table 1) inhibits inflammatory mediators like TNF- α and IL- β , which are significant contributors to AD pathogenesis⁵⁷. Moreover, verbascoside (37, Table 1) enhances memory and spatial cognition by suppressing A β deposition and tau protein accumulation⁴⁹. Additionally, the protective effects of scutellarin (41, Table 1) in AD, both *in vitro* and *in vivo*, are attributed to its antioxidant and anti-inflammatory properties. Scutellarin has been shown to enhance the levels of ACh and reduce levels of ROS in the brain, leading to decreased A β deposition⁵⁸. Similarly, Apigenin (63, Table 1) is known for its potent anti-inflammatory effects involving the downregulation of cytokines and nitric oxide, thereby preserving neurite integrity and cell viability⁵⁰.

In addition, coumarins as intriguing acetylcholinesterase and butyrylcholinesterase inhibitors, have been shown to mitigate oxidative stress and neuroinflammation⁵⁹. These dual actions align closely with the neuroprotective properties of fatty acids, particularly linolenic and oleic acids, which play a crucial role in mitigating AD progression. For instance, linolenic acid (76, Table 1) shows potential in regulating inflammatory responses within the central nervous system, the main site of inflammation in AD. Additionally, it has demonstrated efficacy as a dietary AChE inhibitor, thereby enhancing its neuroprotective properties⁶⁰. On the other hand, oleic acid (78, Table 1), which is abundant in neuronal myelin sheaths, contributes to membrane phospholipid integrity and has been associated with reduced risk of cancer and AD, alongside cholesterol-lowering benefits⁶¹. Notably, lower levels of oleic acid have been observed in individuals with major depressive disorders and AD cases⁵¹.

Despite the significant potential of certain metabolites present in the *C. infortunatum* extract, further studies are needed to isolate and evaluate individual bioactive metabolites or enriched fractions. These findings underscore the multifaceted therapeutic potential of natural compounds from the aerial parts of *C. infortunatum* in combating AD, highlighting the need for continued exploration for the future development of dietary supplements, nutraceuticals, or drugs, and their clinical application.

Conclusion

Based on the results obtained, the aerial parts of *C. infortunatum* have the potential to serve as a safe, natural, and cost-effective herbal remedy for protecting against AD. Through LC-QTOF-MS/MS analysis, a tentative identification of 79 compounds from diverse classes was conducted, representing the first comprehensive metabolite profiling for this plant species. Notably, phenylpropanoids were the most abundant (19 metabolites), followed by phenolic acid and other phenolics (17), flavonoids (15), fatty acids (15), organic acids (9), and coumarins (4).

The oral administration of the extract was safe at a dose as high as 2000 mg/kg over 48 h, showing significant suppression of the *in vitro* AChE activity, with IC₅₀ at 0.18 μ g/mL (compared to 0.08 μ g/mL for the standard drug DON). Furthermore, its anti-AD potential was investigated in SCOP-induced AD rats. The groups treated with 200 mg/kg and 400 mg/kg of the extract showed a significant effect, similar to that of DON. This was evident not only in the NOR test but also across various neurotransmitter and biochemical parameters, including ACh, AChE, DA, and NA, as well as anti-inflammatory mediators (TNF- α and IL-1 β), antioxidant factors (GSH and MDA), and the AD marker A β protein. Histopathological investigation of the hippocampi confirmed previous findings, indicating that groups administered at these doses of the extract exhibited nearly normal hippocampal morphology compared to both the AD-induced group and the group administered 100 mg/kg of the extract.

These results point to a promising correlation between the major metabolites of the extract and its anti-AD activity. Further investigation is required, including in-depth mechanistic studies, extensive data collection, and the isolation of the most promising secondary metabolites or enriched fractions. Future research should also prioritize comprehensive ADMET analysis and bioavailability assessments to fully validate these compounds for clinical use, ensuring both efficacy and safety in drug development.

Data availability

Data is provided within the manuscript or supplementary information files.

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References

- Nichols, E. et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Health* **7**, e105–e125 (2022).
- Gauthier, S., Rosa-Neto, P., Morais, J. A. & Webster, C. World Alzheimer Report 2021: journey through the diagnosis of dementia. *Alzheimer's Dis. Int.* **2022**, 30 (2021).
- Heppner, F. L., Ransohoff, R. M. & Becher, B. Immune attack: the role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* **16**, 358–372 (2015).
- Pereira, A. C. et al. Age and Alzheimer's disease gene expression profiles reversed by the glutamate modulator riluzole. *Mol. Psychiatry* **22**, 296–305 (2017).
- Lee, I. S. et al. Human neural stem cells alleviate Alzheimer-like pathology in a mouse model. *Mol. Neurodegen.* **10**, 1–16 (2015).
- Arora, A. & Bhagat, N. Insight into the molecular imaging of Alzheimer's disease. *J. Biomed. Imaging* **2016**, 2–2 (2016).
- Elmaidomy, A. H. et al. The anti-alzheimer potential of *Tamarindus indica*: an in vivo investigation supported by in vitro and in silico approaches. *RSC Adv.* **12**, 11769–11785 (2022).
- Bhattacharjee, D., Das, A., Das, S. K. & Chakraborty, G. *Clerodendrum infortunatum* Linn.: a review. *J. Adv. Pharm. Healthc. Res.* **1**, 82–85 (2011).
- Gouthamchandra, K., Mahmood, R. & Manjunatha, H. Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from *Clerodendrum infortunatum* L. *Environ. Toxicol. Pharmacol.* **30**, 11–18 (2010).
- Bhuvanendran, S., Kumari, Y., Othman, I. & Shaikh, M. F. Amelioration of cognitive deficit by embelin in a scopolamine-induced Alzheimer's disease-like condition in a rat model. *Front. Pharmacol.* **9**, 665 (2018).
- Gupta, R. & Singh, H. K. Nootropic potential of *Alternanthera sessilis* and *Clerodendrum infortunatum* leaves on mice. *Asian Pac. J. Trop. Dis.* **2**, S465–S470 (2012).
- Otify, A. M. et al. A comprehensive analytical framework integrating liquid chromatography-tandem mass spectrometry metabolomics with chemometrics for metabolite profiling of lettuce varieties and discovery of antibacterial agents. *Food Res. Int.* **172**, 113178 (2023).
- Schymanski, E. L. et al. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* **48**, 2097–2098 (2014).
- Younis, I. Y. et al. Non-targeted metabolomics and chemometrics for saffron (*Crocus sativus* L.) authentication and adulteration detection in relation to its anticholinesterase activity. *Food Chem. Adv.* **2**, 100217 (2023).
- Kidd, E., Banerjee, A., Ferrier, S., Longbottom, C. & Nugent, Z. Relationships between a clinical-visual scoring system and two histological techniques: a laboratory study on occlusal and approximal carious lesions. *Caries Res.* **37**, 125–129 (2003).
- Srivastava, M., Maurya, P., Shanker, K. & Jyotshna & *Clerodendrum Viscosum*: a critical review on phytochemistry, pharmacology, quality assurance, and safety data. *Med. Chem. Res.* **30**, 1–23 (2021).
- Brito, A., Ramirez, J. E., Areche, C., Sepúlveda, B. & Simirgiotis, M. J. HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in Northern Chile. *Molecules* **19**, 17400–17421 (2014).
- Kumar Deb, P., Shilkar, D., Sarkar, B. & UHPLC-ESI-QTOF-MS/MS Based identification, quantification, and Assessment of in Silico Molecular Interactions of Major Phytochemicals from bioactive fractions of *Clerodendrum Glandulosum* Lindl. *Leaves Chem. Biodivers.* **19**, e202200617 (2022).
- Elaskary, H. I., Sabry, O. M., Khalil, A. M. & El Zalabani, S. M. UPLC-PDA-ESI-MS/MS profiling of *Clerodendrum inerme* and *Clerodendrum splendens* and significant activity against *Mycobacterium tuberculosis*. *Pharmacogn. J.* **12**, 1518–1524 (2020).
- Kumar, N. & Goel, N. Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnol. Rep.* **24**, e00370 (2019).
- Lee, J. Y., Lee, J. G., Sim, S. S., Whang, W. K. & Kim, C. J. Anti-asthmatic effects of phenylpropanoid glycosides from *Clerodendron trichotomum* leaves and *Rumex gmelinei* herbes in conscious guinea-pigs challenged with aerosolized ovalbumin. *Phytomedicine* **18**, 134–142 (2011).
- Lin, T. et al. Uses, chemical compositions, pharmacological activities and toxicology of *Clematidis Radix* et *Rhizome*-a review. *J. Ethnopharmacol.* **270**, 113831 (2021).
- Han, L. et al. Structural characterisation and identification of phenylethanoid glycosides from *Cistanthes Deserticola* YC Ma by UHPLC/ESI-QTOF-MS/MS. *Phytochem. Anal.* **23**, 668–676 (2012).
- Chen, Z. Y., Wong, I. Y. F., Leung, M. W. S., He, Z. D. & Huang, Y. Characterization of antioxidants present in bitter tea (*Ligustrum pedunculare*). *J. Agric. Food Chem.* **50**, 7530–7535 (2002).
- Uddin, M. J. et al. Phenylpropanoid and flavonoid glycosides from the leaves of *Clerodendrum infortunatum* (Lamiaceae). *Biochem. Syst. Ecol.* **92**, 104131 (2020).
- Frezza, C. et al. Leucosceptosides A and B: two phenyl-ethanoid glycosides with important occurrence and Biological activities. *Biomolecules* **12**, 1807 (2022).
- Lei, L. et al. New glycosides from *Cistanche salsa*. *Helv. Chim. Acta* **90**, 79–85 (2007).
- Goufo, P., Singh, R. K. & Cortez, I. A reference list of phenolic compounds (including stilbenes) in grapevine (*Vitis vinifera* L.) roots, woods, canes, stems, and leaves. *Antioxidants* **9**, 398 (2020).
- Abd-El-Aziz, N. M., Hifnawy, M. S., Lotfy, R. A., Younis, I. Y. & LC/MS/MS GC/MS/MS metabolic profiling of *Leontodon Hispidulus*, in vitro and in silico anticancer activity evaluation targeting hexokinase 2 enzyme. *Sci. Rep.* **14**, 6872. <https://doi.org/10.1038/s41598-024-57288-4> (2024).
- Otify, A. M., El-Sayed, A. M., Michel, C. G. & Farag, M. A. Metabolites profiling of date palm (*Phoenix dactylifera* L.) commercial by-products (pits and pollen) in relation to its antioxidant effect: a multiplex approach of MS and NMR metabolomics. *Metabolomics* **15**, 1–17 (2019).
- Dey, P., Dutta, S. & Chaudhuri, T. Comparative phytochemical profiling of *Clerodendrum infortunatum* L. using GC-MS method coupled with multivariate statistical approaches. *Metabolomics* **5**, 1000147 (2015).
- Otify, A. M. et al. Unveiling metabolome heterogeneity and new chemicals in 7 tomato varieties via multiplex approach of UHPLC-MS/MS, GC-MS, and UV-Vis in relation to antioxidant effects as analyzed using molecular networking and chemometrics. *Food Chem.* **417**, 135866 (2023).
- Otify, A. M. et al. Bioherbicidal Activity and metabolic profiling of allelopathic metabolites of three *Cassia* species using UPLC-QTOF-MS/MS and molecular networking. *Metabolomics* **19**, 16 (2023).
- Xue, Z. & Yang, B. Phenylethanoid glycosides: Research advances in their phytochemistry, pharmacological activity and pharmacokinetics. *Molecules* **21**, 991 (2016).
- De Rodriguez, S. L. & Ramirez-Garza, R. & Serna Saldivar, S. O. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. *Sci. World J.* **2020** (2020).

36. Dias, M. C., Pinto, D. C. & Silva, A. M. Plant flavonoids: Chemical characteristics and biological activity. *Molecules* **26**, 5377 (2021).
37. Younis, I. Y. et al. Exploring geographic variations in quinoa grains: unveiling anti-alzheimer activity via GC–MS, LC-QTOF-MS/MS, molecular networking, and chemometric analysis. *Food Chem.* **141918** (2024).
38. Youssef, F. S. et al. Metabolomics-based profiling of *Clerodendrum speciosum* (Lamiaceae) leaves using LC/ESI/MS-MS and in vivo evaluation of its antioxidant activity using *Caenorhabditis elegans* model. *Antioxidants* **11**, 330 (2022).
39. Nguyen, T. N. et al. Coumarins from the aerial parts of *Artemisia Iwayomogi* Kitamura. *J. Appl. Biol. Chem.* **63**, 335–338 (2020).
40. Mohsen, E., Younis, I. Y. & Farag, M. A. Metabolites profiling of Egyptian *Rosa Damascena* Mill. Flowers as analyzed via ultra-high-performance liquid chromatography-mass spectrometry and solid-phase microextraction gas chromatography-mass spectrometry in relation to its anti-collagenase skin effect. *Ind. Crop Prod.* **155**, 112818 (2020).
41. Yu, K., Nation, R. L. & Dooley, M. J. Multiplicity of medication safety terms, definitions and functional meanings: when is enough enough? *BMJ Qual. Saf.* **14**, 358–363 (2005).
42. Li, J. et al. Benzoate fraction from *Gentiana Rigescens* Franch alleviates scopolamine-induced impaired memory in mice model in vivo. *J. Ethnopharmacol.* **193**, 107–116 (2016).
43. El-Marasy, S. A., Abd-Elsalam, R. M. & Ahmed-Farid, O. A. Ameliorative effect of silymarin on scopolamine-induced dementia in rats. *Open Access Macedonian J. Med. Sci.* **6**, 1215 (2018).
44. Delibas, N., Ozcankaya, R. & Altuntas, I. Clinical importance of erythrocyte malondialdehyde levels as a marker for cognitive deterioration in patients with dementia of Alzheimer type: a repeated study in 5-year interval. *Clin. Biochem.* **35**, 137–141 (2002).
45. Rabiei, Z. et al. Inhibitory effect of *Thymus vulgaris* extract on memory impairment induced by scopolamine in rat. *Asian Pac. J. Trop. Biomed.* **5**, 845–851 (2015).
46. Helen, L. R., Jayesh, K., Vysakh, A. & Latha, M. J. O. P. Polyphenolic content correlates with anti-inflammatory activity of root bark extract from *Clerodendrum Infortunatum* L. and inhibit carrageenan induced paw edema. *J. Pharmacogn. Phytochem.* **7**, 2032–2041 (2018).
47. Parihar, M. & Hemnani, T. Alzheimer's disease pathogenesis and therapeutic interventions. *J. Clin. Neurosci.* **11**, 456–467 (2004).
48. Hernández-Rodríguez, M., Arciniega-Martínez, I. M., García-Marín, I. D., Correa-Basurto, J. & Rosales-Hernández, M. C. Chronic administration of scopolamine increased GSK3 β P9, beta secretase, amyloid beta, and oxidative stress in the hippocampus of Wistar rats. *Mol. Neurobiol.* **57**, 3979–3988 (2020).
49. Wang, C. et al. Neuroprotective effects of verbascoside against Alzheimer's disease via the relief of endoplasmic reticulum stress in A β -exposed U251 cells and APP/PS1 mice. *J. Neuroinflamm.* **17**, 1–16 (2020).
50. Balez, R. et al. Neuroprotective effects of apigenin against inflammation, neuronal excitability and apoptosis in an induced pluripotent stem cell model of Alzheimer's disease. *Sci. Rep.* **6**, 31450 (2016).
51. Snowden, S. G. et al. Association between fatty acid metabolism in the brain and Alzheimer disease neuropathology and cognitive performance: a nontargeted metabolomic study. *PLoS Med.* **14**, e1002266 (2017).
52. Liu, L. et al. Neuroprotective effects of D-(-)-quinic acid on aluminum chloride-induced dementia in rats. *Evid. Based Complement. Alternat. Med.* **2020**, 1–10 (2020).
53. Habtemariam, S. Protective effects of caffeic acid and the alzheimer's brain: an update. *Mini Rev. Med. Chem.* **17**, 667–674 (2017).
54. Olayinka, J. N., Okosun, M. O., Amadi, M. C. & Ikpen, J. O. Apigenin restores memory function in stressed mice. *Alzheimer's Dement.* **18**, e069135 (2022).
55. Das, S., Haldar, P. K., Pramanik, G. & Suresh, R. Evaluation of anti-inflammatory activity of *Clerodendron Infortunatum* Linn. Extract in rats. *Glob. J. Pharmacol.* **4**, 48–50 (2010).
56. Qiu, H. & Liu, X. Echinacoside improves cognitive impairment by inhibiting A β deposition through the PI3K/AKT/Nrf2/PPAR γ signaling pathways in APP/PS1 mice. *Mol. Neurobiol.* **59**, 4987–4999 (2022).
57. Dimitrova, P. et al. Antiinflammatory properties of extracts and compounds isolated from *Verbascum Xanthophoeniceum* Griseb. *Phytother. Res.* **26**, 1681–1687 (2012).
58. Hu, X. et al. Pharmacological basis for application of scutellarin in Alzheimer's disease: Antioxidation and antiapoptosis. *Mol. Med. Rep.* **18**, 4289–4296 (2018).
59. Abu-Aisheh, M. N. et al. Coumarin derivatives as acetyl- and butyrylcholinesterase inhibitors: an in vitro, molecular docking, and molecular dynamics simulations study. *Heliyon* **5** (2019).
60. Lowry, J. R., Marshall, N., Wenzel, T. J., Murray, T. E. & Klegeris, A. The dietary fatty acids α -linolenic acid (ALA) and linoleic acid (LA) selectively inhibit microglial nitric oxide production. *Mol. Cell. Neurosci.* **109**, 103569 (2020).
61. Torres, M. et al. Lipids in pathophysiology and development of the membrane lipid therapy: new bioactive lipids. *Membranes* **11**, 919 (2021).

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Author contributions

F.A. Conceptualization, Methodology, Investigation, Formal analysis, Validation, Data curation, Visualization, Writing – original draft. M. (A) A. Conceptualization, Supervision, Investigation, Formal analysis, Validation, Visualization, Writing – review & editing. (B) M. E. Methodology, Formal analysis, Validation, Writing – review & editing. L. P. Methodology, Writing – review & editing. A.M. F. Methodology, Writing – review & editing. M. F. A. Methodology. F.B. Supervision, Methodology, Writing – review & editing. I. Y. Y. Conceptualization, Visualization, Formal analysis, Validation, Writing – review & editing. A. M. O. Conceptualization, Investigation, Formal analysis, Validation, Data curation, Visualization, Writing – review & editing. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The experiments adhered to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No 85–23, 2011). Approval was granted by the Research Ethics Committee of the Faculty of Pharmacy of Cairo University (approval code: MP (3118)).

ARRIVE guidelines

The study is reported in accordance with ARRIVE guidelines.

A statement naming the person who identified the plant

The aerial parts of *C. infortunatum* were authenticated by Agriculture Engineer Therese Labib, a plant taxonomy consultant of the Ministry of Agriculture (Giza, Egypt).

Voucher specimen statement

The plant material was collected with permission in compliance with national guidelines from the Agriculture Research Center, Giza, Egypt at “9 Cairo University Road, Giza District, Giza Governorate”. Samples of the plant material were deposited at the herbarium of the Faculty of Pharmacy, Cairo University, Cairo, Egypt (sample No.10.3.2021).

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-82265-2>.

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