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Research Article

IN VITRO ANTIOXIDANT AND MOLECULAR DOCKING STUDIES FOR ANTI-ALZHEIMER POTENTIAL OF ETHANOLIC EXTRACT OF *ALTERNANTHERA SESSILIS* AND *LANTANA CAMARA*

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ABSTRACT

Objectives: Oxidative stress significantly contributes to the advancement of Alzheimer's disease (AD), and antioxidants (AOX) derived from plants are increasingly recognized for their neuroprotective properties. This research examines the *in vitro* AOX properties and anti-Alzheimer potential of ethanolic extracts of *Alternanthera sessilis* (EEAS) and methanolic extract of *Lantana camara* (MELC) through phytochemical screening, AOX assays, gas chromatography–mass spectrometry (GC-MS) analysis, molecular docking (MD), and ADME prediction.

Methods: Preliminary phytochemical screening identified the existence of flavonoids, phenolics, Alkaloids, sterols, and glycosides. The evaluation of AOX activity was led through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and metal chelating assays. GC-MS analysis revealed significant bioactive compounds in both extracts. The phytoconstituents were docked to AD-related targets (Aβ and AChE) to evaluate their binding affinities. ADME properties were assessed utilizing SwissADME.

Results: Indicate that both EEAS and MELC demonstrated considerable AOX activity, with MELC presenting lower IC_{50} values in the DPPH (204.3 µg/mL) and ABTS (327.47 µg/mL) assays relative to EEAS. GC-MS profiling identified compounds with established pharmacological significance. MD revealed multiple compounds exhibiting high binding affinity for AD targets. ADME analysis demonstrated adherence to drug-likeness criteria, indicating potential for oral bioavailability.

Conclusion: The research indicates that *A. sessilis* and *L. camara* exhibit significant AOX and anti-Alzheimer effects. Phytoconstituents may provide a basis for the advancement of neuroprotective agents. Future in vivo validation is necessary to verify their therapeutic potential.

Keywords: *Alternanthera sessilis*, *Lantana camara*, Alzheimer's disease, AChE enzyme, Gas chromatography–mass spectrometry.

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INTRODUCTION

Developing improved dementia disease-modifying medications remains difficult. As with Alzheimer's disease (AD), dementias cause a steady deterioration in higher cognitive processes [1,2]. As cholinergic neurons in the hippocampus and cortex die, acetylcholine levels gradually decrease in AD patients. Malformed plaques and neurofibrillary tangles are also concerns [3]. Inhibiting acetylcholinesterase (AChE), which promotes acetylcholine buildup at the synapse, addresses the cholinergic deficit and is a therapeutic target for AD medication development. Galantamine, rivastigmine, and donepezil are AD medicines that suppress AChE [4].

Synthetic drugs might cause major negative effects and fail to work. They focus on AD symptoms rather than disease progression, making treatment difficult [5]. Thus, novel, safe, and effective drugs are needed. This allows therapeutic plant research. AChE inhibitors from medicinal plants have promise [6]. AChE inhibition may treat different dementias, Parkinson's disease, glaucoma, and myasthenia gravis [7].

Oxidative stress from reactive oxygen species is a major neurotoxic factor in AD. AOX and free radical's imbalance causes oxidative stress. Elevated free radical levels damage biomolecules, including lipids and proteins (prot), causing cellular oxidative damage and overexpression of oncogenes, mutagens, atherogenic processes, or inflammation. Neurodegenerative diseases such as Alzheimer's, myasthenia gravis, glaucoma, and Parkinson's are linked to oxidative stress [8].

Plant-derived AOXs that stop-free radical chain reactions are gaining popularity. Many herbal extract ingredients are AOXs. Medicinal plants may fight aging and cognitive loss by restoring oxidative equilibrium. Polyphenolic substances such as flavonoids and phenolic acids may make plants AOX [9,10]. AOXs and AChE-inhibiting medicinal plants may treat neurodegenerative diseases.

Alternanthera sessilis is a 0.2-1 m annual or perennial herb. Simple, opposite, shortly percolate or sessilis, broadly lanceolate leaves. The base is attenuated and the apex is sharp, blunt, with whole, glabrous or thin, delicate, articulate edges. The lead axils have dense, sessilis, silvery white inflorescences with compressed spikes. Small, flattened and obviate fruits contain the seed, but Shiny and disc-shaped fruits contain dark brown to black seeds. Light-sensitive [11]. Lupeol, α and β-sitosterol, β-sitosterol, stigmasterol, and campesterol have been found in *A. sessilis* (Linn.). Alkaloids (Alks), glycosides (glys), steroids (Ster), terpenoids, tannins (Tan), flavonoids, saponins (Saps), polyphenols, coumarins, and carbohydrates (carbs) were found in hexane, ethyl acetate, ethanol, and aqueous *A. sessilis* (Linn.) leaf extract *A. sessilis* (L.) claims wound-healing qualities. It is used in combination with other medicinal herbs to treat asthma, chest discomfort, hepatitis, bronchitis, analgesia, dysentery, malaria fever, diarrhea, urinary tract infections, and other disorders.

The aromatic shrub *Lantana camara* L. reaches 0.5–2.5 m. Square stems, opposite oval leaves with toothed borders, and rough texture distinguish the plant. The crushed dark green top and pale underleaves smell fragrant. The inflorescence has a dense, terminal, or axillary umbel of little tubular blossoms. These flowers mature from pink to orange, red, yellow, and white. The small, glossy, meaty drupe changes green to black–purple when ripe and has one or two seeds.

Traditional medicine uses *L. camara* despite its tropical and subtropical invasiveness. Research indicates that it includes bioactive elements such as triterpenoids (TTPs) (e.g., lantadene A and B), oleanolic acid, ursolic acid, lantanilic acid, and steroidal substances (β-sitosterol and stigmasterol It contains quercetin, kaempferol, Alks, Saps, and phenolic acids [12]. *L. camara* leaf, flower, and root extracts (hexane, chloroform, ethanol, and aqueous) contained Alks, glys, flavonoids, Ster, Tan, Saps, triterpenes, and carbs [13]. These molecules permit their varied pharmacological actions. *L. camara* is antibacterial, antifungal, antiviral, wound-healing, anti-inflammatory, analgesic, antipyretic, anticancer, antimalarial, AOX, hepatoprotective, and insecticidal. Many cultures use the plant to treat fever, skin eruptions, asthma, cuts, wounds, ulcers, leprosy, respiratory tract infections, rheumatism, gastrointestinal issues, and monthly abnormalities. The leaves are used in skin and wound poultices and internal decoctions [14].

METHODS

Plant material

The leaves of *A. sessilis* and *L. camara* received authentication from Dr. K. Madhava Chetty, who serves as an Assistant Professor in the Department of Botany at Sri Venkateswara University, Tirupati.

Preparation of ethanolic extracts

A. sessilis and L. camara leaves were carefully gathered, washed, and shade-dried (Fig. 1). A mortar and pestle pounded the dried leaves into a coarse powder. About 300 g of this gritty powder was carefully weighed and extracted with ethanol (99%) over a week using cold maceration with intermittent stirring. Following the muslin cloth separation, the solvent-soaked plant material was filtered through a funnel. The solvent was left in a beaker to evaporate at 25°C, concentrating the extract. This extract was placed in a Petri plate and desiccated until use. The dark-black final extract was dispersed in distilled H_2O with carboxy methyl cellulose for oral administration to experimental animals.

Phytochemical estimation of plant extract

Typical qualitative assays were used to screen for phytochemicals such as flavonoids, TTPs, phenolic compounds (PCs), Tan, amino acids (AA), carbs, Saps, and prot in ethanolic extracts of *A. sessilis* (EEAS). The existence of phytosterols was confirmed by the Libermann–Burchard test [15], which produced a bluish–green color, and the Salkowski test, which produced a red color. Ferric chloride was used to identify phenols by their bluish-red coloring. In the Dragendorff, Mayer, Wagner, and Hager tests, Alks were verified by orange, yellow, or reddish-brown precipitates. Flavonoids were identified by green/yellow reactions in ferric chloride and lead acetate assays, and pink to red coloration in Shinoda's test. With ferric chloride, gelatin, and lead acetate, PCs, and Tan displayed blue, green, or white precipitates. The presence of cyanogenic glys was shown by the red staining of picrate paper, a brown ring in Keller–Killani's test, and dark green or yellow coloration in the anthrone and sodium hydroxide tests.

Gas chromatography–mass spectrometry (GC-MS) analysis

GC-MS study with HP-5 MS fused silica column and 5675C Inert MSD with Triple-Axis detector was conducted in a 7890A GC system and MS (GCMSQP2010, Shimadzu). Helium gas has a 1.0-mL/min column velocity as a carrier gas. Other GC-MS parameters: 1.8 mm output time, 16.2 psi pressure, 300°C injection, 250°C ion-source, 300°C interface, and 1 µL injector in split mode with 1:50 split ratio. After 5 min (min) at 36°C, the column temperature (temp) rose 4°C per min to 150 V. Temp was raised to 250°C at 20°C/min for 5 min. Complete elution took 37 min. The relative % of every element was assessed by equating its average peak area to the total areas. The supplier delivered the Microsoft Solution software to control and gather data.

Identification of compounds

The MS was interpreted using the National Institute of Standards and Technology (NIST) database and component retention indices. About 62,000 compound patterns exist in the database. The standard MS of known components in the NIST library (NISTII) was matched to the unknown components of the *A. sessilis and L. camara* fractions.

Planning of the receptors

The basic receptors of AChE enzyme (protein information bank ID: 4EY5) structures were obtained from the protein information bank. All non-protein atoms and all other elective nuclear destinations were isolated from the protein receptor structures by utilizing the PyRx 0.8 computer program [16,17].

Planning of ligands

The ligand planning process encompasses multiple stages, including transformations, corrections, structural modifications, elimination, and optimization of leads. Two-dimensional representations of the ligands and compounds, potentially distinguishable through GC-MS/MS, were generated using ChemDraw and subsequently transformed into three-dimensional molecular formats. The transformation of grin structures from a three-dimensional configuration was executed utilizing the cactus grin converter apparatus. The increase in hydrogen atoms, elimination of heteroatoms, neutralization of charged groups, creation of ionization states and tautomers, filtration, selective chirality, geometry optimization, low-energy ring conformers, and final output modification and preservation enable detailed compound database analysis and the prediction of the most effective bindings dependent on scoring [18,19].

Atomic docking studies

Atomic docking refers to the process by which two particles align and fit together within a three-dimensional space. This method may serve as a crucial tool in auxiliary science and computer-aided design initiatives. A standard dock was utilized as the default configuration for precise docking, with population estimate (n=200), generations (n=70), and no arrangements (n=2). We chose posture ligands with high compliance to reduce free official energy (Autodock tools Version 1.5.7).

The selected iGEMDOCK for protein-ligand docking has an intuitive interface for determining binding site and ligand docking status, post-docking analysis, and visualizing the progression, positioning, and assessment of screened compounds using pharmacological insights and energy-based scoring methodologies. Most docking software requires receptor and ligand setup. The chosen program determines the authoritative position of the restricted ligand and accounts for hydrogen atom impacts on binding sites [20,21].

In silico retention, conveyance, digestion system, excretion, and harmfulness

We did *in silico* ADMET assessments for the components with the capable structural frameworks to identify drug-like lead compounds for further study. ADMET covers critical and advantageous aspects of developing innovative medications with improved pharmacokinetic (PK) and pharmacodynamic properties. ADMET analysis uses admetSAR to assess intestinal absorption, blood–brain barrier penetration, acute oral toxicity, and carcinogenicity of the top 5 phytoconstituents with drug-like characteristics.

LigPlot examination

From common protein information bank data, LigPlot builds schematic two-dimensional representations of protein–ligand interactions. The color or monochrome record describes intermolecular interactions, including hydrogen bonds and hydrophobic interactions. This method is typical for ligands and can be used to detect protein and nucleic acid intelligence. LigPlot study was utilized to know the complex interaction among docked ligands and dynamic site buildups [22].

In vitro AOX screening assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging was assessed utilizing the 1958 Blois technique. A test tube included 6 mL of an ethanolic DPPH solution (33 mg/L) and different plant extract or standard concentrations (2 mL). The reaction mix was incubated at 25°C for an hour. A ultraviolet-

visible spectrophotometer evaluated the remaining DPPH solution's absorbance (abs) at 517 nm. The test was duplicated. Ascorbic acid was standard. The formula measured inhibition as percentage inhibition (I %), with a lesser IC_{50} value indicating stronger AOX capacity [23].

$$I\ \% = (Abs\ control - Abs\ sample)/Abs\ control \times 100$$

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging capacity was evaluated using the methodology of Re R *et al*, 1999 ABTS was initially synthesized at a concentration of 2 mM and potassium persulfate at 70 mM using distilled H_2O. Subsequently, 200 mL of potassium persulfate was combined with 50 mL of ABTS and allowed to stand for 2 h. This approach assessed ABTS radical scavenging. The abs were calculated at 734 nm after adding 0.6 mL of ABTS radical cation and 3.4 mL of phosphate buffer at pH 7.4–1 mL of plant extract or standard. The experiment was duplicated. Ascorbic acid was standard. The inhibition percentage (I %) was estimated with the help of the indicated method. A lower IC_{50} value shows stronger AOX ability [24].

$$I\ \% = (Abs\ control - Abs\ sample)/Abs\ control \times 100$$

Metal chelating assay

The chelate ability of metals was tested by Khan W, 2019. In this experiment, 0.4 mL of ferrozine solution, 0.2 mL of 2 mM ferric chloride, and 10 mL of plant extract or standard were combined and left for 10 min at room temp while being constantly shaken. At 562 nm, the abs were measured. Three duplicates of the experiment were conducted. The standard was Ethylenediaminetetraacetic acid (EDTA). The formula below was utilized to know the percentage inhibition; a lower IC_{50} value denotes a higher AOX capacity.

$$I\ \% = (Abs\ control - Abs\ sample)/Abs\ control \times 100$$

RESULTS AND DISCUSSION

As indicated in Table 1, the initial phytochemical screening of the methanolic extract of *L. camara* (MELC) and the EEAS identified several phytoconstituents, including flavonoids, PCs, TTPs, Tan, Saps, AA, prot, and carbs.

The GC-MS of the EEAS identified several bioactive phytochemicals, as illustrated in Fig. 2, with the corresponding compounds, retention time (RT), molecular weight (MW), molecular formula, and peak area %, as detailed in Table 2. The GC-MS examination of the MELC revealed many phytochemical ingredients, as shown in Fig. 2 and documented in Table 2.

Molecular docking (MD) studies were performed to know the interactions between critical residues of the 4EY5 active site and the phytochemicals derived from EEAS, involving 18 phytocompounds alongside a validated inhibitor. Protein–ligand complexes were evaluated according to their docking scores (binding energies). A comprehensive evaluation was conducted by analyzing the binding energies and critical residue interactions of each compound with the co-crystallized ligand. Table 3 presents the binding energy values for all tested compounds. In a similar vein, the MELC was identified to possess various bioactive compounds, such as Heptane, 4-ethyl-2,2,6,6-tetramethyl-, N, N-Dinitro-1,3,5,7- tetrazabicyclo [3.3.1] nonane, and Spiro[androst-5-ene-17,1′-cyclobutan]. -2′-one, 3-hydroxy-, and (3β,17β)-. The docking results for these components are shown in Table 3. Particularly in relation to AD, the chemical molecule 2H-Pyran-2,6(3H)-dione, dihydro has demonstrated promise as a neuroprotective agent. This substance, a derivative of pyran, is a structural component of many natural products and has been shown to have neuroprotective qualities. Its potential to lessen the cognitive deterioration brought on by AD has been specifically investigated.

Table 1: Phytochemical screening of *Alternanthera sessilis* and *Lantana camara*

Name of the phytochemical	EEAS	MELC
Carbs	+	+
AA	-	+
Prot	+	+
Alks	+	+
Glys	+	+
TTPs	+	+
Saps	+	+
Flavonoids	+	+
PCs and Tan	+	+
Cardiac glys	+	+
Ster	-	-
Gums	-	-

Where, Positive (+) means present and Negative (-) means absent

Fig. 1: Leaves of *Alternanthera sessilis* and *Lantana camara*

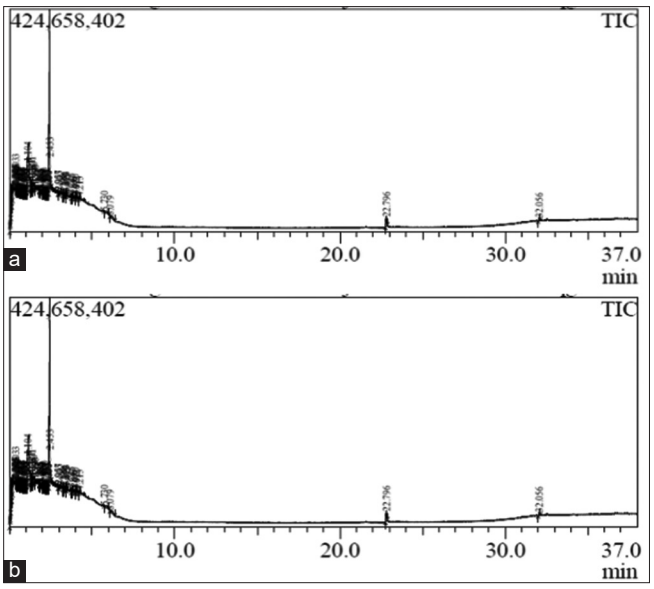


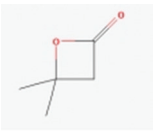
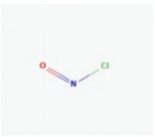
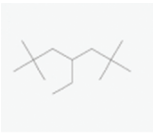
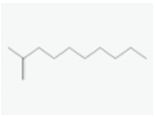
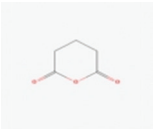
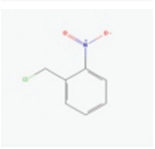
Fig. 2: Gas chromatography–mass spectrometry chromatogram of (a) ethanolic extracts of *Alternanthera sessilis* and (b) methanolic extract of *Lantana camara*

One medication used to treat AD is donepezil. A higher binding affinity that is comparable to or superior to the reference inhibitor is indicated by a lower (more negative) docking score for the relative binding affinity of 2H-Pyran-2,6(3H)-dione, dihydro.

PK properties analysis

The Swiss ADME server facilitated the analysis of the dynamic transport of drug-like molecules into the body by evaluating the ADME

Table 2: Bioactive compounds found in EEAS and MELC

RT	% Area	Name of the compound	Molecular formula	MW	Structure	Biological activity
0.033	6.59	2-Oxetanone, 4,4-dimethyl-	$C_5H_8O_2$	100		Antimicrobial activity, antioxidant activity and anti-cancer activity
4.213	0.18	Difluorinemonoxide	F_2O	54		-
2.957	0.41	Nitrosyl chloride	ClNO	65		-
0.860	0.87	Ethane, 1-chloro-1-fluoro-	C_2H_4ClF	82		-
0.765	0.11	Carbonic chloride fluoride	CClFO	82		-
1.104	20.93	Tetraborane (10)	B_4H_{10}	54		-
1.321	5.22	1-Propene, 3-fluoro-	C_3H_5F	60		-
1.371	2.12	Heptane, 4-ethyl-2,2,6,6-tetramethyl-	$C_{13}H_{28}$	184		antioxidant, antiinflammatory, and antibacterial activity
1.480	2.05	1-Decene, 2-methyl-	$C_{11}H_{22}$	154		-
3.849	0.26	N, N-Dinitro-1,3,5,7-tetrazabicyclo [3,3,1] nonane	$C_5H_{10}N_6O_4$	208		-
2.224	0.18	2H-Pyran-2,6 (3H)-dione, dihydro-	$C_5H_6O_3$	114		Anti-allergic Activity and Antimicrobial Properties
2.453	13.90	Benzene, 1-(chloromethyl)-2-nitro-	$C_7H_6ClNO_2$	171		-

(*Contd...*)

Table 2: (*Continued*)

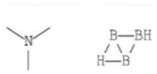
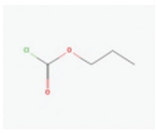
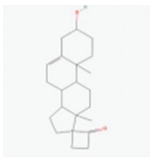
RT	% Area	Name of the compound	Molecular formula	MW	Structure	Biological activity
2.995	0.63	Methanamine, N, N-dimethyl-, compd. With tri borane (7) (1:1)	$C_3H_{16}B_3N$	99		Anticancer activity
3.980	0.32	2-Oxetanone, 4-methylene-	$C_4H_4O_2$	84		HMG-CoA synthase inhibitor
4.022	0.20	Propane, 1-chloro-	C_3H_7Cl	78		-
5.730	1.99	Carbonochloridic acid, propyl ester	$C_4H_7ClO_2$	122		-
6.079	1.64	Propanenitrile, 2,2-dimethyl-	C_5H_9N	83		Antimicrobial Activity
22.796	2.90	Oxirane, decyl-	$C_{12}H_{24}O$	184		-
32.056	0.88	Spiro [androst-5-ene-17, 1'-cyclobutan]-2'-one, 3-hydroxy-, (3. beta, 17. beta.)-	$C_{22}H_{32}O_2$	328		Androgenic Activity, Lipid Metabolism and Hypercholesterolemia, Anti-inflammatory Activity, Anti-inflammatory Activity

Table 3: Docking scores of compounds with 4EY5 and 2LMN

Name of the compound	Docking score (KCal/mol)	
	4EY5	2LMN
2-Oxetanone, 4,4-dimethyl-	−5.3	−7.5
Difluorinemonoxide	−7.3	−7.5
Nitrosyl chloride	−6.3	−6.3
Ethane, 1-chloro-1-fluoro-	−7.2	−8.2
Carbonic chloride fluoride	−6.6	−7.5
Tetraborane (10)	−7.6	−6.5
1-Propene, 3-fluoro-	−3.3	−8.3
Heptane, 4-ethyl-2,2,6,6-tetramethyl-	−6.4	−7.5
1-Decene, 2-methyl-	−8.7	−7.6
N, N-Dinitro-1,3,5,7-tetrazabicyclo [3,3,1] nonane	−7.5	−8.4
2H-Pyran-2,6 (3H)-dione, dihydro-	−8.9	−8.5
Benzene, 1-(chloromethyl)-2-nitro-	−7.6	−8.6
Methanamine, N, N-dimethyl-, compd. With tri borane (7) (1:1)	−7.4	−7.7
2-Oxetanone, 4-methylene-	−7.6	−7.5
Propane, 1-chloro-	−3.3	−8.4
Carbonochloridic acid, propyl ester	−6.4	−7.8
Propanenitrile, 2,2-dimethyl-	−8.1	−6.7
Oxirane, decyl-	−6.2	−7.8
Spiro [androst-5-ene-17, 1'-cyclobutan]-2'-one, 3-hydroxy-, (3. beta, 17. beta.)-		−6.7
Donepezil hydrochloride	−8.9	−8.7

characteristics of the selected 4 components. Every molecule conforms to Veber's rule, Lipinski's rule of 5, Ghose's rule, and Egan's rule. Every molecule contains a maximum of 10 hydrogen bond acceptors and exhibits molar refractivity ranging from 40 to 130, among other characteristics. All compounds exhibited high water solubility and significant lipid solubility. The ADME properties of the 4 designated compounds from EEAS are detailed in Table 4, while those of MELC are outlined in Table 5. Analyzing the likelihood that a drug will be administered orally is known as drug likelihood. By evaluating the drug molecular descriptor values, Lipinski's rule of five determines the drug's likelihood qualities; all of the values are constrained by a factorial of five. For the medicine to be administered orally, its MW should be <500 Daltons, its partition coefficient should be <5, its hydrogen bond donors should be <10, and its hydrogen bond acceptors should be <5. If there are more than two molecular description violations, oral delivery is not feasible.

MD

The phytoconstituents extracted from the EEAS and MELC were assessed for their potential interaction with AD targets, particularly the Aβ protein (PDB ID: 2LMN). Ligand molecules demonstrating the greatest binding capacity to the receptor were identified as prospective lead compounds. The interactions were further evaluated by iGEMDOCK tests, which indicated that the chosen phytocompounds exhibit significant anti-AD activity. Figs. 3 and 4 depict the findings of MD and validation.

Table 4: PK properties of phytoconstituents of EEAS

Name of the compound	Physicochemical properties		Water solubility log S (ESO)	Lipophilic log Po/w (iLOGP)	PKs GI absorption
	Num. H-bond acceptors	Num. H-bond donor			
2-Oxetanone, 4,4-dimethyl-	3	0	−2.63	2.05	High
Difluorinemonoxide	1	0	−1.42	1.40	low
Nitrosyl chloride	2	0	−1.18	0.93	High
Ethane, 1-chloro-1-fluoro-	1	0	−0.91	1.42	Low
Carbonic chloride fluoride	0	0	−4.50	3.44	Low
Tetraborane (10)	6	0	−1.80	1.26	Low
1-Propene, 3-fluoro-	3	0	−0.48	0.82	High
Heptane, 4-ethyl-2,2,6,6-tetramethyl-	2	0	−2.76	1.55	High
1-Decene, 2-methyl-	2	0	−0.54	1.15	High
N, N-Dinitro-1,3,5,7-tetrazabicyclo [3,3,1] nonane	0	0	−1.55	1.72	Low
2H-Pyran-2,6 (3H)-dione, dihydro-	0	0	−4.50	3.44	Low
Benzene, 1-(chloromethyl)-2-nitro-	6	0	−1.80	1.26	Low
Methanamine, N, N-dimethyl-, compd. With tri borane (7) (1:1)	3	0	−0.48	0.82	High
2-Oxetanone, 4-methylene-	0	0	−4.50	3.44	Low
Propane, 1-chloro-	6	0	−1.80	1.26	Low
Carbonochloridic acid, propyl ester	3	0	−0.48	0.82	High
Propanenitrile, 2,2-dimethyl-	0	0	−4.50	3.44	Low
Oxirane, decyl-	6	0	−1.80	1.26	Low
Donepezil hydrochloride	3	0	−0.48	0.82	High

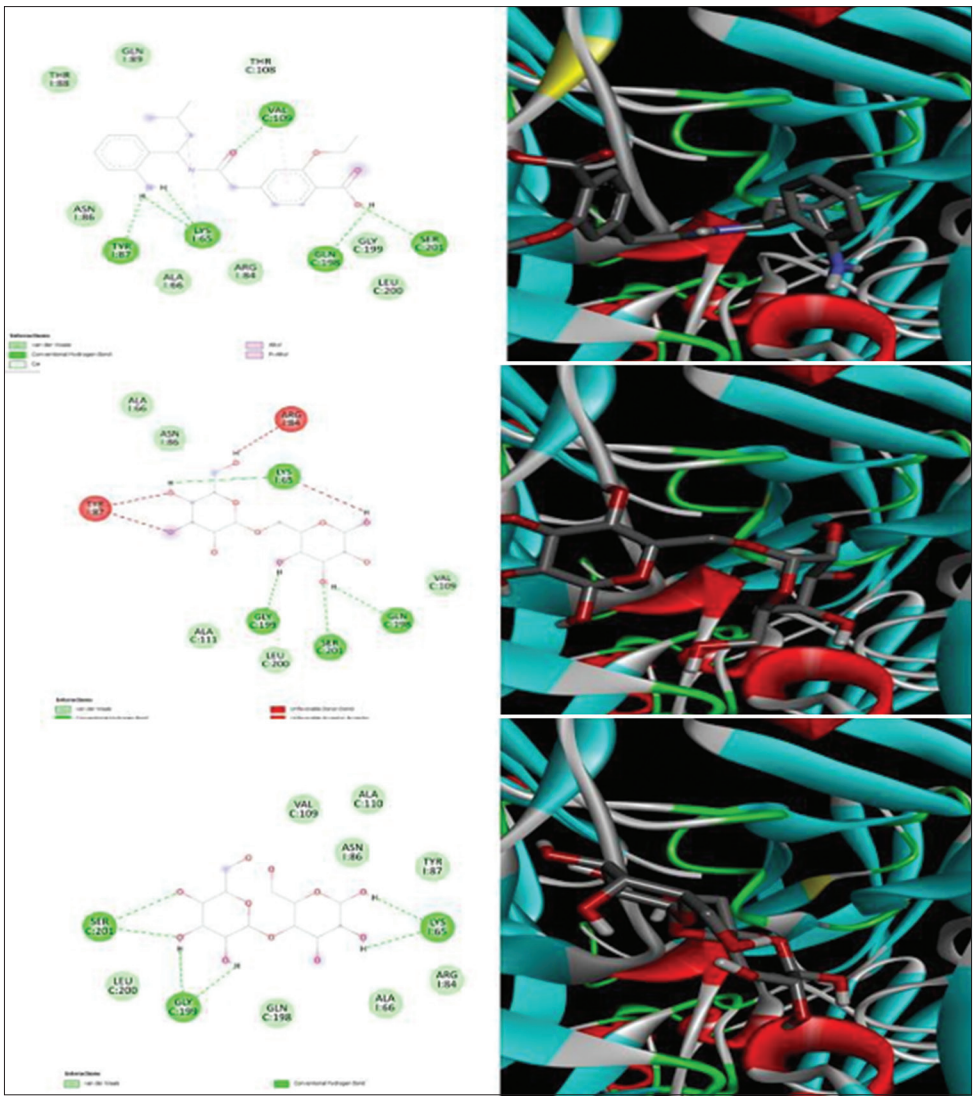


Fig. 3: Interaction of ligands with 4EY5 Docking conformation of 1-Decene,2-methyl 2H-Pyran-2,6(3H)-dione, dihydro- and Donepezil hydrochloride

Table 5: PK properties of phytoconstituents of MELC

Name of the compound	Physicochemical properties		Water solubility log S (ESO)	Lipophilic log Po/w (iLOGP)	Pharmaco kinetics GI absorption
	Num. H-bond acceptors	Num. H-bond donor			
2-Oxetanone, 4,4-dimethyl-	3	0	−2.63	2.05	High
Difluorinemonoxide	1	0	−1.42	1.40	low
Nitrosyl chloride	2	0	−1.18	0.93	High
Ethane, 1-chloro-1-fluoro-	1	0	−0.91	1.42	Low
Carbonic chloride fluoride	0	0	−4.50	3.44	Low
Tetraborane (10)	6	0	−1.80	1.26	Low
1-Propene, 3-fluoro-	3	0	−0.48	0.82	High
Heptane, 4-ethyl-2,2,6,6-tetramethyl-	2	0	−2.76	1.55	High
1-Decene, 2-methyl-	2	0	−0.54	1.15	High
N, N-Dinitro-1,3,5,7-tetrazabicyclo [3,3,1] nonane	0	0	−1.55	1.72	Low
2H-Pyran-2,6 (3H)-dione, dihydro-	2	0	−1.85	1.95	High
Benzene, 1-(chloromethyl)-2-nitro-	3	0	−0.48	0.82	High
Methanamine, N, N-dimethyl-, compd. With tri borane (7) (1:1)	2	0	−2.76	1.55	High
2-Oxetanone, 4-methylene-	2	0	−0.54	1.15	High
Propane, 1-chloro-	3	0	−0.48	0.82	High
Carbonochloridic acid, propyl ester	0	0	−1.55	1.72	Low
Propanenitrile, 2,2-dimethyl-	2	0	−1.85	1.95	High
Oxirane, decyl-	3	0	−0.48	0.82	High
Spiro [androst-5-ene-17, 1'-cyclobutan]-2'-one, 3-hydroxy-, (3. beta, 17. beta.)-	0	0	−1.55	1.72	Low

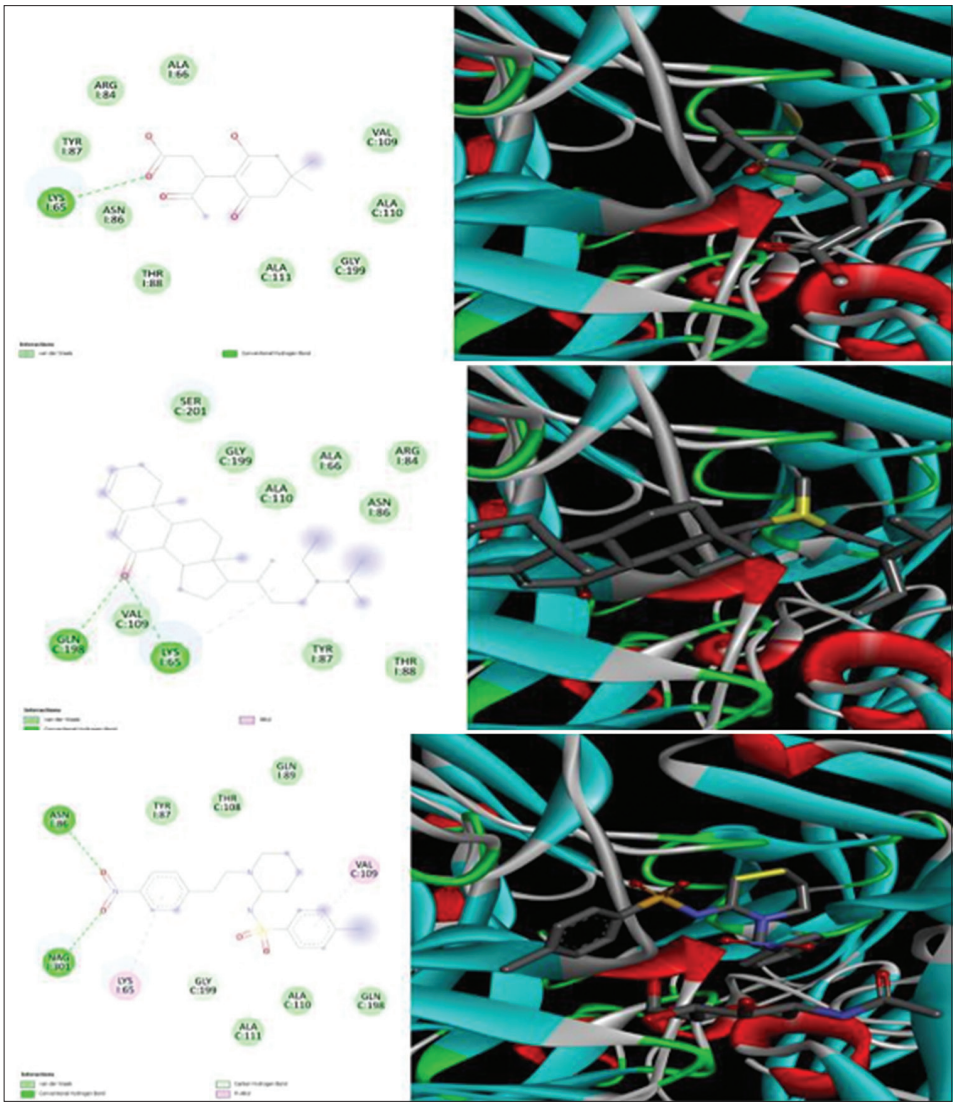


Fig. 4: Interaction of ligands with 2LMN Docking conformation of 2H-Pyran-2,6(3H)-dione, dihydro-, Benzene,1-(chloromethyl)-2-nitro- and Donepezil hydrochloride (Standard)

***In vitro* AOX study**

The AOX ability of the extracts of the leaf was assessed through their efficacy in reducing DPPH radicals, a mechanism that entails the donation of hydrogen atoms or electrons to transform DPPH into its non-radical form, 1,1-diphenyl-2-picrylhydrazine [25]. This reduction produces a discernible hue shift from deep purple to pale yellow, signifying free radical scavenging action [26]. The EEAS and the MELC were assessed through the DPPH test across a concentration range of 100–500 µg/mL. The IC_{50} values, indicating the concentration necessary to block 50% of DPPH radicals, were determined to be 382.33 µg/mL for EEAS and 204.35 µg/mL for MELC. The standard AOX L-ascorbic acid demonstrated IC_{50} values of 296.73 µg/mL and 180.45 µg/mL in the relevant experiments. The findings are depicted in Fig. 5. The EEAS and MELC extract demonstrated a comparable and significant concentration-dependent ability to scavenge free radicals, relative to the standard ascorbic acid (AA). The ethanol and methanol extracts displayed the most pronounced AOX activity compared to L-ascorbic acid, as evidenced by their lowest IC_{50} value at a higher concentration.

ABTS radical scavenging assay

The AOX efficacy of the extracts was assessed through the ABTS radical scavenging assay, which relies on the formation of the $ABTS^+$ radical cation through its interaction with potassium persulfate [27]. This blue–green radical is decolorized when it interacts with AOXs that can donate hydrogen atoms, signifying its neutralization [28]. The ABTS scavenging capacity of the EEAS and the MELC was evaluated against the standard AOX, L-ascorbic acid. Fig. 6 illustrates that an elevation in extract concentration is associated with an increased percentage of radical inhibition. The IC_{50} values, indicating the concentration necessary to block 50% of $ABTS^+$ radicals, were established at 233.01 µg/mL for EEAS and 327.47 µg/mL for MELC, whereas ascorbic acid demonstrated IC_{50} values of 170.92 µg/mL and 276.45 µg/mL, correspondingly.

Metal chelation assay

The metal chelating activity of the test extracts was assessed, and the IC_{50} values are illustrated in Fig. 7. The EEAS exhibited the weakest ferrous ion chelating potential, by an IC_{50} value of 370.87 µg/mL. In comparison, the MELC showed slightly better activity, by an IC_{50} of 306.69 µg/mL. EDTA, used as the standard, demonstrated the highest chelating efficiency, with IC_{50} values of 306.12 µg/mL and 276.45 µg/mL, respectively, as shown in Fig. 7. These results indicate that both plant extracts possess modest metal chelation ability compared to the standard.

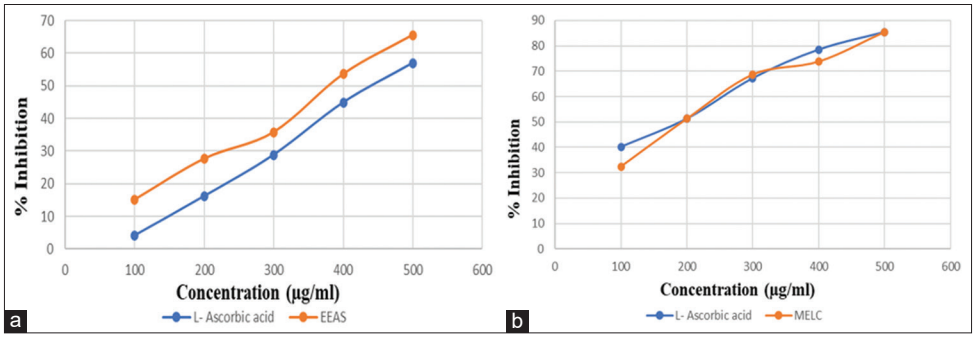


Fig. 5: % Inhibition of 2,2-diphenyl-1-picrylhydrazyl radical scavenging treatment of (a) ethanolic extracts of *Alternanthera sessilis* and (b) methanolic extract of *Lantana camara*

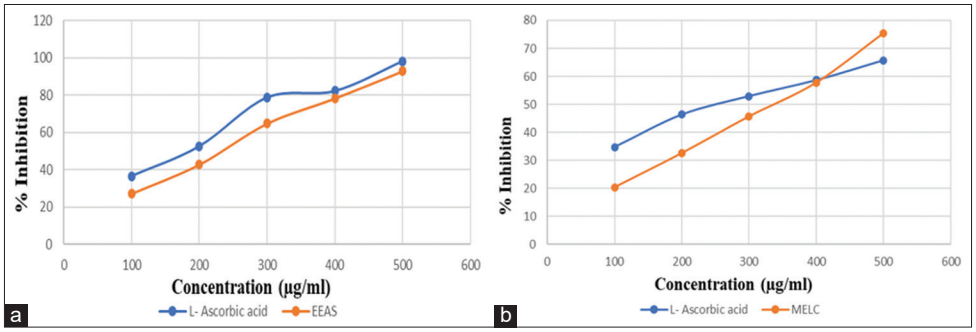


Fig. 6: % Inhibition of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging treatment of (a) ethanolic extracts of *Alternanthera sessilis* and (b) methanolic extract of *Lantana camara*

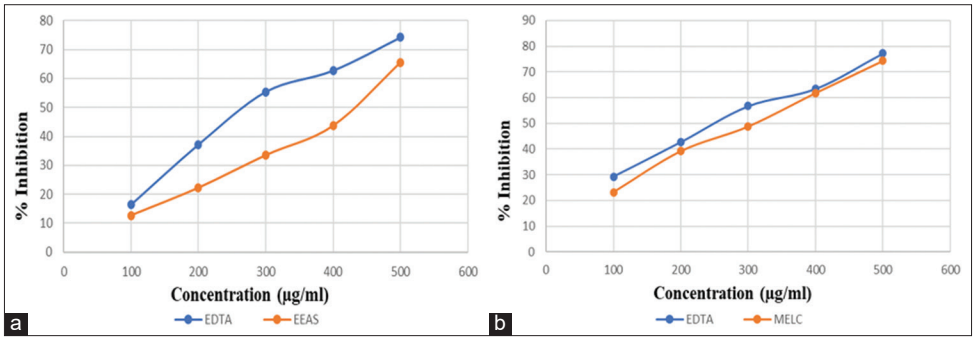


Fig. 7: % Inhibition of metal chelation treatment of (a) ethanolic extracts of *Alternanthera sessilis* and (b) methanolic extract of *Lantana camara*

DISCUSSION

Recent research has investigated plant-based compounds as potential treatments for AD, motivated by the limitations and side effects of existing therapies. AD is linked to reduced acetylcholine levels and increased oxidative stress, underscoring the potential therapeutic benefits of phytochemicals that possess AOX and anticholinesterase activities. Medicinal plant extracts, especially those utilized in traditional Nigerian medicine, encompass bioactive constituents such as flavonoids, Tan, Alks, and Saps. These compounds are recognized for their ability to mitigate neuroinflammation, oxidative stress, and apoptosis while maintaining neurotransmitter equilibrium and mitochondrial functionality. Chromatographic and GC-MS analyses confirmed the prevalence of flavonoids, including quercetin and kaempferol, which demonstrate significant AOX activity through mechanisms such as free radical scavenging, metal chelation, and inhibition of lipid peroxidation. MD studies indicated that these phytochemicals bind effectively to AChE through hydrogen bonds and π–π stacking, implying significant inhibitory potential. ADME analyses indicated that these compounds adhere to Lipinski's rule of five, possess appropriate TPSA and rotatable bond counts, and demonstrate favorable bioavailability. The phytoconstituents exhibited significant neuroprotective and anti-AD properties, supporting their potential as candidates for Alzheimer's treatment [5,29-34].

This work sought to assess the AOX and possible anti-Alzheimer activities of the EEAS and the MELC using *in vitro* tests and MD analysis. The initial phytochemical analysis verified the existence of many bioactive compounds, including flavonoids, phenolics, Alks, sterols, Tan, glycosides, and terpenoids in both extracts, which are recognized for their AOX and neuroprotective properties. The GC-MS analysis corroborated this by identifying a variety of phytocompounds, several of which have been documented to possess therapeutic benefits pertinent to neurodegenerative disorders.

The AOX assays, comprising DPPH, ABTS, and metal chelation techniques, indicated that both EEAS and MELC exhibit moderate to strong radical scavenging activity. In the DPPH experiment, MELC exhibited superior scavenging capacity ($IC_{50:}$ 204.3 µg/mL) relative to EEAS ($IC_{50:}$ 382.33 µg/mL), while both were less efficacious than the standard L-ascorbic acid. A comparable trend was noted in the ABTS assay, wherein MELC demonstrated superior AOX capacity ($IC_{50:}$ 327.47 µg/mL) relative to EEAS ($IC_{50:}$ 233.01 µg/mL), although both were less effective than the reference standard. In the metal chelating experiment, MELC exhibited a superior chelation capability ($IC_{50:}$ 306.69 µg/mL) compared to EEAS ($IC_{50:}$ 370.87 µg/mL); however, it was inferior to that of EDTA. The data indicate that MELC demonstrates a comparatively superior AOX capacity in the evaluated models, potentially because of an elevated content of active polyphenolics or metal-binding components.

MD studies offered additional insights into the neuroprotective capabilities of the discovered drugs. Phytoconstituents from both extracts were docked against targets associated with AD, specifically the Aβ-42 fibril (PDB ID: 2LMN) and AChE (PDB ID: 4EY5). Numerous substances, such as spiro-androstene derivatives and nitrogenous bicyclic molecules sourced from MELC and EEAS, exhibited robust binding affinities, comparable to or surpassing those of conventional inhibitors. The interaction of these chemicals with critical residues in the binding pockets indicates their potential to modulate pathogenic processes linked to AD. The drug-likeness and PK properties (ADME) of the top-docked composites, assessed via SwissADME, demonstrated advantageous qualities, such as high-water solubility, favorable lipophilicity, and adherence to Lipinski's and Veber's criteria. This further substantiates their potential as primary candidates for oral administration.

The findings indicate that extracts of both *A. sessilis* and *L. camara* have considerable AOX activity and include bioactive compounds that may inhibit targets associated with AD. The superior performance of MELC in AOX assays and docking indicates it may be a more promising candidate for further advancement. Both extracts require *in vivo* confirmation and additional mechanistic investigation to confirm their therapeutic efficacy against AD.

CONCLUSION

This work emphasizes the therapeutic potential of *A. sessilis* and *L. camara* as prospective sources of natural AOXs and anti-Alzheimer compounds. Both extracts exhibited significant AOX activity as evidenced by DPPH, ABTS, and metal chelation assays, with *L. camara* displaying somewhat greater efficacy. GC-MS profiling found multiple bioactive chemicals, which were then verified using MD against AD targets (Aβ and AChE), demonstrating substantial binding affinities and advantageous interactions. ADME research verified that the lead compounds exhibit drug-like characteristics and satisfactory PK profiles. These findings endorse the prospective application of these medicinal herbs in formulating neuroprotective treatments. Nonetheless, additional *in vivo* and clinical investigations are important to validate their efficacy and safety.

AUTHORS CONTRIBUTION

All authors contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHOR FUNDING

We have not received any funding.

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