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Original Research Article

Integrated Pharmacological, Antimicrobial, Neuroprotective, and Behavioral Evaluation of Selected Medicinal Plants for the Management of Alzheimer's Disease

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Abstract: Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and medicinal plants that could be used for inhibiting the enzymes that cause the disease, have antioxidant, neuroprotective, and cognitive supporting properties should be investigated. Objective: This study aimed to comparatively evaluate Bacopa monnieri, Withania somnifera, Curcuma longa, and Ginkgo biloba for pharmacological, antimicrobial, cellular neuroprotective, and behavioral effects relevant to experimental AD research. ***Methodology***: The four medicinal plants were investigated for them in-vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity, DPPH radical scavenging activity, and antimicrobial activity against Escherichia coli, Staphylococcus aureus and Candida albicans. The neuroprotective effect was evaluated by the MTT, reactive oxygen species (ROS), and malondialdehyde (MDA) assays on the cellular model of SH-SY5Y cell injury induced by hydrogen peroxide (H2O2). Ten rats per group were used to assess behavioral outcomes in a streptozotocin induced AD-like rat model in the Morris water maze, novel object recognition and open-field tests. ***Results***: Ginkgo biloba demonstrated the strongest AChE inhibition (IC_{50} = 104.0 µg/mL), whereas C. longa exhibited the greatest DPPH radical-scavenging activity (88.3%). C. longa produced inhibition zones of 14.9 mm, 17.5 mm, and 13.8 mm against E. coli, S. aureus, and C. albicans, respectively. In injured SH-SY5Y cells, C. longa increased viability to 79.6%, compared with 55.2% in the injury control, representing a 44.2% relative increase. In behavioral testing involving rats rather than human patients, its novel object recognition index reached 64.5%, compared with 43.5% in AD-model controls, while escape latency decreased to 25.9 seconds from 45.8 seconds. ***Conclusion***: Curcuma longa showed the most promising overall experimental profile, but additional testing is required before it can be applied in clinical settings.

Keywords: Alzheimer's disease; Curcuma longa; Bacopa monnieri; Withania somnifera; Ginkgo biloba; neuroprotection; antioxidant activity; antimicrobial activity; cognitive function.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder that causes memory loss, cognitive decline, changes in behavior and a progressive loss of functional independence. It is the most prevalent type of dementia and has significant clinical and neurological complexity [1, 2]. The pathophysiology includes deposition of amyloid-beta (Aβ) plaques, hyperphosphorylation of tau proteins, oxidative stress, neuroinflammation, mitochondrial dysfunction, and impairment of cholinergic neurotransmission [3,4]. These mechanisms are linked to each other and lead to synaptic dysfunction, neuronal injury and impairment of learning and memory. Conventional pharmaceutical treatments may reduce some symptoms or modify specific disease-related processes, but overall these treatments are not very effective and therefore, research into other therapeutic strategies is needed that can target more than one disease pathway [5].

Medicinal plants have various bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolic acids and glycosides which can affect multiple mechanisms involved in AD [6,7]. The extracts of Bacopa monnieri, Withania somnifera, Curcuma longa and Ginkgo biloba have been investigated with regard to their antioxidant, anti-inflammatory, cholinergic and neuroprotective properties [8,9]. They contain phytochemicals that could be useful in regulating acetylcholinesterase activity, decreasing oxidative damage, modulating inflammatory signaling, and impacting amyloid-related processes. The effects reported, however, are dependent on plant species, extraction method, phytochemical composition, concentration and experimental model. Therefore, the pharmacological results should be evaluated in conjunction with suitable controls and safety evaluation experiments, not as proof of clinical effectiveness based on traditional use [6,8].

An additional pharmacological dimension is offered by antimicrobial evaluation; as selected plant extracts may inhibit microbial growth, but do not necessarily account for the efficacy in AD. Neuroprotective assays can look at cell viability, signs of an injury by oxidation, and many other indicators of protection of neurons, and behavioral experiments can assess learning, memory, locomotor activity and exploratory response, in the pertinent experimental models [3,10]. Combination of these approaches can afford a wider characterization of plant extracts and aid in defining their antimicrobial activity apart from neurological activity. However, interpretation of behavioural outcomes is important as alterations in locomotor or anxiety-like behaviours or overall activity can affect apparent cognitive function [11,12]. In conclusion, the combined pharmacological, antimicrobial, and neuroprotective evaluation with behavioral evaluation under a structured framework would be beneficial for comparing selected medicinal plants and would help identify candidates to be further explored.

Research Objective

To comparatively evaluate the pharmacological, antimicrobial, neuroprotective, and behavioral effects of extracts of Bacopa monnieri, Withania somnifera, Curcuma longa, and Ginkgo biloba, focusing on acetylcholinesterase and butyrylcholinesterase inhibition, antioxidant activity, microbial growth inhibition, cellular viability and oxidative stress, and learning-, memory-, and locomotor-related outcomes in relevant experimental models of Alzheimer's disease.

MATERIALS AND METHODS

Study Design and Experimental Framework

This comparative experimental study looked at *Bacopa monnieri*, *Withania somnifera*, *Curcuma longa*, and *Ginkgo biloba* in the pharmacological, antimicrobial, neuroprotective, and behavioral areas. All of the following assessment methods were used: cholinesterase inhibition, antioxidant activity, microbial growth inhibition, SH-SY5Y cell responses, and learning-, memory-, and activity-related outcomes.

Selection and Identification of Medicinal Plants

Based on their described traditional use, phytochemical profile and established pharmacological or neuroprotective effects, *Bacopa monnieri*, *Withania somnifera*, *Curcuma longa* and *Ginkgo biloba* were selected.

Collection, Processing, and Authentication of Plant Materials

The plant materials were collected from herbal markets and suppliers of medicinal plants in Islamabad, Pakistan, in March 2025 to February 2026. The materials were authenticated by a qualified botanist of NIGAB, NARC, Islamabad. Parts of the plant used were leaves of *Bacopa monnieri*, roots of *Withania somnifera*, rhizomes of *Curcuma longa* and leaves of *Ginkgo biloba*. Materials were cleaned, dried, powdered and stored suitably prior to extraction.

Preparation of Plant Extracts

The plant parts were placed in 1,000 mL of 70% ethanol, macerated for 72 hours, and shaken up from time to time at 25 ± 2°C. This mixture was filtered and the extraction repeated twice using fresh solvent. The combined filtrates were concentrated in a rotary evaporator and were dried to get crude extract.

$$\text{Extraction yield (\%)} = \frac{\text{Mass of dried extract}}{\text{Mass of dry plant powder}} \times 100$$

The dried extracts were stored in light-proof and airtight containers at 4°C and the extraction yield was calculated from the mass of the starting plant material and the recovered extract mass. Extracts were reconstituted in a compatible one of the vehicles and the vehicle concentration used was uniform among the groups

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before testing.

Preliminary Phytochemical Screening

The alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides were qualitatively screened using standard tests. Only quantitative assays of total phenolics and flavonoids were done, and reference standards were employed for these analyses.

Pharmacological Evaluation

Pharmacological testing included acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and DPPH radical-scavenging activity. Background signal and possible extract interference were taken into account by using appropriate assay controls and blanks.

Acetylcholinesterase Inhibitory Activity

The Ellman colorimetric method was used to assess AChE inhibition, using extract concentrations of 25, 50, 100, 200 and 400 µg/mL, acetylcholinesterase, DTNB and acetylthiocholine iodide. Substrate was added after 10 mins of pre-incubation at 25°C and absorbance at 412 nm was recorded. The reference inhibitor was donepezil. Blank corrected readings were used to calculate percentage inhibition against enzyme control; IC_{50} estimates were made from the concentration–response data when such data were present and agreed with the results.

$$\text{Inhibition } (\%) = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Here, A(Sample) and A(Control) denote the blank-corrected responses for extract-treated and enzyme-control conditions, respectively. Lower IC_{50} values indicate stronger inhibition under the assay conditions.

Antioxidant Activity

Antioxidant activity was assessed using the DPPH radical-scavenging assay. Extract dilutions were tested according to the assay protocol, and absorbance was measured using a calibrated spectrophotometer or microplate reader. Ascorbic acid or Trolox served as the reference antioxidant, as specified in the experiment.

$$\text{Scavenging } (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

EC_{50} was estimated by regression only when adequate concentration–response data were available. DPPH findings were interpreted as in vitro radical-scavenging activity, not as direct evidence of antioxidant effects in the human brain or disease modification.

Butyrylcholinesterase Inhibitory Activity

The Ellman colorimetric method, with phosphate buffer (0.1 M, pH 8.0) was used for BChE inhibition with the substrate butyrylthiocholine iodide, under the same conditions as the AChE assay. This resulted in an enzyme pre-incubation period of 10 min at 25°C prior to the addition of the substrate and absorbance was read at 412 nm, with concentrations of extract ranging from 25–400 µg/mL. The reference inhibitor used was donepezil. The percentage inhibition was determined by blank corrected readings and the IC_{50} value was calculated by four parameters nonlinear regression of the concentration response data. Each enzyme assay was carried out three times with technical triplicates; the results are shown as mean ± SD.

Antimicrobial Evaluation

The antimicrobial activity was determined at Metropole Laboratories, Islamabad, by agar-well diffusion method. Standardized microbial suspensions of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were plated on Mueller–Hinton agar and Sabouraud dextrose agar (yeast). The plant extract was added to the wells (6 mm diameter), 100 µg/mL (100 µL) and plates were incubated for 24 h for bacteria and 48 h for yeast at 37°C and 25°C, respectively. Inhibition-zone diameters (mm) were determined three times and expressed as mean ± SD. The solvent control was 70% ethanol and Ciprofloxacin (10 µg) was the reference for the bacteria and Fluconazole (10 µg) was used as reference for C. albicans. Strain identifiers were taken from supplier certificates.

Minimum Inhibitory and Microbicidal Concentrations

The two-fold broth dilution method was used to determine the MIC. Standardized microbial suspensions were inoculated into each of the concentrations of extract (50 to 0.78 µg/mL) and then incubated under the above conditions. MIC was determined as the lowest concentration where no visible growth occurred. Experiments were repeated three times.

Neuroprotective Evaluation

Human SH-SY5Y neuroblastoma cells were cultured in DMEM with 10% FBS and 1% penicillin–streptomycin at 37 °C in 5% CO_2 for assessment of the neuroprotective activity. H_2O_2 (200 µM) was used to induce oxidative injury for 24 h. All four extracts showed no cytotoxicity in preliminary MTT assay (extracts non-toxic up to 200 µg/mL), and thus a working concentration of 100 µg/mL was chosen for all extracts. The experimental groups comprised of a group of animals without any treatment, an injury model group (H_2O_2 only), vehicle control as a negative control, a positive neuroprotective control group (quercetin at 10 µM) and an extract + injury group. Quercetin and extracts were used simultaneously with H_2O_2. Three separate experiments were conducted with all cell experiments.

Assessment of Cell Viability and Cytotoxicity

After 24 hours of exposure, cell viability was determined by the MTT assay, formazan crystals were solubilized and absorbance measured at 570 nm (reference 630 nm), and viability expressed as percentage of the untreated control (background-corrected). Assay interference was

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ruled out by the inclusion of cell-free extract blanks. Intracellular reactive oxygen species (ROS) were determined using a fluorescent probe of 2',7'Dichlorofluorescein diacetate (DCFH-DA) and malondialdehyde (MDA) was quantified by Thiobarbituric Acid Reactive Substituents (TBARS) assay, both were expressed per untreated control. The values of MTT, ROS, and MDA readings were not interpreted as the definite evidence of neuronal protection in vivo, but as the indicators of the cell metabolic activity and oxidative status.

$$\text{Cell viability } (\%) = \frac{A_{\text{treated}}}{A_{\text{control}}} \times 100$$

Background-corrected absorbance values were used for the calculations. In experiments involving induced cellular injury, the effects of plant extracts were evaluated by comparing extract-treated injury groups with the injury-only group. MTT measurements were interpreted as indicators of cellular metabolic activity and viability rather than conclusive evidence of neuronal protection.

Evaluation of Oxidative Stress and Neuroprotective Effects

Intracellular reactive oxygen species (ROS) and malondialdehyde (MDA) were used to evaluate oxidant stress. A fluorescent probe was used to determine ROS levels and a biochemical assay was used to measure MDA levels. Assay conditions, normalization procedures, experimental controls and evaluation of possible extract-induced signal interference were uniform for the relevant treatment groups.

Behavioral Evaluation

Learning and memory were assessed using the Morris water maze (MWM) and novel object recognition (NOR) tests in the experimental Alzheimer's disease model (n = 10 animals per group).

Morris water maze: escape latency was recorded across four consecutive training sessions (S1–S4), and time spent in the target quadrant was recorded during the probe trial following training. Donepezil served as the reference treatment.

Novel object recognition: after habituation and familiarization, exploration time for familiar and novel objects was recorded following a 1 h retention interval, and the recognition index was calculated as T_novel / (T_familiar + T_novel) × 100. Total exploration time was monitored to exclude general-activity effects.

Open field: locomotor and exploratory activity were recorded over 5 min (distance traveled, rearing, central/peripheral time). Behavioral data were collected by video tracking with the scorer blinded to group allocation.

Establishment of the Experimental Alzheimer's disease Model

Adult male Wistar rats (8-10 weeks old, weighing 200-250 g) were used. Its induction of the AD-like model was performed with streptozotocin (STZ; 3 mg/kg, intracerebroventricular injection) by a two-injection scheme with an interval of 48 hours between each injection. The criteria used for evaluation of model establishment included impaired Morris water maze performance, decreased novel object recognition index, and biochemical changes. Animals were randomly divided into groups: normal control group (n = 10), AD-model control group (n = 10), donepezil group (n = 10), and plant-extract group (n = 10).

Assessment of Learning and Memory

The Morris water maze and novel object recognition were selected for testing learning and memory in a standardized manner, in accordance with the experimental protocol.

Morris Water Maze

Escape latency during training and time spent in the target quadrant in the probe trial of the Morris water maze was recorded. These include standardized configuration of the pool, location of the platform, environmental conditions, training schedule, procedure for the probe trials, and tracking procedures. Within animal correlation was taken into account and repeated training measurements were analyzed. The level of swimming ability, motor impairment and task engagement were taken into account when interpreting performance to isolate potential cognitive effects from variations in physical activity.

Novel Object Recognition

Novel object recognition was evaluated by measuring the amount of time spent exploring familiar and novel objects and determining the recognition index by the formula as per the study protocol. All experimental groups had the same habituation, familiarization, retention interval, placement of objects, exploration criteria, and exclusion criteria. Differences in general activity were also taken into account in total exploration time. Consistent recognition-index formula and denominator used for all animals.

Assessment of Locomotor and Exploratory Activity

Activity was measured in a five-minute open field test conducted in standardized conditions of locomotion and exploratory activity observed by direct observation or video recording. The distance traveled, number of grid crossings, rears and time spent in the central and peripheral areas were recorded. The arena size, lighting, animal handling, tracking, scoring, and arena cleaning procedures were common to each group. Open field performance was read as general activity/exploration and taken into account when assessing the water maze and novel object recognition tests.

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Animal Welfare and Ethical Approval

Animal procedures were done in compliance with institutional and national practice. Before starting animal experiments, ethical approval was granted by the respective institutional ethics committee of NIGAB, NARC, Islamabad. Animals were handled in a manner to minimize discomfort, as per approved protocol.

Experimental Replication and Quality Control

The biological and technical replicates were separated during the experiments. Standardization of instrument calibration, preparation of reagents, use of experimental controls, handling of samples, and data recording were standardized. For each outcome, numbers of independent experiments, biological replicates, technical replicates and number of animals analyzed were recorded. Consistent prespecified exclusion criteria and procedures for invalid measurements were used. Randomization and assessment by blinding were reported.

Statistical Analysis

All the data were analyzed and organized in MS Excel 2016 and IBM SPSS Statistics version 27. Data are presented as mean ± SD. Three independent in-vitro assays and ten animals per group of behavioural assays were used. Descriptive summaries were used to summarize the yields and qualitative phytochemical profiles in the extraction process. Nonlinear regression to determine IC_{50} and EC_{50} values and antimicrobial activity expressed as inhibition-zone diameters and MIC values. Comparative data were presented as mean differences (95% confidence intervals [CIs] and p values). A p value of less than 0.05 was used as the criterion of statistical significance. Escape latency in the Morris water maze was analyzed using repeated-measures or mixed-effects analysis, which were appropriate for this data.

RESULTS

Table 1 show that among the four plants, *Ginkgo biloba* leaves gave the highest extraction yield (18.10%), followed by *Bacopa monnieri* (16.80%), *Curcuma longa* (14.30%), and *Withania somnifera* (12.60%). Qualitative screening confirmed that all four extracts contained flavonoids, phenolics, terpenoids, and glycosides, while saponins were unique to *B. monnieri* and *W. somnifera*, and tannins were absent only in *C. longa*.

Table 1. Extraction yield and qualitative phytochemical profile of selected medicinal plants

Plant extract	Plant part	Starting powder (g)	Dried extract (g)	Extraction yield (%)	Phytochemicals detected
Bacopa monnieri	Leaves	100	16.8	16.80	Alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides
Withania somnifera	Roots	100	12.6	12.60	Alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides
Curcuma longa	Rhizomes	100	14.3	14.30	Flavonoids, phenolics, terpenoids, glycosides
Ginkgo biloba	Leaves	100	18.1	18.10	Alkaloids, flavonoids, phenolics, tannins, terpenoids, glycosides

Yield (%) = (dried extract mass ÷ starting powder mass) × 100. Qualitative tests per standard protocols (Dragendorff, Shinoda, $FeCl_3$, gelatin, foam, Salkowski, Keller–Kiliani, Bornträger). | n = 3 batches per plant.

G. biloba was the most potent acetylcholinesterase inhibitor (IC_{50} = 104.0 µg/mL; 81.0% inhibition at 400 µg/mL), and *C. longa* showed the strongest radical-scavenging activity (DPPH EC_{50} = 75.0 µg/mL; 88.3% scavenging), whereas *W. somnifera* was consistently the least potent in both assays (AChE IC_{50} = 175.0; DPPH EC_{50} = 112.0 µg/mL). All extracts were considerably weaker than the references donepezil (AChE IC_{50} = 0.08 µg/mL) and ascorbic acid (DPPH EC_{50} = 18.0 µg/mL; Table 2).

Table 2. In-vitro pharmacological activity: cholinesterase inhibition and DPPH radical scavenging (mean ± SD, n = 3 independent experiments)

Extract / reference	AChE inhibition at 400 µg/mL (%)	AChE IC_{50} (µg/mL)	BChE inhibition at 400 µg/mL (%)	BChE IC_{50} (µg/mL)	DPPH scavenging at 400 µg/mL (%)	DPPH EC_{50} (µg/mL)
B. monnieri	77.9 ± 2.3	120.5	68.4 ± 2.4	166.0	86.1 ± 1.9	83.5
W. somnifera	68.8 ± 2.5	175.0	60.3 ± 2.2	219.0	79.8 ± 2.1	112.0
C. longa	75.4 ± 2.4	129.5	72.6 ± 2.3	150.0	88.3 ± 1.8	75.0
G. biloba	81.0 ± 2.2	104.0	74.5 ± 2.1	138.0	86.8 ± 1.7	78.0
Donepezil (AChE reference)	94.6 ± 0.8	0.08	—	—	—	—
Ascorbic acid	—	—	—	—	93.2 ± 0.6	18.0

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(DPPH reference)						

Ellman method (pH 8.0, 412 nm); butyrylthiocholine iodide as BChE substrate; DPPH at 517 nm. IC_{50}/EC_{50} by four-parameter nonlinear regression. Lower values indicate greater potency; values are not comparable across different assays.
C. longa exhibited the strongest and broadest antimicrobial activity, producing the largest inhibition zones against *E. coli* (14.9 mm), *S. aureus* (17.5 mm), and *C. albicans* (13.8 mm) and the lowest MICs (6.25, 3.125, and 6.25 µg/mL, respectively). *W. somnifera* was the weakest agent overall, requiring the highest MIC against *E. coli* (25.0 µg/mL). All extracts remained less active than ciprofloxacin and fluconazole, and the solvent control produced no inhibition (Table 3).

Table 3. Antimicrobial activity: agar-well diffusion (inhibition zone, mm) and minimum inhibitory concentration (MIC, µg/mL) (mean ± SD, n = 3 replicates)

Extract / control	*E. coli* zone (mm)	*E. coli* MIC	*S. aureus* zone (mm)	*S. aureus* MIC	*C. albicans* zone (mm)	*C. albicans* MIC
B. monnieri	12.6 ± 0.8	12.5	15.9 ± 0.7	6.25	11.4 ± 0.9	12.5
W. somnifera	10.8 ± 0.7	25.0	14.3 ± 0.8	12.5	13.0 ± 0.8	12.5
C. longa	14.9 ± 0.6	6.25	17.5 ± 0.7	3.125	13.8 ± 0.7	6.25
G. biloba	13.4 ± 0.7	12.5	16.3 ± 0.8	6.25	12.3 ± 0.8	12.5
Ciprofloxacin (10 µg; bacterial reference)	23.8 ± 0.5	—	25.3 ± 0.4	—	—	—
Fluconazole (10 µg; fungal reference)	—	—	—	—	21.9 ± 0.5	—
Solvent (70% ethanol) control	0.0	—	0.0	—	0.0	—

Agar-well diffusion, 6-mm wells, 100 µg/mL extract, 37°C/24 h (bacteria), 25°C/48 h (yeast). MIC by broth/two-fold dilution method..
H_2O_2 injury reduced SH-SY5Y cell viability to 55.2% of control while elevating ROS (2.16) and MDA (2.03) levels (Table 4). *C. longa* provided the greatest protection, restoring viability to 79.6% and reducing ROS and MDA to 1.34 and 1.39 units, respectively — approaching the positive control quercetin (82.8% viability, 1.27 ROS, 1.32 MDA) — while *W. somnifera* was the least effective of the four extracts (70.2% viability).

Table 4. Neuroprotective outcomes in H_2O_2-injured SH-SY5Y cells (mean ± SD, n = 3 independent experiments)

Experimental group	Cell viability (% of control)	ROS (relative units)	MDA (relative units)
Untreated control	100.0 ± 4.2	1.00 ± 0.08	1.00 ± 0.07
Injury model (200 µM H_2O_2)	55.2 ± 5.0	2.16 ± 0.15	2.03 ± 0.14
Vehicle control	56.4 ± 4.9	2.10 ± 0.15	1.97 ± 0.13
Quercetin 10 µM (positive control)	82.8 ± 4.5	1.27 ± 0.11	1.32 ± 0.10
B. monnieri 100 µg/mL + injury	77.1 ± 4.8	1.40 ± 0.12	1.46 ± 0.11
W. somnifera 100 µg/mL + injury	70.2 ± 5.1	1.61 ± 0.14	1.65 ± 0.12
C. longa 100 µg/mL + injury	79.6 ± 4.6	1.34 ± 0.10	1.39 ± 0.09
G. biloba 100 µg/mL + injury	78.2 ± 4.6	1.38 ± 0.11	1.43 ± 0.10

MTT at 570 nm after 24 h; ROS by DCFH-DA fluorescence; MDA by TBARS assay, normalized to untreated control. Concentrations selected from preliminary cytotoxicity (non-toxic ≤ 200 µg/mL).
In the experimental AD model, the AD-model control showed marked impairment (escape latency 45.8 s in session 4, target-quadrant time 19.2 s, recognition index 43.5%). *C. longa* produced the strongest behavioral recovery (session-4 latency 25.9 s, target-quadrant time 32.8 s, recognition index 64.5%), comparable to donepezil (26.5 s, 33.7 s, 63.9%), while *W. somnifera* showed the weakest improvement among the extracts (34.6 s, 27.6 s, 56.1%). Open-field distances confirmed that cognitive differences were not explained by locomotor deficits (Table 5).

Table 5. Behavioral outcomes in the experimental Alzheimer's disease model (mean ± SD, n = 10 animals/group)

Group	Escape latency S1 (s)	S2 (s)	S3 (s)	S4 (s)	Target-quadrant time (s)	NOR index (%)	Open-field distance (m/5 min)
Normal control	41.8 ± 6.1	32.5 ± 5.3	26.4 ± 4.7	21.6 ± 4.1	38.9 ± 5.3	69.6 ± 6.2	18.6 ± 2.4
AD-model control	55.6 ± 7.2	51.4 ± 6.9	49.1 ± 6.4	45.8 ± 6.1	19.2 ± 4.5	43.5 ± 5.8	12.9 ± 2.1
Donepezil (reference)	47.6 ± 6.6	38.1 ± 5.8	31.2 ± 5.1	26.5 ± 4.7	33.7 ± 5.0	63.9 ± 5.6	17.1 ± 2.2
B. monnieri	49.0 ± 6.8	39.8 ± 6.0	33.5 ± 5.3	28.4 ± 4.9	32.0 ± 4.7	62.2 ± 5.4	16.4 ± 2.0

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W. somnifera	51.6 ± 7.0	45.1 ± 6.4	39.3 ± 5.9	34.6 ± 5.4	27.6 ± 4.8	56.1 ± 5.9	15.1 ± 2.3
C. longa	47.5 ± 6.5	36.9 ± 5.6	30.7 ± 5.0	25.9 ± 4.6	32.8 ± 4.9	64.5 ± 5.3	16.7 ± 2.1
G. biloba	48.2 ± 6.7	38.3 ± 5.7	32.1 ± 5.2	27.2 ± 4.8	31.5 ± 4.6	63.0 ± 5.5	16.3 ± 1.9

Morris water maze: 4 training sessions plus probe trial (target-quadrant time); NOR index = Tnovel/(Tfamiliar + Tnovel) × 100; recognition index data from 1-h retention trial.

Table 6 shows that *Curcuma longa* demonstrated significant improvements across several experimental outcomes, including increased SH-SY5Y cell viability by 24.4 percentage points (95% CI: 17.0–31.8, p = 0.001), reduced ROS levels by 0.82 relative units (p = 0.001), and decreased Morris water maze escape latency by 19.9 seconds (p = 0.001) compared with the respective control groups. It also improved the novel object recognition index by 21.0 percentage points (p = 0.001) and increased the inhibition zone against *E. coli* by 4.1 mm (p = 0.003). These findings suggest promising antioxidant, neuroprotective, antimicrobial, and behavioral effects under the tested experimental conditions.

Table 6. Comparative Statistical Analysis of Pharmacological, Antimicrobial, Neuroprotective, and Behavioral Outcomes of Selected Medicinal Plant Extracts

Outcome	Comparison	effect estimate	95% CI	p-value
AChE inhibition	*C. longa* vs. *W. somnifera*	6.6 percentage points	2.1 to 11.1	0.008
BChE inhibition	*G. biloba* vs. *W. somnifera*	14.2 percentage points	8.0 to 20.4	0.002
DPPH scavenging	*C. longa* vs. *W. somnifera*	8.5 percentage points	3.7 to 13.3	0.004
E. coli inhibition zone	*C. longa* vs. *W. somnifera*	4.1 mm	2.0 to 6.2	0.003
SH-SY5Y cell viability	*C. longa* vs. injury control	24.4 percentage points	17.0 to 31.8	0.001
ROS level	*C. longa* vs. injury control	−0.82 relative units	−1.05 to −0.59	0.001
MDA level	*C. longa* vs. injury control	−0.64 relative units	−0.86 to −0.42	0.002
Morris water maze latency, S4	*C. longa* vs. AD-model control	−19.9 seconds	−27.0 to −12.8	0.001
Target-quadrant time	*C. longa* vs. AD-model control	13.6 seconds	8.2 to 19.0	0.001
Novel object recognition index	*C. longa* vs. AD-model control	21.0 percentage points	13.5 to 28.5	0.001
Open-field distance	*C. longa* vs. AD-model control	3.8 m	1.2 to 6.4	0.006

DISCUSSION

The yield of extraction varied between the four 70% ethanol extracts (12.60% in the case of Withania somnifera to 18.10% in the case of Ginkgo biloba). Several classes of phytochemicals identified through qualitative screening that may be related to biological activity. However, there are no indications of the concentration or identity of the active compounds in the extraction yield and qualitative screening. The differences in the observed pharmacological profiles could be due to differences in extract compositions which requires further chemical characterization.

In terms of cholinesterase inhibition and antioxidant activity, the cholinesterase inhibitory activity of G. biloba was the lowest reported (AChE IC_{50} = 104.0 µg/mL) while Curcuma longa had the highest antioxidant activity (DPPH EC_{50} = 75.0 µg/mL). The results corroborate the need of further studies of their specific activities. Curcumin is known to have antioxidant and potential anti-amyloid properties, and systematic reviews have found inconsistent cognitive effects with G. biloba preparations [13,14]. However, the activity of crude extracts derived from laboratory tests does not guarantee clinical activity and the values reported must be checked back to the original experimental records.

C. longa was also shown to have the most effective antimicrobial activity with reported MIC values of 6.25 µg/mL against Escherichia coli, 3.125 µg/mL against Staphylococcus aureus, and 6.25 µg/mL against Candida albicans. In-vitro antibacterial activity of C. longa rhizome extracts against S. aureus has been documented previously (Gupta et al., 2015) and is relevant. Variation between studies may be due to differences in extract composition, microbial strains or assay procedures [15]. This suggests antimicrobial activity under the conditions tested, but has not been shown to have a direct therapeutic effect on Alzheimer's disease.

In the model of cellular injury induced by H_2O_2, the viability decreased to 55.2% along with ROS and MDA of 2.16 and 2.03 respectively. The treatment of C. longa led to a viability rate of 79.6%, ROS 1.34 and MDA 1.39 approaching the results reported by quercetin group, which had a viability of 82.8%. This result is similar to that obtained by studies of the antioxidant activity of curcumin in experimental models of AD [13]. These results indicate protection under the test conditions, rather than complete neuronal rescue, as MTT, ROS and MDA are specific cellular endpoints.

Behavioral outcomes indicated an improvement in the C. longa group, as the group escaped the Morris water maze in 25.9 seconds in session 4, and spent 32.8 seconds in the target quadrant and 64.5% of the time in the NOR index when compared with 45.8 seconds, 19.2 seconds, and 43.5 seconds, respectively, for the AD-model

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control. The donepezil group had comparable values of 26.5 seconds, 33.7 seconds, and 63.9%. The similarities described above are not an indicator of equivalence with donepezil, and a statistical test is required to confirm this. Previous reviews also highlight uncertainty about the ability to discern clinical cognitive effects from plant-based interventions [16,17].

Our results show that treatment with Curcuma longa showed a 24.4 percentage increase in cell viability in the SH-SY5Y cell line, a 0.82 relative decrease in reactive oxygen species (ROS), a 19.9 second decrease in escape latency during Morris water maze, and a 21.0 percentage increase in the novel object recognition index relative to the respective controls. Such results are consistent with previous preclinical studies that showed that curcumin could have a protective role against oxidative stress and behavioural effects in the Alzheimer's disease experimental models. Hamaguchi et al. (2010) also performed a systematic review on the possible neuroprotective properties of curcumin in models of Alzheimer's disease [13]. Differences in composition of the extracts and experimental protocols, however, preclude direct comparisons.

In summary, the profile of this antioxidant and antimicrobial profile was the most favorable for C. longa, while the strongest reported AChE inhibition was observed for G. biloba, overall. However, the lower activity of W. somnifera in these assays should not be extended to all other possible biological effects as they can vary between different preparations and experimental models [18]. Reproducible assays, dose–response analysis, and clear statistical reporting should be a priority for future studies.

Strength and Limitations

The most significant advantage of this study is its comprehensive comparative analysis of four medicinal plants (Bacopa monnieri, Withania somnifera, Curcuma longa, and Ginkgo biloba) in different domains of pharmacological, antimicrobial, neuroprotective, and behavioural aspects of Alzheimer's disease. An evaluation of acetylcholinesterase and butyrylcholinesterase inhibition, antioxidant activity, antimicrobial effect, cellular viability, oxidative stress markers, learning, memory, and locomotor activity gives a more comprehensive view on their potential biological activity than a single-assay approach. Comparative framework is further enhanced by the addition of reference treatments, vehicle controls, multiple experimental endpoints and behavioral assessment. There are, however, a few restrictions to bear in mind. Crude extracts do not allow for the identification of specific bioactive components and their respective roles in the effects observed. The in vitro experiments were performed with only three independent experiments, and the behavioral evaluation was done with ten animals per group, which may decrease the statistical power and generalizability of the findings. Both the hydrogen peroxide-induced model of cellular injury and the streptozotocin-induced Alzheimer's disease model exhibit some features of oxidative injury and cognitive impairment but not the full complexity of human Alzheimer's disease. In addition, the results of the antimicrobial studies are limited in their direct relevance to the management of Alzheimer's disease, and the lack of detailed inferential statistical output, lack of dose–response evaluation in cellular and behavioral studies, and lack of a detailed toxicity evaluation limit the strength of the conclusions.

CONCLUSION

The present study aims to compare the effect of Bacopa monnieri, Withania somnifera, Curcuma longa and Ginkgo biloba with respect to the complementary pharmacological, antimicrobial, neuroprotective and behavioral endpoints relevant to the investigation of potential therapeutic approaches for Alzheimer's disease. In light of the data presented, Curcuma longa had the best overall profile due to its highest levels of DPPH radical-scavenging activity, relatively higher levels of antimicrobial activity, higher levels of cell viability in SH-SY5Y cells, lowest levels of oxidative stress markers, and better learning and memory outcomes in the experimental model. Ginkgo biloba, on the other hand, showed the highest ability to inhibit acetylcholinesterase, indicating a possible different therapeutic effect. Bacopa monnieri also exhibited favorable cellular and behavioral effects while Withania somnifera exhibited relatively lower activity on some of the endpoints under the conditions tested. The results indicate different and possibly complementary biological properties in the selected medicinal plants but don't prove efficacy in humans or disease modification. Additional research with standardized extracts, identification of active constituents, optimization of dose, full safety evaluation, mechanism of action and adequately powered, replication independent studies is required to confirm these findings and to assess if any of these plants could play a role in the future development of evidence-based drugs for Alzheimer's disease.

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